APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1977, p. 1244-1246
Vol. 33, No. 6

Printed in U.S.A.

Spoilage Association of Chicken Leg Muscle

T. A. McMEEKIN

Faculty of Agricultural Science, The University of Tasmania, Hobart, Tasmania, 7001, Australia

Received for publication 27 January 1977

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 chill-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% wt/vol) using a Colworth Stomacher (A. J. Seward and Co. Ltd., London). Serial dilutions were prepared and plated in 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 10^4 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, 4.2×10^3, 8.5×10^3, 7.3×10^4, 4.7×10^6, and 9.2×10^8 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,

TABLE 1. *Distribution of flora during storage at 2°C*

No. of days stored at 2°C	No. of isolates	% of population						
		Flavobacteria	*Acinetobacter/Moraxella*	*Pseudomonas* group I	*Pseudomonas* group II	*Pseudomonas* group III/IV	*A. putrefaciens*	Others
0	47	32	26	4	4	21	2	11
4	46	35	44	2	2	2	4	11
8	48	12	53	18	11	0	4	0
12	46	2	28	41	24	0	6	0
16	48	0	17	47	32	0	4	0

TABLE 2. *Distribution of psychrophiles and off-odor producers during storage at 2°C.*

No. of days stored at 2°C	Psychrophiles (% flora)	Off-odor producers	
		% Flora	% Psychrophiles
0	22	2	9
4	60	10	17
8	85	15	17
12	92	15	15
16	100	21	21

and a consistently small fraction of the flora has been implicated as described for fish substrates (1, 7). The selection for off-odor producers during chill storage of chicken breast muscle (11) may be explained by postulating that off-odor producers present among the relatively few types isolated initially (after 7 days at 2°C) exhibited the fastest growth rates.

Sulfide-like off-odors have been described in a wide variety of chill-stored flesh foods, including fish (4, 6, 7, 10, 13), shellfish (9), meat (12, 14), poultry (11, 12), and bacon (5). The organism most commonly responsible has been *A. putrefaciens*, which produces hydrogen sulfide, methyl mercaptan, and dimethyl sulfide by degradation of the sulfur-containing amino acids cysteine and methionine (8). Characteristically, it is isolated only in small numbers from fresh products but increases rapidly to form a large proportion of the flora at spoilage (2, 4, 7, 9). *A. putrefaciens* is absent from the spoilage association of chicken breast muscle (2, 11), but the higher pH of leg muscle allowed rapid growth, although the proportion remained low throughout the storage period. Sulfide-like odors were also produced on leg muscle by some fluorescent pseudomonads, and this property has been recorded for *Pseudomonas fluorescens* growing on fish (7, 13).

Some *Pseudomonas* group II strains produced the fruity-ester-like odors described for spoiling fish (3), fish muscle (7), and chicken 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.

ACKNOWLEDGMENTS

I thank the Australian Chicken Meat Research Committee for financial support, and Glenila Poultry Service, Hobart, for the supply of chickens.

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