5:7 Effects of storage in vacuum-packages, with CO_2, on the shelf life of beef mince - microbial observations

MADDEN, R.H. and MOSS, B.W.

Agriculture and Food Science Research Centre, Department of Agriculture for Northern Ireland, Newforge Lane, Belfast BT9 5PX

Introduction

As vacuum-packaging of primal cuts increases in popularity (Marriot et al., 1977) and replaces traditional sales of carcasses it also produces some problems. Frequently contracts placed for large quantities of beef in vacuum-packs do not account for the entire carcass. Hence the meat processors can dispose of primal cuts but have the problem of disposal of the forequarter meat, and other less popular cuts. The work described below had the objective of studying suitable storage methods for beef mince (ground beef) so that forequarter meat could be processed to give this simple, added-value product. Given adequate storage and shelf lives the beef could either be steadily released onto the local market or offered to the customers purchasing the primal cuts.

For the purpose of this study it was decided that the packaging systems used should be as similar as possible to those currently in use in Northern Ireland, or be suitable for implementation without major capital expenditure on the part of the processors. Hence vacuum-packaging was selected and this was supplemented by the addition of solid CO_2. Solid CO_2 pellets are available commercially and the anti-microbial effects of CO_2 are well known and are summarised by Clark and Takacs (1980). CO_2 pellets have the advantage of being simple to use, requiring no capital expenditure, and being much quicker to use than back-flushing techniques. Since vacuum-packaging is frequently the rate-limiting step in processing plants back-flushing could cause unacceptable delays by extending the time taken for packaging, with a consequent loss of quality by the meat due to extended storage in the boning-out hall.

Materials and Methods

The meat used was boned-out and packaged in a commercial abattoir, and was less than 48 hours old (post-kill). About 30 kg of forequarter meat was obtained and cut into 100-500 g pieces to resemble small pieces of trim. The pieces were then thoroughly mixed and treated as shown in Fig 1. Mincing was through a 4 mm plate.

The aliquots (2 kg) of mince and trim which were packaged with CO_2 had 4 g of solid CO_2 ("dry ice") added to the barrier bag prior to the addition of the meat. The CO_2 evolved caused significant swelling of the bags but within 24 hours it dissolved into the meat and it was impossible to distinguish the packages with CO_2 from those without the gas. The entire experiment was performed twice.

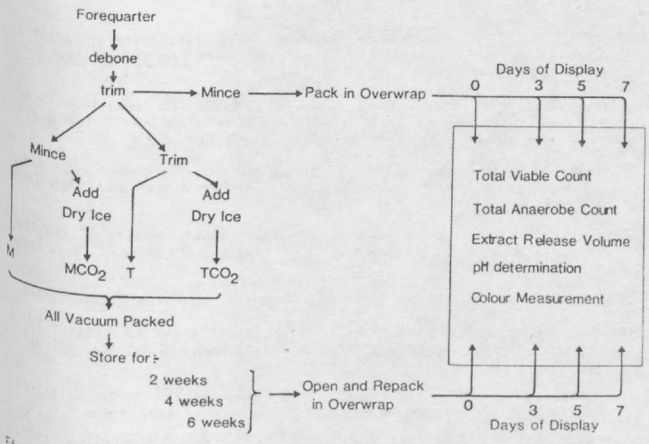

Fig. 1 Outline of experimental procedure

Analyses performed consisted of:
Total viable count (TVC) - catalase-positive colonies on nutrient agar (Oxoid) incubated at 22°C for 3 days. This enumerates those organisms likely to cause aerobic spoilage.

Total anaerobe count (TAC) - catalase-negative colonies on brain-heart infusion yeast-extract agar (BHIYE) (Baird and Patterson, 1982) incubated for 3 days at 22°C in an anaerobic cabinet (Forma Scientific Model 1024, Marietta, Ohio, USA) using an atmosphere of 85% N_2, 10% H_2 and 5% CO_2 (all vol/vol). This enumerates principally the lactic acid producing flora which will be referred to under the general name of "meat lactics".

Extract release volume - the method of Jay (1964) was used. High ERV's indicate fresh meat, falling as the meat undergoes aerobic spoilage.

pH - A 1:10 (wt/vol) homogenate of the meat in distilled water was subjected to a pH measurement.

Colour measurement - A Pye-Unicam SP8-200 linked to an Apple II microcomputer was used to measure and store colour measurements. Subsequently the collected results were analysed on the mainframe computer.

All samples were stored at 1-2°C.

Results and Discussion

The results were subjected to analysis of variance (Lawes A.T., 1980) and the following statistically significant interactions between the parameters were noted (Table 1). The figures in brackets denote confidence as a percentage. "Method" applies to the treatment before packaging, i.e. whether the meat is minced or left as trim.

TAC and TVC were expressed as $\log_{10}$ (colony forming units) g^{-1} of meat for the analyses and when mentioned below they will be assumed to be in these units unless otherwise stated.

TVC, storage, display, CO_2	(99.9)		
TAC, storage, display, CO_2	(99.9)	TAC, method, CO_2	(95)
TAC, method, display	(99.9)	TAC, method, storage	(99)
pH, storage, CO_2	(95)	pH, method, CO_2	(95)
ERV, storage, display	(95)		

Table 1 Parameters showing statistically significant interactions (confidence in brackets as percentage)

Considering the microbial populations studied it can be seen from the above table that complex interactions between the effects of storage, display and CO_2 addition are found. Since storage was from a period of from 0-6 weeks it is not surprising to find that significant changes in TVC and TAC occurred. Similarly samples displayed for up to 7 days would be expected to change in terms of TVC and TAC and it would be unlikely for samples of fresh mince to show a similar pattern of microbial change to samples stored for 6 weeks in a vacuum-pack. Thus storage and display parameters would be expected to appear as significant in the statistical analyses performed.

The next parameters of interest are CO_2 (its presence or absence) and method. It can be seen that the TVC is affected by CO_2 as is the TAC but the method significantly affects the TAC only. The effects of the method during storage are not unexpected since the growth of anaerobes will be affected by nutrient availability which would be expected to be higher in the minced meat since a higher surface area will be exposed for colonisation. However the TVC will enumerate organisms requiring oxygen for growth and these will grow where the oxygen concentration is highest - immediately adjacent to the packaging film. Therefore the TVC is more likely to reflect the available surface area of the package than the form of the meat in the package.

Figure 2 illustrates several of these points. It can be seen that CO_2 slows the growth of the anaerobes in trim and that this effect is most marked after two weeks but then declines until after 6 weeks no difference is visible. Thus CO_2 reduced the rate of growth of the anaerobes but not their ultimate population density. Initially mince with CO_2 shows a similarly reduced rate of growth to trim with CO_2 but after 4 weeks the effect diminishes and ultimately the TAC is about 5-fold higher than in trim. This supports the hypothesis that a higher surface area, as in mince, provides a better environment for the proliferation of anaerobes. Thus any adverse effects on shelf life caused by the anaerobes will be exacerbated by mincing prior to packaging.

Figure 2 also shows that after 4 weeks of storage the TAC is near its maximum hence little change in numbers can be expected when these samples are put on

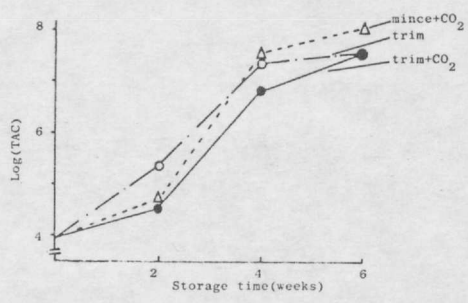

Fig.2 Effects of CO_2, treatment, and storage on the TAC.

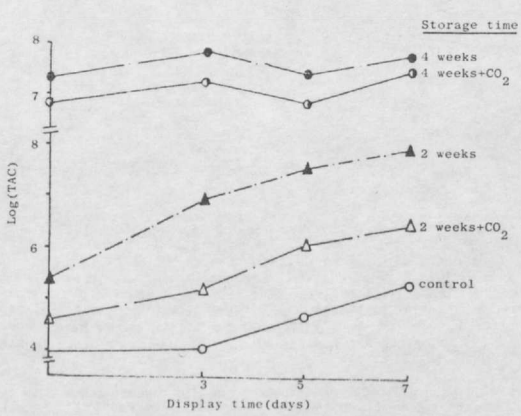

Fig.3 Effects of storage and CO_2 on the TAC of trim, when subsequently minced and displayed.

display. This was found to be the case and the results are shown in Fig 3, which shows the results obtained with trim. After 4 weeks of storage the TAC is slightly higher in the absence of CO_2 and this differential is maintained over the display period of 7 days, during which time only a slight increase in numbers occurs.

However when the results after 2 weeks of storage are compared with those of the control marked differences can be seen. The control shows a lag phase of 3 days duration which is probably due to microbial adaptation to the chill temperature, low temperatures being known to extend lag phase (Olson and Nottingham, 1980). The trim lacking CO_2 shows no such lag phase, presumably due to the microbial population having already adapted to the environment presented by the chilled meat during the period of storage.

Intermediate between these two results are those obtained from the trim packaged with CO_2. Although the meat was subjected to a major change prior to display - being minced and repacked in aerobic conditions - a clear difference between the displayed samples is seen. Initially the CO_2 treated sample had a TAC only 16% of that of the untreated sample but after 7 days on display this had fallen to 3%. From Fig. 3 it can be seen that the major reason for this is the absence of a lag phase in the absence of CO_2 whilst in the CO_2-treated sample the TAC appears to exhibit a lag phase.

Such "residual" preservation effects, where microbes show reduced growth even after being removed from the atmosphere causing the growth reduction, have been reported before. Silliker et al. (1977) found such residual effects after storage of meat in CO_2 when studying pork and beef but neither of these meats were in a comminuted state and their results related purely to organisms enumerated, aerobically, on Plate Count Agar. The quantity of CO_2 used by Silliker et al. is difficult to compare with this work since they used 55 gallon (US) steel drums filled with atmospheres containing 50% CO_2, hence about 90 ℓ of CO_2 would be present with their meat samples. Nonetheless Silliker et al. observed these effects with aerobes and the above results show that anaerobes can be similarly affected. This is important since the TAC showed much more rapid growth on the trim pieces than those reported for beef slices. For example Sutherland et al. (1975) found that counts of $10^7/cm^2$ were not reached until after 9 weeks of storage whilst in this study such a count was obtained (per gramme) after 2 weeks storage followed by 3 days of display (Fig. 3). Such rapid growth of anaerobes, which were largely lactic acid bacteria, could lead to sour spoilage of the mince (Sutherland et al., 1975).

Conversely the production of lactic acid, and the resultant fall in pH, has been shown to inhibit Gram-negative psychrotrophs (Gill and Newton, 1982) and also fermentative Gram-negative bacteria (Grau, 1981), both of which are organisms capable of causing spoilage of beef. Despite the high numbers of anaerobes found during this study no samples appeared to be sour-spoiled on opening the vacuum-packages but the colour results, to be discussed later, suggest that lower numbers of anaerobes may be preferable.

Considering the TVC results during display, Fig. 4, these again show residual preservation effects from the CO_2, as reported by Silliker et al. (1977). If the TVC after 2 weeks storage with CO_2 is compared with the control both show a lag phase lasting 3 days. The curves are virtually identical. In the absence of CO_2 no lag phase is found, paralleling the results observed with the TAC. However in contrast with the TAC little growth of aerobes is seen during storage, as would be expected in a vacuum-package (Dainty et al., 1979). Thus the CO_2 residual preservation effect again appears to act by extending the lag phase of the bacteria, the effect noted by Silliker et al. (1975).

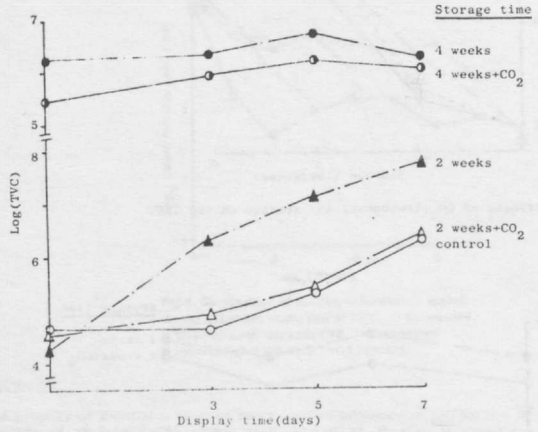

Fig.4 Effects of storage CO_2 and display on the TVC.

However during storage of more than two weeks a second effect acting on the TVC was noted. It can be seen from Fig. 4 that after 4 weeks of storage with CO_2 the TVC is about 10-fold greater than that found after 2 weeks. But during display growth is slight, whilst the sample lacking CO_2 shows virtually no growth at all. The results for samples stored for 6 weeks are not plotted since they are very similar to those found after 4 weeks. Thus aerobic growth apparently ceases at a TVC of roughly 10^6. In our laboratory we have observed TVC values 10^9-10^{10} on mince and similar results have been reported (Edwards et al., 1983).

The cessation of growth of the aerobes could be due to two effects:

a) nutrients being metabolised by the anaerobes leaving little readily metabolisable material for the aerobes.

b) production of inhibitory compounds by the anaerobes.

The work described here does not allow either of these possible reasons to be supported or rejected, and in fact the observations may result from a combination of the two. Dubois et al. (1979) isolated lactic acid bacteria from mince which could inhibit potential aerobic spoilage organisms while Simonetti et al. (1982) found that some Clostridium sp. were inhibited by lactobacilli. This previous work showed that lactic acid bacteria can produce compounds inhibitory to other organisms and that the inhibition was not simply due to lactate or reduced pH.

Further work would be required to investigate the possibility of nutrient limitation but since most aerobes associated with the aerobic spoilage of beef would be Pseudomonas sp. (Sutherland et al., 1975; Edwards et al., 1983) then a lack of soluble carbohydrate would be expected to result in the organisms attacking alternative nutrients such as the proteins and fats. This would rapidly lead to off-odours such as putrescine being produced (Edwards et al., 1983). Such off-odours were not evident during this work.

Overall, therefore, it was seen that solid CO_2 addition could slow the growth of both aerobic and anaerobic bacteria on beef pieces when vacuum-packaged and stored at 1°C. This effect was most marked after 2 weeks storage and residual effects, consisting of an extended lag phase, were apparent when the meat was unpacked, minced and placed in overwrap packs, on display, at 1°C. During subsequent storage the bacteria in CO_2 treated packs increased in numbers until a population slightly less than that in untreated packs was seen. Vacuum-packaged mince supported a higher population of anaerobes than did vacuum-packaged trim. The population of aerobes was not affected by the form of the meat in the vacuum packages.

From a microbiological viewpoint, therefore, beef intended for the production of mince was best stored vacuum-packaged in the presence of CO_2 and minced after storage.

At the time of writing only preliminary results are available from the colour analyses. These showed that all stored samples produced a brighter and redder mince than the control. The samples showed a reduction in both of these values during display and the reduction was most rapid in the samples stored the longest. CO_2 addition, and mincing after storage, appeared to reduce the rate of decay of the brightness and redness of the mince. Hence the colour results support the microbiological results in that samples with the fewest microbes showed the best colour properties, and hence CO_2 addition to meat stored in vacuum-packs was beneficial and would give the longest shelf life of the systems studied.

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