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Spoilage of Vacuum-Packaged Dark, Firm, Dry Meat at Chill Temperatures

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The flora of vacuum-packaged dark, firm, dry meat included three organisms not usually found on vacuum-packaged meat, *Yersinia enterocolitica, Enterobacter liquefaciens,* and *Alteromonas putrefaciens. Y. enterocolitica* did not affect the meat quality. Production of spoilage odors by *E. liquefaciens* could be prevented by addition of glucose or citrate to the meat. Greening of meat by *A. putrefaciens* was not prevented by addition of glucose, as the organism degraded cysteine with the release of H_2S even when glucose was present. To prevent greening, growth of *A. putrefaciens* must be inhibited by reducing the meat pH to less than 6.0.

Dark, firm, dry (DFD) meat derived from the carcasses of stressed animals is characterized by a high ultimate pH (15). It spoils more rapidly than normal meat of ultimate pH 5.5, and this has been assumed to be due to more rapid growth of spoilage bacteria at the elevated pH. However, many spoilage bacteria are unaffected by pH in the range of 5.5 to 7.0. Recently, it has been shown that the onset of spoilage in meat at normal pH is delayed until the available glucose has been consumed. Only then are amino acids attacked, with the production of spoilage odors (5). DFD meat is deficient in glucose, so amino acids are attacked without delay and spoilage becomes evident at far lower bacterial cell densities than normal. The aerobic shelf life of DFD meat is extended by addition of glucose, but not by reduction of the pH (12).

Vacuum-packaged DFD meat also spoils rapidly, with the development of a typical green discoloration. This is due to production by the spoilage flora of H_2S, which converts the muscle pigment to green sulfmyoglobin (13). To determine whether it is possible to control spoilage of vacuum-packaged DFD meat, the factors responsible for the spoilage process were examined.

MATERIALS AND METHODS

Organisms. Bacteria were isolated from spoiled vacuum-packaged DFD beef stored at 2°C for 6 weeks. They were identified to the generic level from the tables of reactions in Cowan's *Manual for Identification of Medical Bacteria* (3) and to the species level from reactions listed by Hanna et al. (7), Johnson et al. (8), and Lee et al. (10). Cultures were maintained on nutrient agar slopes.

Growth of bacteria on meat slices. The bacteria were grown in pure culture on slices of normal or DFD beef longissimus dorsi muscle obtained by using aseptic techniques (5). The composition and pH of DFD meat slices were modified by addition of 5 μl of the following solutions per g (wet weight): 10% (wt/vol) glucose; 1.3 M disodium citrate, pH 5.0; or 1.3 M disodium citrate–1.5 M lactic acid, pH 4.2.

After inoculation, slices were individually packaged by heat sealing in nylon–polythene laminate under a vacuum of 635 mm of Hg. The laminate is a commonly used commercial vacuum-packaging film with an oxygen permeability of 300 ml/m^3 per 24 h per atmosphere at 25°C and 100% relative humidity. Packs were stored at 10°C and examined daily for appearance, odor, and bacterial counts. Exudate in packs was examined for sulfmyoglobin by the method of Nicol et al. (13).

Growth of bacteria in meat juice medium. Bacteria were grown in diluted meat juice medium under a nitrogen atmosphere at 30°C. The medium was sparged with oxygen-free nitrogen and inoculated with cells from early-log-phase cultures growing anaerobically in the medium used for continuous culture of *Alteromonas putrefaciens.* The preparation, sampling, and analysis of cultures have been described previously (5, 6).

Growth of *A. putrefaciens* in continuous culture. *A. putrefaciens* was grown at pH 7.0 in a simple salts medium (4) supplemented with 1 g of glucose per liter, 1 g of Casamino Acids per liter, and 0.2 g of yeast extract per liter. Aerobic, oxygen-limited, and anaerobic cultures were grown at 30°C at the maximum dilution rate and at 20% of the maximum rate. Cysteine and methionine solutions were added to culture samples, and H_2S evolution was detected with lead acetate paper.

RESULTS

Four major types of bacteria were isolated from 10 packs of vacuum-packaged DFD meat.

They were identified as *A. putrefaciens, Enterobacter liquefaciens, Yersinia enterocolitica,* and *Lactobacillus* sp.

Pure cultures of a strain of each species were grown on normal and DFD meat in vacuum packages. Only *A. putrefaciens* caused visible greening of DFD meat. It was unable to grow on meat of normal pH. *E. liquefaciens* grew at a reduced rate on normal meat, but produced spoilage odors and traces of sulfmyoglobin on both normal and DFD meat. The other species had no obvious effect upon meat quality, and their growth rates were unaffected by the type of meat on which they grew. However, maximum numbers of *Y. enterocolitica* were about 100-fold higher on DFD than on normal meat (Table 1).

When grown anaerobically in meat juice medium, *E. liquefaciens* showed some utilization of serine when glucose and glucose 6-phosphate were the major substrates (Fig. 1). Other amino acids (lysine, arginine, threonine) were not attacked until glucose was exhausted. *A. putrefaciens* also utilized serine in the presence of glucose, and initially serine appeared to be the preferred substrate (Fig. 2). On exhaustion of glucose, *A. putrefaciens* attacked the same amino acids as *E. liquefaciens,* but glucose 6-phosphate was not utilized.

Samples taken from continuous cultures were used to determine whether *A. putrefaciens* could produce H_2S in the presence of glucose. All cultures were found to have high residual glucose concentrations in the spent culture medium. Anaerobic cultures and the oxygen-limited culture growing at the submaximal rate evolved H_2S without delay when cysteine was added, but H_2S was not produced from methionine even after prolonged incubation. Aerobic and rapidly growing oxygen-limited cultures did not produce H_2S.

To examine the effect of glucose addition and pH reduction on the spoilage of vacuum-packaged meat, slices of DFD meat were treated with glucose, citrate, or citrate-lactate solutions to give meat of pH 6.7, 6.0, and 5.6, respectively. The slices were vacuum-packaged and stored at 10°C. After 5 days, control and glucose-treated packs were spoiled due to greening. Citrate-treated packs showed greening after 8 days, but those packs treated with citrate-lactate buffer showed no greening after 14 days. Spoilage odors were detected only in the control packs.

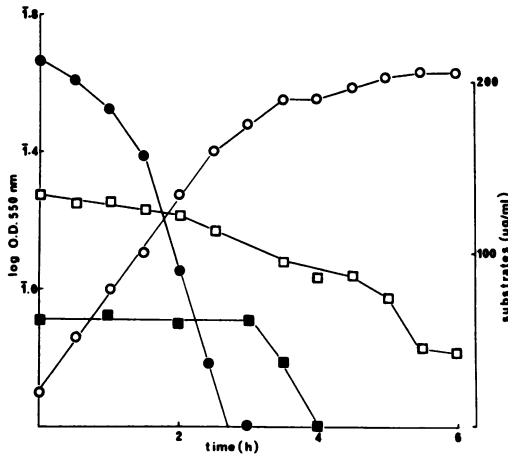

FIG. 1. *Growth of E. liquefaciens in anaerobic meat juice medium-phosphate buffer (7:3, vol/vol) at 30°C. Symbols:* ○, *optical density at 550 nm* ($O.D._{550}$); ●, *glucose;* ■, *glucose 6-phosphate;* □, *serine.*

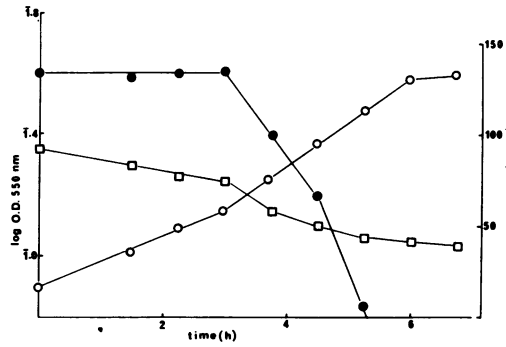

FIG. 2. *Growth of A. putrefaciens in anaerobic meat juice medium-phosphate buffer (1:1, vol/vol) at 30°C. Symbols:* ○, *optical density at 550 nm* ($O.D._{550}$); ●, *glucose;* □, *serine.*

TABLE 1. *Growth at 10°C of pure cultures of spoilage bacteria on vacuum-packaged meat*

Bacterium	DFD meat, pH 6.5				Normal meat, pH 5.7			
	Generation time (h)	Maximum no./cm²	Visible greening	Spoilage odor	Generation time (h)	Maximum no./cm²	Visible greening	Spoilage odor
A. putrefaciens	3.3	2.5×10^7	+	−			−	−
E. liquefaciens	3.8	1.6×10^8	−	+	4.9	7.9×10^7	−	+
Y. enterocolitica	4.3	6.3×10^7	−	−	4.3	7.2×10^5	−	−
Lactobacillus	4.6	5.0×10^7	−	−	4.6	3.2×10^8	−	−

DISCUSSION

Under anaerobic conditions, the dominant bacteria on normal chilled meat are lactobacilli. These organisms utilize only glucose and arginine for growth and appear to inhibit competing species by production of an antimicrobial agent (11). However, the anaerobic spoilage flora of DFD meat includes species which do not usually occur in significant numbers on normal meat. *Y. enterocolitica* was consistently found in high numbers on vacuum-packaged DFD meat, whereas it has been reported to occur in less than 10% of packs containing normal meat (7). The sensitivity of *E. liquefaciens* (*Serratia liquefaciens*) and *A. putrefaciens* to reduced pH and their occurrence on vacuum-packaged high-pH meat has been observed by other workers (1, 14). It appears that a high pH and absence of glucose are necessary to allow these species to compete successfully with the lactobacilli.

Large numbers of *Y. enterocolitica* do not seem to have any effect on meat quality, but the presence of this organism may still be undesirable, as some strains can be pathogenic. However, the relationship between such pathogenic strains and the common nonpathogenic strains is uncertain (2).

Greening is a result of H_2S production by *A. putrefaciens,* utilizing cysteine which presumably derives from protein degradation and glutathione. Glutathione and cysteine are rapidly oxidized under aerobic conditions, but this would not occur in the anaerobic environment of vacuum packages (9). Degradation of some amino acids by *A. putrefaciens* is prevented by glucose, but serine appears to be utilized preferentially to glucose, and cysteine is attacked even when glucose is present.

Although *E. liquefaciens* can also produce H_2S, its ability to do so is weak, and it does not seem to make any significant contribution to visible greening. However, it readily produces spoilage odors (14) and appears to be the major cause of this type of spoilage in vacuum-packaged DFD meat. Like *A. putrefaciens, E. liquefaciens* utilizes serine in the presence of glucose, but spoilage odors are not produced until glucose is exhausted and other amino acids are attacked.

To prevent greening, it is necessary to stop the growth of *A. putrefaciens.* This can be achieved by reducing the pH to below 6.0. However, *E. liquefaciens* continues to grow when the pH is reduced, so addition of a preferentially utilized carbohydrate substrate (glucose or citrate) is also necessary to prevent development of spoilage odors.

The control of spoilage in vacuum-packaged DFD meat requires considerably greater modification of the meat composition than is necessary to prevent early spoilage under aerobic conditions. Such modifications are now being examined to determine whether it is possible to prevent early spoilage of vacuum-packaged DFD meat under practical conditions without adversely affecting the quality of the product.

LITERATURE CITED

1. **Barnes, E. M., and C. S. Impey.** 1968. Psychrophilic spoilage bacteria of poultry. J. Appl. Bacteriol. **31**:97–107.
2. **Carter, P. B., C. F. Varga, and E. E. Keet.** 1973. New strain of *Yersinia enterocolitica* pathogenic for rodents. Appl. Microbiol. **26**:1016–1018.
3. **Cowan, S. T.** 1974. Manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge.
4. **Gill, C. O.** 1975. Effect of growth temperature on the lipids of *Pseudomonas fluorescens.* J. Gen. Microbiol. **89**:293–298.
5. **Gill, C. O.** 1976. Substrate limitation of bacterial growth at meat surfaces. J. Appl. Bacteriol. **41**:401–410.
6. **Gill, C. O., and K. G. Newton.** 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. **43**:189–195.
7. **Hanna, M. O., D. L. Zink, Z. L. Carpenter, and C. Vanderzant.** 1976. *Yersinia enterocolitica*-like organisms from vacuum-packaged beef and lamb. J. Food Sci. **41**:1254–1256.
8. **Johnson, R., R. R. Colwell, R. Sakazaki, and K. Tamura.** 1975. Numerical taxonomy study of the *Enterobacteriaceae.* Int. J. Syst. Bacteriol. **25**:12–37.
9. **Lea, C. H., L. J. Parr, and H. F. Jackson.** 1969. Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophilic spoilage bacteria at 1°C. Br. Poult. Sci. **10**:229–238.
10. **Lee, J. V., D. M. Gibson, and J. M. Shewan.** 1977. A numerical taxonomic study of some *Pseudomonas*-like marine bacteria. J. Gen. Microbiol. **98**:439–451.
11. **Newton, K. G., and C. O. Gill.** 1978. The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. **44**:91–95.
12. **Newton, K. G., and C. O. Gill.** 1978. Storage quality of dark, firm, dry meat. Appl. Environ. Microbiol. **36**:375–376.
13. **Nicol, D. J., M. K. Shaw, and D. A. Ledward.** 1970. Hydrogen sulfide production by bacteria and sulfmyoglobin formation in prepacked chilled beef. Appl. Microbiol. **19**:937–939.
14. **Patterson, J. T., and P. A. Gibbs.** 1977. Incidence and spoilage potential of isolates from vacuum-packaged meat of high pH value. J. Appl. Bacteriol. **43**:25–38.
15. **Tarrant, P. V.** 1976. The occurrence of dark cutting beef, paper B8, 1–6. *In* Proceedings, 22nd European Meeting of Meat Research Workers, Malmö, vol. 1.