5:1 The microbiology of vacuum-packaged pork

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Introduction

Vacuum-packaged beef is a major export commodity for Austalia. Trade in this product is possible because of a storage life under commercial conditions of ca. 10 weeks. Under laboratory conditions, storage lives of up to 15 weeks at 0°C have been reported (Newton and Rigg, 1979; Egan, 1983). To achieve a long shelf life only meat of low pH (<6.0) must be packaged and the packaging material must be of low gas permeability. When spoilage does occur it is due to the development of flavours and/or odours commonly described as sour, acid or cheesy. These are caused by the growth of lactic acid bacteria (Dainty et al., 1979; Egan and Shay, 1982; Egan, 1983).

If meat of high pH (>6.0) is packaged, spoilage is usually more rapid and characterized by changes in appearance, especially greening of the weep. When packs are opened putrefactive odours are detected. Spoilage of this type is caused by Gram negative bacteria especially Alteromonas putrefaciens (which is a strong producer of hydrogen sulphide) and psychrotrophic Enterobacteriaceae (Nicol, Shaw and Ledward, 1970; Bem, Hechelmann and Leistner, 1976; Taylor and Shaw, 1977; Patterson and Gibbs, 1977; Gill and Newton, 1979; Newton and Gill, 1980; Erichsen, Molin and Möller, 1981).

Although vacuum-packaged pork is not exported, limited amounts are used in the local hotel and restaurant trade. Processors report a shelf life of only 2-3 weeks under commercial conditions at temperatures up to 5°C, and this is considered inadequate even for local use. Since there is a higher incidence of high pH meat with pork than there is with beef, the packaging of meat of high pH may be a major factor causing the inadequate shelf life.

In contrast to beef there have been very few studies of the microbiology of vacuum-packaged pork. Hermansen (1980) reported that the spoilage of high pH pork was putrefactive and caused by Alteromonas putrefaciens. In contrast, low pH meat spoiled due to the development of a sweet-sour odour caused by B.thermosphacta and lactic acid bacteria. This worker has recently suggested a shelf life under commercial conditions of 1-3 weeks at 2-4°C for vacuum-packaged retail cuts of pork (Hermansen, 1983).

We have examined the growth of the microbial flora on the lean, fat and skin surfaces of vacuum-packaged pork stored at both 0°C and 5°C. Using the laboratory taste panel, the shelf life of vacuum-packaged pork of low pH has been estimated and found to be only about half of that of beef stored under similar conditions. The shelf life of vacuum-packaged pork is discussed in relation to that of beef.

Materials and Methods

Most procedures have been described in detail previously (Campbell et al., 1979; Egan and Shay, 1982). Pork loins and beef striploins were obtained from local processors. Pig carcasses were 1-2 days post mortem and meat samples were selected on the basis of surface pH immediately after boning. In most experiments loins were "skin-off".

Samples for microbiological analysis were obtained by excision using a sterile cork borer of known cross-sectional area. The samples were of total area 10 cm^2 except those used in experiments in which the meat was presented for taste panel evaluation. In such cases, a sample of 5 cm^2 was excised from each of the three packs of each treatment being tested and the three samples from the same treatment were pooled. Samples were homogenized in 90 ml of 0.1% (w/v) peptone water for one min using a Model 400 Stomacher. Total viable counts were determined by plating suitable dilutions on Tryptone Soya Agar (Oxoid CM131) supplemented with 0.5% (w/v) yeast extract and 0.2% (w/v) glucose (TSYG agar). B.thermosphacta was enumerated using STAA agar (Gardner, 1966). To determine the count of Gram negative bacteria samples were plated on peptone agar (Grau, 1983). On this medium they grow as large colonies and the growth of lactic acid bacteria and B.thermosphacta is greatly restricted. For confirmation, colonies were tested for Gram reaction using the method of Buck (1982). Lactic acid bacteria were enumerated using MRS agar (Oxoid). However, in the presence of large numbers of B.thermosphacta this medium may give a false result, since some strains form colonies on it and may give a negative catalase test. Thus to confirm the count of lactic acid bacteria a representative number of colonies were patched onto TSYG agar and the patches tested for catalase activity. The Petri dishes were incubated at 25°C and colonies counted after two and three days. Duplicate sets of both the TSYG agar and the peptone agar were incubated for a suitable time at the temperature of the storage experiment.

Loins were cut into three portions of approximately equal size and placed in plastic bags. The packaging film used consisted of a layer of polyvinylidene chloride coated on both sides with ethylene-vinylacetate copolymer and had an oxygen permeability of 20-30 ml/m^2/24h/atm measured at 25°C and 75% rh (W gauge Barrier Bag, W.R. Grace & Co.). Meat was vacuum-packaged by heat sealing using a Supervac chamber-type evacuator operated at maximum vacuum. Packs were not heat shrunk and were stored in the dark at ca. 90% rh.

Each taste panel experiment consisted of two treatments, but samples were presented in a three-way test, since duplicate stored samples were compared to a frozen control. To reduce possible variability due to differences in the meat samples presented to the taste panel, three separate loins were used at each time point. Thus each time point comprised three separate tasting sessions. At any one session, the three packs from the same muscle were compared (two stored test samples and one frozen control).

After sampling for microbiological analysis the fat layer was removed from each sample and the ends were sliced off and discarded. The meat was cut into slices ca. 2 cm in thickness, dry roasted at 230°C for 20 min and cut into cubes of side ca. 2 cm for presentation to the taste panel. All other details have been previously described (Egan, Ford and Shay, 1980; Egan and Shay, 1982).

Results

The growth of the microbial flora on the lean and skin surfaces of vacuum-packaged pork of low pH (5.5-5.8) stored at 0°C is examplified by the results shown in Fig. 1. Psychrotrophic lactic acid bacteria were the dominant component on the fat as well as the lean and skin surfaces. On the lean surface, these organisms reached a maximum population typically in the range of 2-5 x $10^7/cm^2$ after 4-5 weeks of storage and comprised more than 99% of the organisms present.

Whilst B.thermosphacta reached a maximum population of only ca. $10^4/cm^2$ on the lean (Fig. 1b), it reached about 5 x $10^5/cm^2$ on the fat (data not shown) and exceeded $10^6/cm^2$ on the skin (Fig. 1a). The population of gram negative bacteria remained low on all three types of tissue (ca. $10^4/cm^2$) but typically showed quite large fluctuations.

A trained analytical taste panel of 15 members was used to determine the shelf life of vacuum-packaged pork stored at 0°C and the results of a typical experiment are shown in Fig. 2a. The pork samples gradually deteriorated on storage mainly due to the development of a changed or "off" flavour which first became apparent after about four weeks. This "off" flavour increased in intensity during further storage and the flavour of the stored samples became significantly different from that of the frozen controls after 6 weeks ($P<0.01$). At this time the flavour of the meat was variously described as bitter, sour or acid. A changed or "off" aroma was also noted by the tasters, but the flavour change was regarded as the major defect.

Beef of pH 5.5-5.8, vacuum-packaged and stored under similar conditions, was presented to the same taste panel for evaluation. Again spoilage was predominantly due to a change in meat flavour but with the beef it only became significant after 11 weeks storage at 0°C ($P<0.05$).

The development of the microbial flora on vacuum-packaged pork of high pH was then studied. Results demonstrating growth on the lean surface are shown in Fig. 3. The total number of viable organisms present on pork of pH 6.1-6.7 stored for 4-5 weeks at 0°C was typically somewhat higher than found for meat of low pH (cf. Fig. 1). Lactic acid bacteria were again the dominant component of the flora reaching ca. $10^8/cm^2$. B.thermosphacta and the Gram negatives were also present in higher numbers than on meat of low pH (Fig. 3b).

When the high pH meat was stored at 5°C, not only did the bacteria grow more rapidly but the total count was noticeably higher than at 0°C (typically 2-5 x $10^8/cm^2$). There were also significant increases in the populations reached by the Gram negatives and B.thermosphacta, which grew to $10^8/cm^2$ and $10^6/cm^2$ respectively. Growth of these groups continued even in the presence of a population of lactic acid bacteria in excess of $10^8/cm^2$ (Fig. 3a). A considerable proportion of the gram negative isolates were capable of producing hydrogen sulphide but detailed studies of these organisms have not yet been done. Table 1 lists the maximum population of the various groups of bacteria found on the lean surface of vacuum-packaged pork.

TABLE 1: Highest numbers of bacteria on the lean surface of vacuum-packaged pork* stored for up to 6 weeks.

pH	Temperature (°C)	Total viable count+	Lactic acid bacteria	B.thermosphacta	Gram-ve bacteria
5.4-5.8	0	7.9	7.9	4.2	4.8
	5	8.0	7.9	4.5	6.2
6.1-6.7	0	8.7	8.5	7.5	6.7
	5	8.6	7.9	7.2	8.0

* Packaging film oxygen permeability 20-30 ml/m^2/24h/atm measured at 25°C and 75% r.h.

\+ Log_{10} number per cm^2. Data taken from a total of 12 experiments.

The lactic acid bacteria did not grow to as high numbers on the fat and skin surface of high pH meat as they did on the lean. Typical numbers reached were 1-2 x $10^7/cm^2$. B.thermosphacta reached ca. $10^7/cm^2$ on the fat and skin surfaces at both temperatures. The populations of gram-negative bacteria were again more variable but were considerably higher at 5°C than at 0°C.

In most experiments, spoilage of high pH vacuum-packaged pork was due to the development of undesirable colour defects. Greening of the weep often occurred, and this resulted in a general discolouration which was most noticeable on the fat surface. When green packs were opened a smell of hydrogen sulphide was usually noted. Greening of high pH pork usually occurred after 4-5 weeks when stored at 0°C and after on 2-3 weeks at 5°C.

Discussion

Lactic acid bacteria were the dominant component of the flora of low pH pork stored at 0°C. The low numbers of gram negative bacteria present suggests that these organisms are unlikely to be significant in spoilage. Similarly B.thermosphacta is unlikely to be a cause of spoilage since it reached a population in excess of $10^6/cm^2$ only on the skin. Only very low numbers of yeasts were detected (typical populations 10^2-$10^3/cm^2$. Overall the microbiology was very similar to that reported for low pH beef stored under similar conditions (Dainty et al., 1979; Gill and Newton, 1978; Egan, 1983).

The taste panel results show that pork spoiled due to the development of a flavour defect after about six weeks storage. Vacuum-packaged beef also spoils due to the development of "off" flavours described as sour, acid or cheesy (Shay and Egan, 1982; Egan, 1983). This "off" flavour development is believed to be caused by the lactic acid bacteria. However the taste panel results indicate a shelf life for pork of only about half that for beef stored under similar conditions. There may be several factors contributing to this. The initial count of psychrotrophs on pork is commonly higher than on beef (unpublished data) and in addition, the growth rate of the bacteria

appears to be slightly faster on the pork. However it is difficult to explain the difference in shelf life solely on a microbiological basis. Studies of the endogenous chemical and biochemical changes which occur in the muscle during storage may be needed to help understand this result.

Our microbiological results show that vacuum-packaged pork of high pH provides a much more permissive environment for bacterial growth than does meat of low pH. Thus the situation with pork is similar to that reported for beef (Taylor and Shaw, 1977; Gill and Newton, 1979; Erichsen, Molin and Möller, 1981). B.thermosphacta grew to much higher populations on vacuum-packaged high pH pork than it did when the pH was low. Populations in excess of $10^7/cm^2$ were present in some experiments and this population is likely to be significant in spoilage (Egan and Grau, 1981). For this organism the amount of growth was more dependent upon meat pH than on temperature (Table 1). Since its' population also increases with increasing packaging film permeability (data not shown), the factors controlling its' growth on vacuum-packaged pork are the same as those for beef (Campbell et al., 1979).

The population of Gram negative psychrotrophs on vacuum-packaged pork increased when either meat pH or storage temperature increased. The temperature effect was particularly significant with populations of $10^8/cm^2$ occurring on high pH pork at 5°C (Table 1). These organisms are likely to be the major cause of the putrefactive odours and colour changes which cause the spoilage of vacuum packaged high pH pork, particularly at 5°C.

For export from Australia an assured shelf-life of at least 6-8 weeks at 0°C is required. The relationship between the estimate of shelf life obtained using the taste panel and consumer shelf life is difficult to quantitate, although we know that our panel is more discriminating than the average local consumer. However it seems unlikely that even low pH pork could be exported without encountering problems.

Additional procedures may be useful in extending the shelf life of vacuum-packaged pork. Storage in atmospheres of carbon dioxide shows considerable promise (Enfors, Molin and Ternström, 1979; Blickstad and Molin, 1983) and sanitizing carcasses or cuts with solutions of organic acid may be of use (Cacciarelli et al., 1983; van Netten and Mossel, 1980). A number of potential pathogens have been reported as being able to grow on vacuum-packaged beef and pork of high pH. These include Yersinia enterocolitica, Aeromonas hydrophila and Erysipelothrix-like bacteria (Gill and Newton, 1979; Erichsen, Molin and Möller, 1981; Myers et al., 1982). We have also detected these organisms on vacuum-packaged pork. They may present a problem particularly on high pH meat stored at 5°C and further studies to clarify their significance are required.

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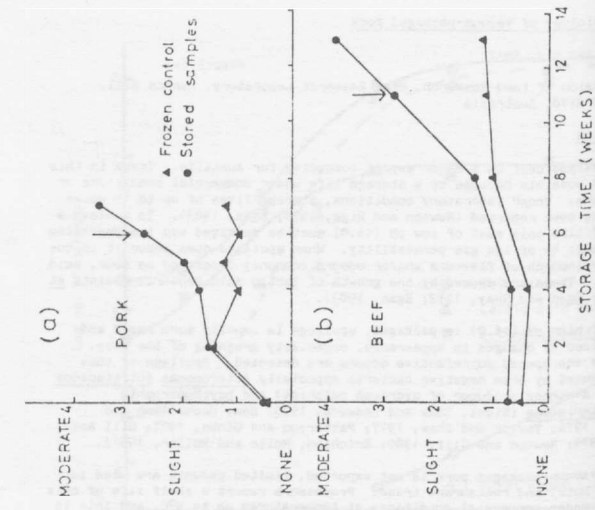

FIG. 2: Taste panel evaluation of the development of changed or off-flavour in low pH (5.5-5.8) vacuum-packaged pork and beef stored at 0°C.

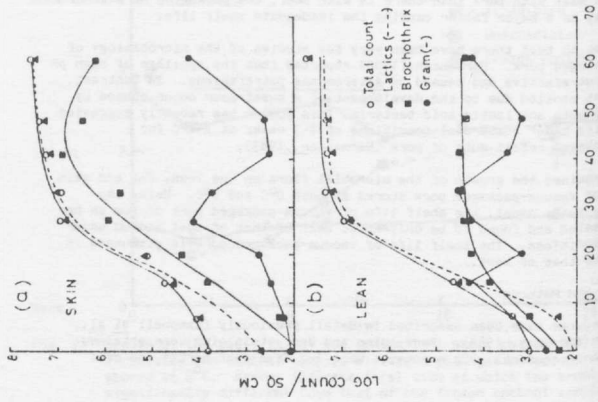

FIG. 1: Growth of the bacterial flora on the lean and skin surfaces of low pH (5.5-5.8) vacuum-packaged pork stored at 0°C.

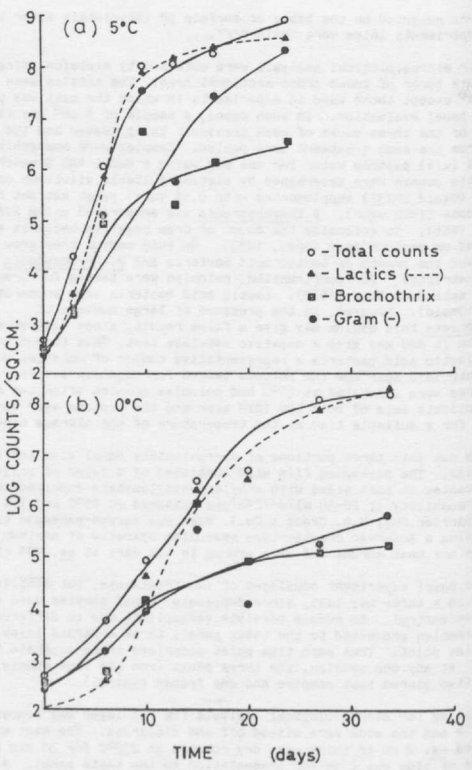

FIG. 3: Growth of the bacterial flora on the lean surface of high pH (6.1-6.7) vacuum-packaged pork stored at 0°C and 5°C.