Spoilage Association of Chicken Leg Muscle

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The ability of pure cultures of bacteria isolated from spoiling chicken leg muscle to produce strong off-odors was tested by using sterile leg muscle sections. Changes in the flora during storage and the incidence and identity of organisms capable of producing strong off-odors were noted.

The development of techniques that allow the excision of sterile muscle sections has provided a means of characterizing that portion of the microflora responsible for the organoleptic changes associated with the spoilage of chilled stored flesh foods. Information of this nature is available for chicken breast muscle (11), but it is known that the spoilage association of breast muscle is different from that of chicken leg muscle (2). The work described follows changes in the flora of chicken leg muscle during storage at 2°C, and organisms capable of producing strong off-odors on this substrate have been characterized using sterile muscle sections.

MATERIALS AND METHODS

Origin and isolation of strains. The leg skin of freshly processed, eviscerated chickens was removed, and the underlying muscles were excised and stored in sterile petri dishes at 2°C. The muscle was not homogenized in order to preserve the cellular and physical integrity of the substrate.

At each sampling time (0, 4, 8, 12, and 16 days) approximately 15 g of muscle was selected at random and homogenized in 135 ml of saline (0.9% w/v) using a Colworth Stomacher (A. J. Seward and Co. Ltd., London). Serial dilutions were prepared and plated on nutrient agar (Oxoid), and the numbers of organisms present were recorded after 3 days at 22°C. Approximately 50 colonies were removed from an appropriate dilution plate at each sampling time and purified by streaking on nutrient agar.

Production of off-odors on chicken leg muscle. A modification of the technique described by McMeekin (11) was used to excise sterile sections of chicken leg muscle. After treatment with crystal violet and brilliant green, the legs were frozen. This allowed much easier manipulation and excision of muscle sections. The use of unprocessed (laboratory plucked) carcasses that carried much lower loads of psychrophiles also facilitated excision of sterile sections. Sterility checks were as described by McMeekin (11).

The 235 isolates were grown on nutrient agar for 48 h at 22°C. Cells were washed from the plates with sterile Ringer solution, and the suspension was used immediately to inoculate sterile muscle sections. The initial concentration was approximately 10⁴ cells/g, and sections were incubated at 2°C for 14 days. Sensory examinations were carried out at 7 and 14 days.

Characterization of isolates. The scheme of Shewan et al. (15) was used to identify the isolates. Strains identified as Acinetobacter/Moraxella were previously termed Achromobacter.

RESULTS

Numbers and incidence of different bacteria during spoilage of naturally contaminated muscle. On days 0, 4, 8, 12, and 16 of storage, 4.2 x 10⁶, 8.5 x 10⁶, 7.3 x 10⁶, 4.7 x 10⁶, and 9.2 x 10⁶ organisms were recovered. An analysis of the flora at each sampling time is shown in Table 1. All of the Pseudomonas groups I and II and Alteromonas putrefaciens isolates were psychrophilic (produced colonies on nutrient agar after 14 days at 2°C). Sixty percent of the flavobacteria and 9 of 10 Pseudomonas III/IV types isolated were not psychrophilic. Among the Acinetobacter/Moraxella types, a selection for psychrophiles was evident as storage progressed, and this was also evident for the flora as a whole (Table 2).

The incidence of off-odor producers is shown in Table 2. Expressed as a percentage of the psychrophilic flora, this remained uniformly low throughout the storage period, with a maximum of 21% off-odor producers recorded after 16 days of storage. All 10 A. putrefaciens isolates produced pungent sulfide-like odors, as did 8 of 54 fluorescent pseudomonads. Ten Pseudomonas group II types (28%) produced "fruity-ester"-type odors, as did two Acinetobacter/Moraxella strains. Two of the latter caused fishy odors.

DISCUSSION

Previous reports have demonstrated that the development of strong off-odors characteristic of spoiling flesh foods at chill temperatures occurs as a result of the growth and metabolism of a restricted group of psychrophiles (7, 11). This has been confirmed for chicken leg muscle,
breast muscle (11) inoculated with *P. fragi*. Fruity odors were also produced by two *Acinetobacter/Moraxella* strains that grew well on chicken leg muscle at 2°C but are unable to compete with *Pseudomonas* group I and II types on breast muscle (2, 11).

Thus *Pseudomonas* group I and II types developed most rapidly on spoiling chicken leg muscle stored at 2°C and eventually dominated the spoilage association. *Acinetobacter/Moraxella* strains also grew rapidly, but after 8 days decreased in proportion in relation to the pseudomonads. Although *A. putrefaciens* remained a constantly small fraction of the flora, this organism must be considered an important part of the spoilage association as all isolates were psychrophilic and produced strong off-odors when grown on chicken leg muscle.

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**LITERATURE CITED**