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Microbial Spoilage of Whole Sheep Livers

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When livers treated with antibiotics to inhibit microbial growth were held at 10°C, the initial high pH (6.4) declined as lactic acid accumulated throughout the storage period of 10 days. The glycogen content also declined, but the glucose concentration in the tissues remained high. When livers were allowed to spoil at 10°C, distinct but variable floras developed within the tissues, in the drip, and on the upper surface. Deep-tissue floras were composed of anaerobic and facultative organisms (*Lactobacillus, Enterobacter, Aeromonas*); surface floras were generally dominated by strictly aerobic organisms (*Pseudomonas, Acinetobacter*); drip floras contained variable proportions of organisms of all three types, but the facultatively anaerobic *Enterobacter* were usually present as a major component. Spoilage occurred after 4 to 6 days with the development of visible discrete colonies on the upper surface without spoilage odors being evident. Changes in tissue and drip composition due to microbial activity could be detected only when spoilage had reached an advanced stage.

Microbial spoilage of meat has been extensively studied, and the process is now understood in considerable detail (6, 11), but little attention has been given to the spoilage of other tissues from meat animals that are used for human consumption. There is a complete absence of published data on the spoilage of all internal organs with the exception of liver, which has been the subject of two reports (3, 12). Unfortunately, similar storage conditions were not used in both studies, and this may have contributed to differences between the results which led the authors to markedly different conclusions. We have therefore examined the process of microbial spoilage in lamb livers with the objective of determining how the environment offered by liver affects the development of microbial spoilage in this organ.

MATERIALS AND METHODS

Composition of livers. Ten fresh lamb livers were obtained from an abattoir. Two samples of 2 g were removed from each liver. One sample was homogenized with 5 ml of distilled water, and the pH was determined with a glass electrode. The second sample was homogenized with 8 ml of 3.5% perchloric acid, and 0.2-ml portions of the homogenate were used for the estimation of glycogen by the amyloglucosidase method of Keppler and Decker (8). The remaining homogenate was centrifuged. The supernatant was decanted, neutralized with 20% KOH, and analyzed for glucose, lactic acid, and ammonia by methods previously used with meat (4).

Autolytic changes in liver composition during storage. The outer tissues from two fresh livers were removed with sterile instruments and discarded. The remaining tissues were cut into 5-g portions under aseptic conditions. Portions were placed in individual sterile petri dishes and treated with 0.5 ml of an antibiotic solution containing (per ml) tetracycline, 500 μg; polymyxin B, 500 μg; and cycloheximide, 250 μg. The dishes were wrapped in plastic film and stored at 10°C. Two samples from each liver were removed at intervals of 2 days, and the compositions were determined as described above. Samples from the water homogenate were spread on nutrient agar plates, which were incubated at 30°C for determination of the extent of microbial growth.

Initial examination of spoilage. Two livers were obtained at each of four points during preparation and packing of livers at an abattoir: (i) after evisceration and inspection, (ii) after removal of the gall bladder and trimming, (iii) after washing, and (iv) after packing in plastic tubs holding about six livers. Each liver was collected in a separate plastic bag. A 5-cm^2 area of the upper surface, delimited by a template, was swabbed with a moist and then a dry cotton-wool swab. The swabbed surface was seared with a hot spatula, and a deep tissue sample of about 1 g was removed aseptically. Swabs and tissue samples were each shaken with 10 ml of peptone water, and serial dilutions of the resultant suspensions were spread on nutrient agar. The livers were placed in shallow trays, loosely wrapped with plastic film, and incubated at 10°C. This incubation temperature gives a conveniently rapid development of spoilage floras that do not differ substantially from those that grow at lower temperatures (5, 10). Surface swabs, deep tissue samples, and samples of drip were collected at 2, 4, and 7 days for bacteriological examination.

Agar plates were incubated at 25°C for 2 days. Bacterial cell densities were determined by counting colonies on plates of suitably diluted samples. Estimates of initial and final flora compositions were obtained by identifying and enumerating individual colony types in the initial and final floras. Bacteria

TABLE 1. pH and concentrations in 10 fresh livers of glycogen, glucose, lactic acid, and ammonia

Component	Avg concn and range
Glucose	2.73 (0.68–6.33) mg/g
Glycogen	2.98 (0.70–5.43) mg/g
Lactic acid	4.14 (3.42–5.87) mg/g
Ammonia	7.52 (6.44–8.30) μmol/g
pH	6.41 (6.26–6.63)

were identified to the genus level by the criteria of Cowan and Steel (2).

Detailed examination of spoilage. Two tubs of livers were obtained from an abattoir. One liver was removed from the center of each tub and incubated as for the initial examination. The surface, deep tissue, and drip of each liver were examined daily for total bacterial counts. The pH and concentrations of glucose and ammonia were also determined. The compositions of initial and final floras were identified by picking at random and identifying 50 colonies from plates used for enumeration of the floras.

RESULTS

Composition of fresh livers. There were wide variations in the glucose and glycogen concentrations in livers. The concentrations of lactic acid and ammonia were more consistent, and the pH was in the range of 6.2 to 6.6 in all cases (Table 1).

Autolytic changes in liver composition. The glucose concentration in livers fluctuated during storage but remained at a high level throughout. The glycogen concentration declined, and the lactic acid concentration increased with time. The ammonia concentration began to increase markedly after 4 days of storage. Accumulation of lactic acid and ammonia would have opposite effects upon the pH, but the pH declined throughout the period of storage (Fig. 1). There was some growth of both bacteria and yeasts during storage, but total numbers were less than 10^4/g in the final samples on day 10.

Initial examination of spoilage. Livers examined shortly after they were removed from the carcass carried bacteria on the surface at a density of $10^3/cm^2$. After trimming and washing, surface contamination was reduced by an order of magnitude. Deep-tissue contamination was less than surface contamination, all deep-tissue samples yielding 50 to 100 bacteria per g.

Livers were spoiled by the appearance of visible bacterial colonies on the upper surfaces. When the colonies were small, no spoilage odors were evident, but these developed as the size of the colonies increased. The time taken for colonies to appear on the eight livers varied between 4 and 6 days. Bacterial growth on the surfaces commenced without appreciable lag in those livers that showed early development of visible colonies, but in cases where the appearance of visible colonies was delayed, there was little increase in total numbers during the first 2 days. The time to appearance of visible colonies was not related to the point of processing at which the livers were collected, as the three livers which were spoiled on day 4 were each obtained at different points. Time was required for drip to accumulate, so there was no initial measurement of the bacterial density of the drip. Growth in the drip appeared to parallel growth on the exposed surface. There was a lag of 4 days before numbers of bacteria in the deep tissues increased significantly (Fig. 2). Although the cell densities on the surface, in the drip, and in the deep tissue are not directly comparable, it seems that after 7

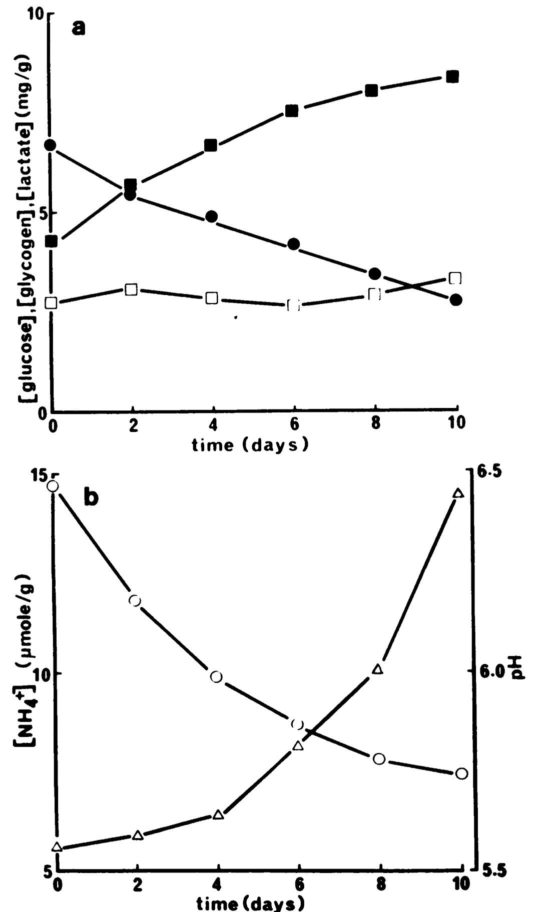


FIG. 1. (a) Changes in the concentrations of glycogen (●), glucose (□), and lactic acid (■). (b) Change in the concentration of ammonia (△) and pH (○) during storage at 10°C of liver slices treated with antibiotics to inhibit microbial growth.

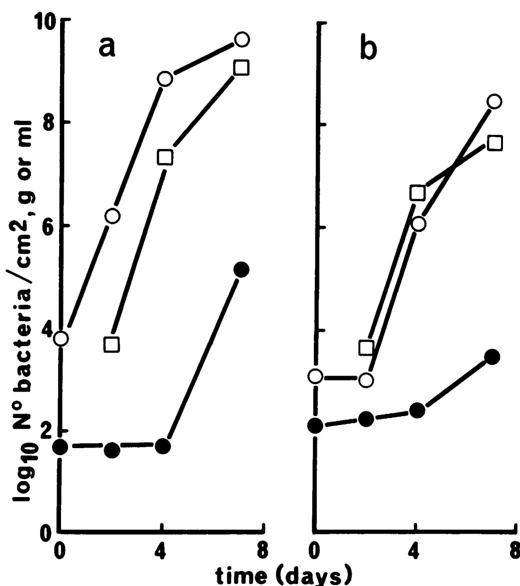


FIG. 2. Growth of bacteria on two trimmed, unwashed livers. Increase in bacterial cell densities: at the surface, number per cm^2 (○); in deep tissue, number per g (●); and in the drip, number per ml (□). Initial surface flora compositions: (a) *Bacillus*, 19%; *Micrococcus*, 42%; *Pseudomonas*, 14%; *Moraxella*, 25%; (b) *Bacillus*, 44%; *Micrococcus*, 47%; *Pseudomonas*, 1%; *Moraxella*, 7%.

days of storage, bacterial densities on the surface (10^8 to $10^9/cm^2$) and in the drip (10^8 to 10^9/ml) were similar and several orders of magnitude greater than the cell densities in the deep tissue (10^3 to 10^5/g).

The initial surface floras varied considerably in composition, but in seven of the eight livers the initial floras were dominated by mesophilic organisms, *Bacillus*, *Micrococcus* and, in one case, *Staphylococcus*. Other organisms present as substantial fractions (≥5%) of some floras were *Moraxella* sp. (four cases; 25, 7, 7, and 5%), *Brochothrix thermosphacta* (two cases; 64 and 23%), *Pseudomonas* sp. (two cases; 14 and 7%), *Acinetobacter* sp. (one case; 12%), and *Enterobacter* sp. (one case; 5%). In all cases, the final surface floras were dominated by *Pseudomonas*, *Acinetobacter*, or both. In two cases *Moraxella* formed about 5% of the flora, and in the one case where *Enterobacter* had contributed substantially to the initial flora, it also formed a major portion (42%) of the final flora. Other genera were not observed in the final surface floras. It is notable that the three livers on which visible colonies had appeared by day 4 were those which had initial floras containing substantial fractions of the organisms which dominated the final floras.

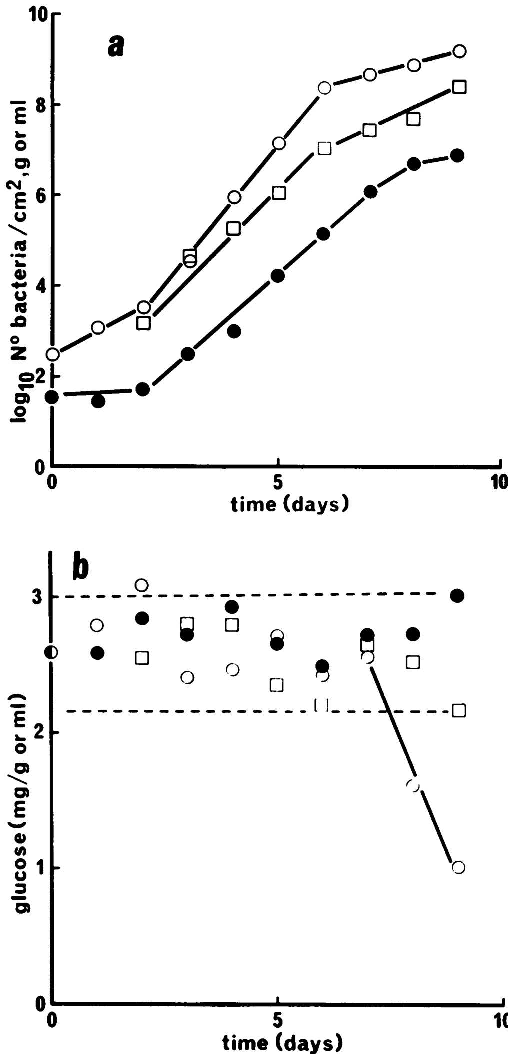
The final drip floras were composed only of the genera *Pseudomonas*, *Acinetobacter*, and *Enterobacter*. However, unlike the surface floras, *Acinetobacter* was present in only two cases (49 and 22%). *Pseudomonas* dominated four of the floras, but in the other four, *Enterobacter* was the major component.

Enterobacter also dominated three of the deep-tissue final floras; three floras were dominated by *Aeromonas*, and two were dominated by *Lactobacillus*. The presence of other genera was not observed.

Detailed examination of spoilage. Both livers gave similar results. Surface colonies were visible on day 4, and strong spoilage odors were evident by day 7. The changes in the composition of the deep tissues followed the pattern observed for autolytic changes throughout the whole period of the experiment, with the pH declining and the glucose content remaining approximately constant. The composition of the surface tissues altered in the same manner during the first 7 days although the rate of pH decline was more rapid. During the last 2 days of storage there was a large decrease in the surface glucose concentration and the pH started to increase (Fig. 3). The increase in pH was apparently due to enhanced ammonia production at the surface, as on day 9 the ammonia concentrations in internal and external tissues were 16.0 and 22.9 μmol/g, respectively. There was a large decline in the drip pH after surface spoilage had become evident, but the glucose concentration remained high (Fig. 3). Detailed analysis of initial and final floras confirmed that the floras of the surface, drip, and deep tissue developed in the manner indicated by the preliminary experiments, although the initial floras of both livers contained a high proportion of spoilage organisms (Table 2).

DISCUSSION

Although the perception of a state of spoilage in any food is essentially a subjective evaluation which will vary with consumer expectations, any obvious symptoms of microbial activity are usually unacceptable. How microbial activity is first detected will depend upon the composition of the food and the nature of the spoilage flora. Many organisms associated with low-temperature spoilage, such as *Pseudomonas* and *Enterobacter*, produce malodorous compounds when degrading amino acid, and in high-protein foods offensive odors may be the first indication of spoilage. However, such organisms will preferentially utilize simple carbohydrates, such as glucose, if they are present and while doing so do not produce offensive by-products (4, 9). The maintenance of a high glucose concentration in liver therefore allows the spoilage bacteria to



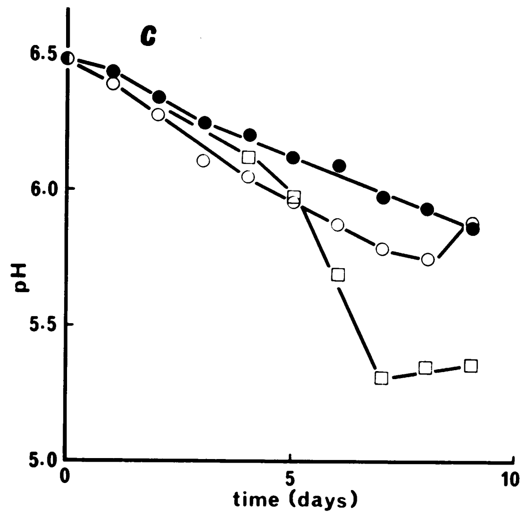


FIG. 3. (a) Growth of bacteria, (b) changes in glucose concentration, and (c) pH at the surface (○), in the deep tissues (●), and at the drip (□) of a liver spoiling at 10°C. The flora compositions are those of liver I in Table 2.

form visible colonies on exposed surfaces before any other evidence of spoilage develops.

The organisms mainly responsible for this visual spoilage of livers, *Pseudomonas* and *Acinetobacter*, are those which also predominate in the spoilage floras of meat and fish (7). On meat the pseudomonads can usually outgrow competing species at 10°C and below (5), and on livers the *Acinetobacter* group would seem to have a similar growth rate advantage. Other organisms only occurred in the final surface spoilage flora when they were present as a substantial part of the initial psychrotrophic flora, presumably because the relatively large numbers would tend to compensate for the growth rate advantage of the dominant organisms. However, the long lag phase and slow growth rate of *B. thermosphacta* (10) probably accounts for the absence of this psychrotroph from the spoilage floras even when large numbers were initially present. The relatively early appearance of visible colonies on livers with a high proportion of spoilage organisms in the initial floras suggests that the initial colony-forming units were large in comparison with those present on livers, which remained visually acceptable for longer periods.

The differing compositions of the spoilage floras on the exposed surface, in the drip, and in the deep tissues can be accounted for by the differences in aerobic status of these areas. The strict aerobes dominate the surface where oxygen is readily available, whereas only facultative and anaerobic organisms can grow in the deep tissue where oxygen is absent. The drip will be anaerobic where it bathes the undersurface of the liver, but aerobic growth should be possible at liquid-air interfaces. These partially anaerobic conditions will favor development of the facultatively anaerobic *Enterobacter*, and the low oxygen tension probably accounts for the general absence of *Acinetobacter*, the growth of which is favored by highly aerobic environments (1).

The low oxygen tension in the drip would also account for the large decrease in drip pH as a result of formation of organic acids by the dense microbial populations. *Enterobacter* would produce acids by fermentation, *Pseudomonas* would convert glucose to gluconate and 2-oxogluconate but would be able to further degrade only a portion of these products because of the restricted availability of oxygen (13). However,

TABLE 2. Composition of initial and final floras, determined from 50 randomly selected colonies from each flora, of two livers collected after packing and stored for 9 days at 10°C

Flora	Composition of flora (%)							
	Pseudomonas	*Acinetobacter*	*Moraxella*	*Enterobacter*	*Aeromonas*	*Lactobacillus*	*Micrococcus*	*Bacillus*
Liver I								
Initial surface flora	8	8	6	14	—	—	62	2
Final surface flora	56	6	2	26	6	—	4	—
Final drip flora	62	2	4	16	—	16	—	—
Initial deep-tissue flora	—[a]	—	—	94	—	2	2	—
Final deep-tissue flora	—	—	—	86	—	14	—	—
Liver II								
Initial surface flora	26	20	4	26	—	2	18	—
Final surface flora	44	42	—	14	—	—	—	—
Final drip flora	58	10	—	30	2	—	—	—
Initial deep-tissue flora	—	—	6	78	—	12	2	—
Final deep-tissue flora	—	—	—	84	2	14	—	—

[a] —, None.

it is not clear why the pH of deep tissues and drip did not rapidly equilibrate.

Our microbiological results were in broad agreement with the observations of Gardner (3), who found aerobic spoilage of pig livers was usually due to surface growth of strictly aerobic gram-negative organisms (*Alcaligenes* sp. and *Pseudomonas* sp.), whereas the deep tissues developed smaller floras dominated by lactobacilli. However, when the availability of oxygen was restricted by packaging the livers in plastic bags, Gardner (3) observed that surface floras too were dominated by lactobacilli. This later result paralleled the findings of Shelef (12), who found that the spoilage floras of diced beef livers packed in covered beakers were dominated by lactic acid bacteria. With these storage conditions, Shelef (12) observed a large decline in pH similar to that which we found to occur only in the drip.

Although livers from different species were used for each of the three studies, the results appear to be compatible, any differences arising from the different storage conditions employed. It seems that the liver composition imposes little significant selective effect on the initial flora so the spoilage flora can be very variable in composition depending on the relative numbers of the various psychrotrophic organisms in the initial flora. Livers stored in air will generally spoil as a result of the development on exposed surfaces of visible colonies of rapidly growing aerobic organisms, such as the pseudomonads. When storage conditions restrict the oxygen supply, the strict aerobes are displayed by facultative and anaerobic organisms. Dense floras of this type can cause a fall in pH, beyond that resulting from autolysis, by production of organic acids from the substantial amounts of glucose available in livers.

Further work will obviously be necessary to amplify these findings and to determine the conditions of treatment and storage which will optimize the shelf life of fresh livers.

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