PACKAGING FRESH AND CURED MEATS
SESSION K: CHANGES DURING STORAGE

G.A. GARDNER,
Ulster Curers' Association, 2 Greenwood Avenue,
Belfast BT4 3JL,
N. Ireland.

It is not my intention to attempt a comprehensive review of the microbiology of packaged meats with reference to changes in microflora and shelf life, but to highlight some of the reasons why we are interested in such aspects, briefly mentioning the factors which are known to influence microbiological changes in packaged meats, some of which can be introduced in the laboratory at the time of analysis.

Researchers are interested in changes during storage from the fundamental microbial ecological aspect and how they relate to deteriorative changes, e.g. the biochemical aspects of spoilage. Such data can and has led to the adoption of various preservative procedures, resulting in increased stability of meat products, thus reducing loss and wastage due to spoilage.

This leads to the subject of microbial associations. These are generally specific for each type of meat product, thus giving criteria by which to assess changes in the technology of meat and meat product production. Also, one can recognise the important spoilage species for a particular meat under known production and storage circumstances, determine sources of contamination, and hence instigate changes in procedure to minimize the risk of contamination.

As our knowledge and understanding of microbial and associated biochemical changes which occur in meat storage are increased, bacteriological tests and chemical indices for use in quality control programmes and in shelf-life predictions can be devised. In addition, such data is extremely valuable in the investigation of customer complaints regarding keeping quality at factory level.

The fate (i.e. death, survival, or growth) of bacteria which are important in public health - Salmonella, Staphylococcus and clostridia - must also be considered. Such data is essential in the evaluation of new production procedures, e.g. vacuum packaging, so that one may be assured or otherwise that such changes will not lead to a higher risk product, as far as foodborne pathogens are concerned.

In summary, studies in changes during storage can lead to continuous improvements in the stability and wholesomeness of all meat products for human consumption.

There are a number of factors which are known to affect microbiological changes, both quantitatively and qualitatively in packaged meats.

1) There is some indication that the origin of the meat is important, i.e. there are differences in the spoilage floras between beef, lamb and pork. Thus principles which apply to one meat may not necessarily be applicable to the others.

2) Microbial growth is also influenced by the type of tissue examined - musculature, adipose tissue, skin, connective tissue, and even tissue exudates. In some work on packaged liver (Gardner, 1971) spoilage occurred in the "drip" within the pack before the liver tissue spoiled. Also, bacterial growth on the fat of sliced vacuum packed bacon is more rapid than on the meat. But it is not until the counts on the meat reach high levels that spoilage is evident.

3) When considering meat, the inherent physical and chemical state of the muscle has a profound influence, e.g. pH and eH. Some muscles even within a single slice of bacon will spoil at different rates with different microfloras.

4) Temperature also has a profound influence, e.g. storage temperature - whether the meat is frozen, refrigerated or non-refrigerated. One must also include heat treatments such as used for pasteurised hams. In relation to storage temperature, some interesting results would be found with varied temperature conditions. What is needed is to simulate in the laboratory what is likely to happen in practice, by employing a combination of both refrigerated and non-refrigerated conditions in the one treatment. We have such an example in paper K3 of this session.

5) The available water or equilibrium relative humidity of the meat will also affect the microbial changes. This applies particularly to cured, salted, dried and smoked meats. But in packaged meat a water impermeable film is invariably used to prevent product weight loss. This will mean that the available water on the surface of the tissues will be much higher than on unpackaged material, where some drying would have occurred, thus creating conditions more conducive to microbial growth.

6) The gaseous environment in which the meat is held is also important. As well as gas composition within the packs, the volume of headspace must be considered, e.g. vacuum packing. The inhibitory effects of carbon dioxide have been recognised for many years, and this has an influence on the microbiological picture. In vitro studies have shown that high concentrations of oxygen have an inhibitory effect on some microorganisms, but we need more data onwhether this applies to meat packing. Nitrogen packing has changed the microbiology of the meat, but whether this is due to a direct effect of nitrogen or the absence of some other gases is not yet fully established.

7) There are numerous additives which are used in meat product manufacture, all of which are known to influence the microbiology. These include nitrite, possibly nitrate, ascorbic acid, gluco-delta-lactone, sulphite, polyphosphates and smoke components.

8) Finally, there is the possible presence of bacterial interactions within the microflora development during storage. Such phenomena of microbial antagonism have been demonstrated many time in the in vitro situation. There is an obvious need for further study of what occurs on meat.

There are undoubtedly other factors, which contribute to the microbial ecology of meat spoilage, and more research is still needed for their elucidation.

Before discussing the papers in this section, it is important to remember that microbial changes as measured in the laboratory are in many instances a function of the techniques employed. These include the media, dilution procedure, temperature and time of incubation of the plates.

For example, the fresh meat organism Microbacterium thermosphactum has assumed importance in recent years because of two factors, medium and incubation temperature. In glucose-free media and at temperatures over 30° this species is no longer catalase positive (Davidson, Mobbs & Stubbs, 1968) and would be classified as belonging to the Lactobacillaceae. Formerly only few workers incubated plates at 25°C or less. Secondly, in my own work (Gardner, 1973) I have recognised an important species of cured meat spoilage as belonging to the genus Vibrio. This organism will not grow well in media with less than 2% sodium chloride and rapidly dies in salt-free diluents. Thus all laboratory procedures should be included in published work, so that, even retrospectively, differences of opinion may be resolved.

The first paper in this session (K1) by the Russian authors Koulikovskaya, Balandina and Piskaryov is entitled "Morphological changes in the psychrophilic bacteria during chilled meat nitrogen storage". Using electron microscopy, various morphological changes were studied, when a species of Pseudomonas fluorescens, a common spoilage organism of meats stored in air, was subjected to holding in an atmosphere of 99% nitrogen at 0°C.

Plates of meat-peptone agar were inoculated with the organism, incubated at 25° for 48 hrs, transferred to the atmosphere of 99% nitrogen, and examined after 3, 6 and 9 days both qualitatively for morphology of cells and also quantitatively by determining the number of viable cells.

The normal (control) cells were rod shaped, 2 μ long by 0.5 μ wide, with 1-3 polar flagellae. The cell cytoplasm has a high electron-optical

density. During storage, granulations within the cytoplasm of varying densities appeared; detachment of the outer membranes from the protoplasm and eventually the breakdown of the cells was observed. This was accompanied by a loss in viability. The authors conclude that these changes are brought about by oxygen starvation, which resulted in the breakdown of the high molecular weight compounds to smaller molecules within the cells, a lysis phenomenon, resulting in complete autolysis of the cells.

This work does, in part, give some indication of why the normal Pseudomonas type spoilage microflora does not usually develop on vacuum packed beef. However, more quantitative data is needed to show that the effects described can be wholly attributed to the nitrogen, e.g. what was the other 1% of the atmosphere? Coyne (1932) found that all species of Achromobacter, Flavobacterium, Micrococcus, Pseudomonas, Aerobacter, Bacillus and Proteus isolated from fish were able to grow as well in an atmosphere of nitrogen containing $< 0.3\%$ of O_2 as they would in air. Partmann, Frank & Gutschmidt (1970) concluded after much experimentation in the packaging of beef, veal and pork that "small oxygen concentrations (1%) in the presence of nitrogen offered scarcely any advantage compared with storage in air". Ledward, Nicol & Shaw (1971) showed that there was no inhibition of the growth of a Pseudomonas at 5°C in an atmosphere containing 0.8% oxygen. At 0.2% O_2 they found 75% inhibition and in an oxygen free system, total inhibition. Therefore oxygen requirement is quite low for these bacteria.

Some additional information on the survival of the organism would be useful in assessing the results from a practical point of view. Have the authors any indication that there would be a difference between their work on a meat-peptone agar and meats? Does pH have an effect? Does CO_2 exert any influence? Is there a relative humidity influence?

Roth & Clark (1972) and Patterson & Sutherland (1973) showed that when aged vacuum packed beef was subsequently stored in air, spoilage could be brought about by the normal pseudomonad type flora. This implies that the organisms had survived in the vacuum pack and, although initially constituted only a small proportion of the total microflora, could rapidly outgrow the other species present.

It is also known that some species of Pseudomonas can grow and cause spoilage in vacuum packed beef. Nicol, Shaw & Ledward (1970) demonstrated that Pseudomonas mephitica could grow in vacuum packed beef at 1-2°C in an atmosphere of ca. 1% oxygen, particularly when the pH of the meat was greater than 6.0, and cause "greening". This was shown to be due to hydrogen sulphide production by the organism, which reacted with the myoglobin to produce a green pigment, sulphmyoglobin. Lapin & Koburger (1974) found a similar situation with Pseudomonas putrefaciens causing spoilage of shrimp

can cause spoilage of vacuum packed bacons, particularly under conditions of low salt cure, high pH meat and non-refrigerated storage conditions. Normally the spoilage of vacuum packed bacon is similar to that of vacuum packed uncured meats - a predominantly Lactobacillus flora.

There is an obvious need for further work on the mechanisms of inhibition of Pseudomonads in vacuum packed meats. The ecological conditions are extremely complex and individual factors difficult to isolate. Much in vitro work may not apply directly to an in vivo situation. However, further experimentation along the lines indicated in this paper will undoubtedly add to our knowledge and understanding of the problem.

The second paper in the session (K2) by Naumann & Balasundaram of the University of Missouri is entitled "Extending the package life of fresh beef through sanitation and formulated gaseous atmospheres".

The authors prepacked beef steaks from loins which had been previously treated for 1 min at 57°C with a solution of 4% acetic acid, and subsequently stored them in four atmospheres.

1) Air, which they refer to as ambient air treatment.
2) Enclosed in a gas impermeable pouch in - (a) Air. (b) 85% air 15% CO_2. (c) 85% oxygen, 15% CO_2.

The steaks were stored at -1.1°C and subjected at various time intervals (up to 20 days) to visual appraisal and microbiological evaluation, using a standard plate count procedure.

The aim was to measure the effect of reduced bacterial load on the raw material for prepacking, the effect of elevated levels of carbon dioxide and oxygen on the colour and bacteriological stability of the meats.

From the results given in Table 1 the effect of the acetic acid treatment is very marked (ca. 0.04% survival). However, steaks prepared from these loins had relatively more bacteria than those prepared from the control (high count) loins. I have calculated that the counts on steaks were in the order of 15% of that of the treated loins, while only 0.19% of the controls. The authors noted this in their paper, and we would be interested to hear if they have any further data on this point. As such a treatment has obvious commercial application, some additional information on the appearance of the loins or prime cuts would be useful, e.g. are there any discolouration problems as noted by Biemuller, Carpenter & Reynolds (1973), who worked with pork carcases? Can such a system be used before vacuum packing large cuts of meat destined for further breakdown elsewhere? In 1965 Mountney & O'Malley found that when acetic acid was used with poultry meat, it had a marked influence in extending shelf life, but was unacceptable because of its pungent odour. Is there a flavour effect on the beef loins or steaks?

Also, I feel we should ask the authors if they have any qualitative data on the microflora. Is acetic acid at 55-60°C selective in its effect? In other words, how does the microflora change by the treatment? Are, for example, potential spoilage species such as lactobacilli, M. thermosphactu and the Gram negative species such as the Pseudomonas-Achromobacter group sensitive or resistant? Such a treatment could change the types of bacteria which will cause spoilage, and further work in this area would be very valuabl

Turning now to the effect of the gaseous environment on subsequent keeping qualities, the authors found that shelf life in all aspects examined was markedly superior, when the steaks were stored in 15% CO_2 and 85% O_2. This confirms earlier work in their laboratory (Naumann, Gonzales & Yeh, 1971) and also that of Clark & Lentz (1972) and some earlier work of Georgala & Davidson (1970), which has been patented. There is a need for more data on the changes in the species of bacteria which grow on the meats and cause spoilage. Are the authors in a position to tell us whether the types of spoilage are different, depending on the gaseous environment chosen?

It has been shown many times that carbon dioxide has an inhibitory effect on the growth of meat spoilage bacteria. This, however, is selective, e.g. Gram negatives being more sensitive than Gram positives such as M. thermosphactum or the lactic acid bacteria. Extensions in keeping quality are merely a reflection of the differences in growth rates, i.e. the lactobacilli will grow more slowly than the pseudomonads, and their souring type of spoilage is less easily detected and not so objectionable as the putrid off-odours caused by the Gram negatives.

Paper K3 deals with the survival of potential food poisoning bacteria on vacuum packed meat. The work of the authors involved the inoculation of 3 strains of Staph. aureus (including one which produces Enterotoxin A), 2 strains of Salmonella (S. muenster and S. dublin), and 1 strain of Clostridium welchii, each at 2 levels onto small pieces of beef, which were vacuum packed and stored for up to 8 weeks at 0-2°C. Some stored vacuum pack were repackaged in an oxygen-permeable film and stored for a further 3 days at 15°C. A key reference, Patterson & Sutherland (1973), is missing from the paper and it can be found in the Proceedings of the 19th European Meeting of Meat Research Workers, Paris, 1, 327.

In summary, they found that all strains could survive for varying times in vacuum packed beef, but showed no potential for growth either in the vacuum or subsequent aerobic high temperature storage. They do state that "after 8 weeks in the vacuum package there was evidence of multiplicatio of the test organism (Cl. welchii) at 15°C, provided the meat was kept in the evacuated bag". However, on examining the results in Table 3, I find it difficult to accept this point. Variation between samples probably [illegible]

The growth or survival of potentially pathogenic bacteria in vacuum packed meats will be influenced by a large number of factors, as discussed earlier, but perhaps the most important of these would be storage temperature. I am sure the authors would agree that it would have been highly unlikely that any of the species would grow on meat at 0-2°C. Nevertheless, what is interesting is that there was no evidence of growth, when the meats were subsequently stored at 15°.

Angelotti, Foter & Lewis (1961) found that the minimum growth temperature in foods for Salmonella was 6.7°C, for S. aureus 5.6°C, and for Cl. welchii 15°C. Enterotoxin production by S. aureus did not occur below 18°C. Shaw & Nichol (1969) studied the growth of a Salmonella oranienburg on slices of beef in various concentrations of oxygen, carbon dioxide and nitrogen. The lower limit for growth was 8°C, and they concluded that only by reducing the temperature to below 7°C can the growth of Salmonella be completely inhibited on chilled meat.

Thus under refrigeration (i.e. below 4°C) there is no likelihood of a food poisoning hazard with packaged meats, but it must not be forgotten that neither the organisms nor their toxin will be destroyed.

At this point we would have a number of questions.

1) Can the test species used in this paper grow in 3 days at 15° on artificial media or even more relevant, can they grow on meat even under other storage conditions? Also, we can ask whether the organisms are of meat origin or have any connection with the meat environment.

2) Was, for example, the pH of the meat too low for rapid growth? In other aspects of meat bacteriology the pH of the musculature has a profound effect on phenomena of survival, growth and spoilage.

3) The authors postulate, citing a number of references, that growth of the pathogens may well have been inhibited by the normal spoilage flora. It would be interesting to hear what the microbiology in terms of the non-pathogenic flora of the meats was during the 15° storage trial, e.g. were the slices spoiled or did they show any signs of deterioration? In other words, some quantitative data is needed. The inhibition of pathogens by food spoilage bacteria has been well demonstrated, but there is an urgent need for a more detailed and critical study of the mechanisms involved.

Although it will take some time and work to finally establish if vacuum packing of beef will increase the risk of meat-borne food poisoning, the information to date suggests that the situation is no worse than with unpackaged beef. Vacuum packed beef can still be regarded as a source of salmonellae and staphylococci, which might contaminate other foods, and the position of Cl. welchii does not change, in that its importance in cooked meats, particularly reheated meats and gravies, has been well established.

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