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EFFECTS OF FOUR SPECIES OF BACTERIA ON PORCINE MUSCLE.
1. Protein Solubility and Emulsifying Capacity

SUMMARY—A technique was used to obtain aseptic porcine muscle, portions of which were inoculated with cultures of Pediococcus cerevisiae, Micrococcus luteus, Leuconostoc mesenteroides *and* Pseudomonas fragi. *The inoculated samples were compared with aseptic controls throughout a 20-day storage period at temperatures of 2 and 10°C. All 4 organisms grew at 10°C, but only* P. fragi *and* L. mesenteroides *grew at 2°C. The solubilities of the various protein fractions were affected by inoculation treatment. This was exemplified by correlation coefficients ranging from −.37–0.50. The coefficients indicated the interrelationships affected by storage conditions and bacterial growth. Protein solubility studies revealed a loss in the water-soluble fraction during storage of the controls and the* M. luteus- *and* L. mesenteroides-*treated samples. Samples inoculated with* P. fragi *evidenced an initial loss, followed by an increase. The solubility of meat proteins in a salt solution increased during the first 8 days of storage, then decreased or remained relatively constant for all samples. In comparison with controls, samples inoculated with* P. fragi *increased in salt-soluble protein solubility during the first 8 days, whereas those inoculated with* L. mesenteroides *decreased during the latter part of storage. Insoluble protein generally increased except for* P. fragi-*inoculated samples, which decreased. Nonprotein nitrogen (NPN) increased for all treatments and controls during the 20-day storage period. NPN extracted from the samples inoculated with* P. fragi *increased greatly. The pH increased with growth of* M. luteus *and* P. fragi *and decreased with growth of* P. cerevisiae *and* L. mesenteroides. *The emulsifying capacity was not influenced by the growth of* M. luteus *or* P. cerevisiae. *However, the emulsifying capacity of samples inoculated with* L. mesenteroides *decreased, whereas that of samples inoculated with* P. fragi *increased.*

INTRODUCTION

RECENT studies concerning the effects of microorganisms on muscle during storage have used general inocula in which the species have not been controlled. The purpose of this study was to inoculate porcine muscle with specific cultures and determine the effects of these organisms on some of the properties of the muscle.

Borton et al. (1968a) found that inoculation of porcine muscle with a mixed culture decreased emulsifying capacity, whereas Ockerman et al. (1969) reported an increase in emulsifying capacity of bovine muscle inoculated with a mixed culture. The reason for such discrepancies in results seemed to be due to differences in the predominant types of organisms present in the inocula.

Kirsh et al. (1952) found that nonpigmented *Pseudomonas* and *Aerobacter* microorganisms dominated the flora of fresh ground beef. They also found lactic acid-producing organisms and some cocci organisms. Their findings have been substantiated by numerous other reports including those of Ayres (1955), Wolin et al. (1957) and Halleck et al. (1958). Kitchell (1962) indicated 39% of the microorganisms on fresh pork were micrococci, whereas in products with a high salt content such as hams or bacon, 89–100% of the organisms were micrococci. Shank and Lundquist (1963) found lactic acid bacteria were the primary spoilage agents of vacuum-packaged processed meat products. Such organisms were also found when meat was stored under aerobic conditions.

In this study, the organisms used were chosen to represent the groups normally found on meat and meat products. *Pediococcus cerevisiae* and *Leuconostoc mesenteroides* represented the lactic acid-producing organisms. *Micrococcus luteus* was chosen as representative of the salt-tolerant micrococci and has also been found on fresh beef as reported by Stringer et al. (1969). After preliminary investigations with 2 or 3 species of *Pseudomonas* organisms, *Pseudomonas fragi* was chosen to represent this psychrophilic, proteolytic group, as it grew more readily at the pH of porcine muscle.

MATERIALS & METHODS

Sample procurement

8 180–230-lb hogs were slaughtered at monthly intervals with conventional procedures, except the sticking area of the neck was thoroughly scrubbed with a hexachloraphene bactericidal soap. The scalding, dehairing and evisceration were accomplished normally with additional sanitary precautions (i.e., frequent knife sterilization and hand washing). The unsplit carcass was then rinsed with alcohol and flamed before being placed in a 1–3°C cooler. After cooling for 24 hr, the shoulders were removed, the remaining portion of the carcass placed on a kraft-paper-covered table so that the dorsal midline was easily accessible, and rinsed with alcohol.

Using sterilized equipment, the longissimus dorsi muscle was removed following a procedure similar to that reported by Borton et al. (1968b). A cut was made along the dorsal midline of the backfat cover and 2 cuts made perpendicularly to it, 1, 5–8 cm from the scapula and the second over the tuber coxae. The backfat was stripped and rolled back to expose the longissimus dorsi muscle which was sliced into 3-cm slices, with 1 slice being removed at a time and placed in 1 of 3 containers. The 2 longissimus dorsi muscles were excised in this manner and divided equally into the 3 containers.

Inoculation of the sample

The sample from 1 container was ground through a 2-mm grinder plate with 10 ml of sterilized water being added. The sample was reground and designated as the control. The muscle slices from a second container were treated the same as the control sample except 10 ml of a 1/100 dilution of a 48-hr culture of either *P. cerevisiae, L. mesenteroides, M. luteus* or *P. fragi* were used to inoculate the sample. After regrinding the first inoculated sample, the grinder was cleaned and resterilized. The inoculation procedure was repeated for the second culture. After grinding and inoculation, each of the samples was divided aseptically into 13 jars each containing 60–70 g of sample. 1 jar of each sample was used as the day 0 sample, with 6 of the remaining jars being stored at 2°C and 6 at 10°C. The samples were then analyzed after 2, 4, 8, 12, 16 and 20 days of storage.

Bacterial numbers

The method outlined by the American Public Health Association (1958) was used for determination of the number of bacteria per gram of sample. 11 g of sample were blended in a sterile blender with 99 ml of sterilized water. After appropriate dilution, 1.0 or 0.1 ml was pipetted into sterile disposable petri dishes to which APT agar was added. The number of colonies which had grown on each plate was counted after incubation at 25°C for 48–72 hr.

Protein solubility

The extraction procedure outlined by Helander (1957) was used. It included extraction of the water-soluble protein with 0.03 M K PO_4 buffer, pH 7.4, extraction of the salt-soluble protein with 1.1 M KI, 0.1 M K PO_4 buffer, pH 7.4 and precipitation of the water-soluble protein with 10% trichloracetic acid to obtain a nonprotein nitrogen (NPN) fraction. The micro-Kjeldahl procedure outlined by the American Instrument Company (1961) was used to determine the nitrogen content of the samples and the extraction aliquots.

RESULTS & DISCUSSION

Bacterial growth

The amount of microbial growth which took place in each treatment group is shown in Figures 1–4. The samples were not sterile, as growth was noted on

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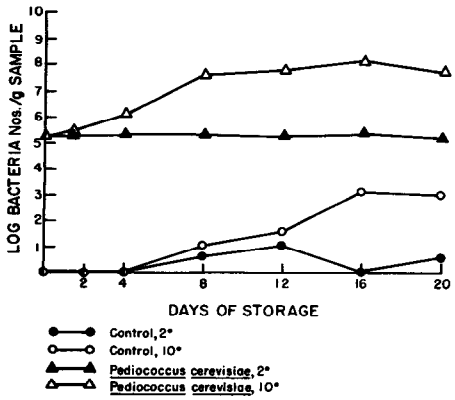

Fig. 1—Log of bacterial numbers per gram of control and Pediococcus cerevisiae-inoculated porcine samples stored at 2 and 10°C for 20 days.

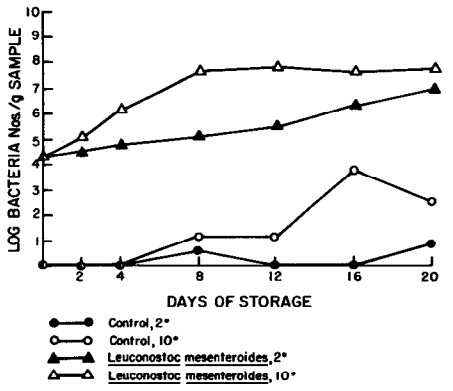

Fig. 2—Log of bacterial numbers per gram of control and Leuconostoc mesenteroides-inoculated porcine samples stored at 2 and 10°C for 20 days.

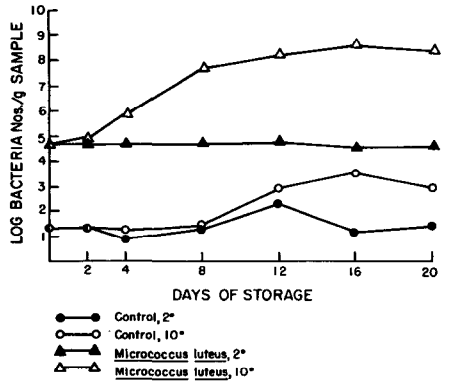

Fig. 3—Log of bacterial numbers per gram of control and Micrococcus luteus-inoculated porcine samples stored at 2 and 10°C for 20 days.

control samples during the 20-day storage period. However, counts were seldom over 10,000 organisms per gram, which was quite low, as counts of 1–95 million organisms per gram of fresh ground beef were reported by Kirsh et al. (1952). Growth for the control samples was slightly higher on those stored at 10°C than on those stored at 2°C.

P. cerevisiae and *M. luteus* organisms grew on porcine muscle when incubated at 10°C but not when incubated at 2°C (Fig. 1 and 3). In both cases the amount of growth did not exceed 100 million organisms per gram when stored at 10°C and there was no loss of viability at 2°C. *L. mesenteroides* grew at both storage temperatures, with the growth at 10°C being more rapid than that at 2°C (Fig. 2). Growth of these organisms at 10°C reached a peak of approximately 50 million after 8 days of storage and remained at that level throughout the remainder of the storage period. There was a continual increase in the number of organisms found on samples stored at 2°C during the 20-day storage period. *P. fragi* grew the fastest and showed the greatest increase in number of organisms of any species studied. The growth of this organism at 2°C was about 4 days slower than that recorded on the samples stored at 10°C (Fig. 4). The amount of growth approached 10 billion organisms per gram for samples stored at both temperatures. There was some decrease in bacterial counts for this organism during the latter days of storage at 10°C (days 12–20).

pH

Since the pH of meat has been reported to influence the extractability of meat proteins (Scopes, 1964) and emulsifying capacity (Swift and Sulzbacher, 1963), the influence of the 4 organisms on the pH of the samples is shown in Figure 5. The samples inoculated with the acid-producing organisms *(P. cerevisiae* and *L. mesenteroides)* and stored at 10°C had a lower pH than the control samples. However, at 2°C, growth of *L. mesenteroides* did not affect the pH. The samples inoculated with *M. luteus* and stored at 10°C evidenced an increasing pH but not to the same extent as found for the samples inoculated with *P. fragi* and stored at 2 and 10°C.

Protein solubility

The solubility of the various protein fractions of 2 control samples is shown in Figures 6 and 7. There was a significant decrease ($P \leqslant .01$) in the amount of water-soluble protein nitrogen during the 20-day storage period at both storage

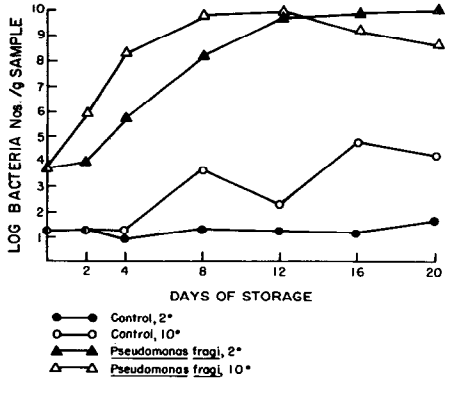

Fig. 4—Log of bacterial numbers per gram of control and Pseudomonas fragi-inoculated porcine samples stored at 2 and 10°C for 20 days.

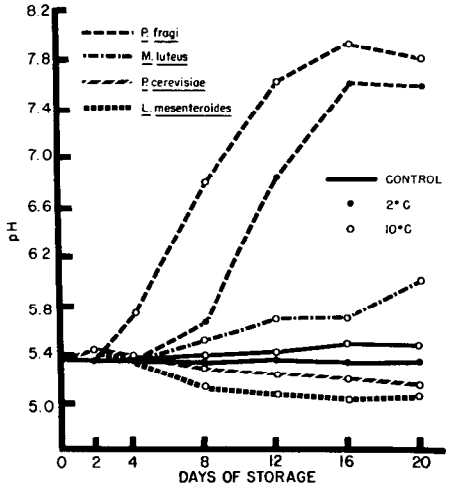

Fig. 5—pH of control and inoculated porcine samples stored at 2 and 10°C for 20 days.

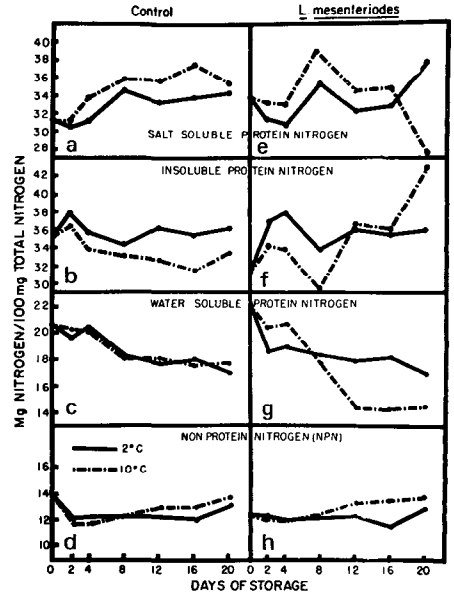

Fig. 6—Solubility of the various protein fractions of Leuconostoc mesenteroides-inoculated (e–h) and related control (a–d) porcine muscle stored at 2 and 10°C for 20 days.

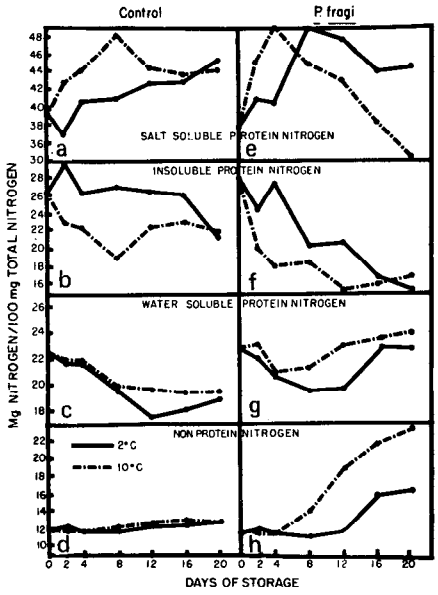

Fig. 7—Solubility of the various protein fractions of Pseudomonas fragi (e—h)-inoculated and related control (a—d) porcine muscle stored at 2 and 10°C for 20 days.

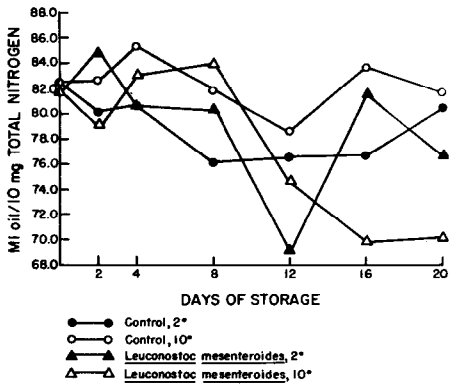

Fig. 8—Emulsifying capacity of control and Leuconostoc mesenteroides-inoculated porcine muscle samples stored at 2 and 10°C for 20 days.

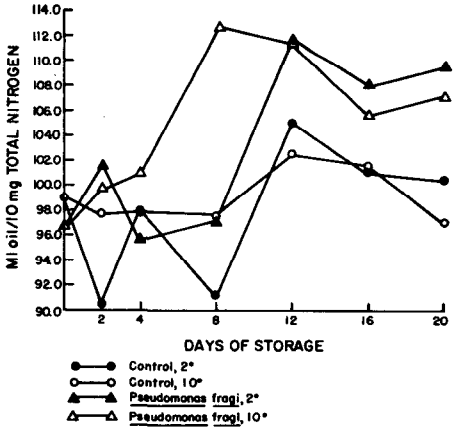

Fig. 9—Emulsifying capacity of control and Pseudomonas fragi-inoculated porcine muscle samples stored at 2 and 10°C for 20 days.

temperatures. The solubility decreased during the first 8 or 12 days and then remained relatively constant during the remainder of the storage period. These results are in agreement with those of Sayre and Briskey (1963) and McLoughlin (1963). The amount of extractable salt-soluble protein nitrogen increased during the first 8 days of storage, after which it remained constant or decreased for the control sample stored at 10°C, whereas that of the sample stored at 2°C increased or remained constant throughout the storage period. These results were in general agreement with those reported by McIntosh (1967). The amount of insoluble protein nitrogen decreased significantly ($P \leqslant .01$) during storage of the control samples. The mean over-all nonprotein nitrogen (NPN) found in control samples stored at 10°C was significantly higher ($P \leqslant .01$) than that found in those stored at 2°C, with the difference between the 2 means being 0.5 mg NPN/100 mg of total nitrogen. An increase ($P \leqslant .01$) in NPN was also noted during the 20-day storage period. The samples inoculated with *P. cerevisiae* and *M. luteus* evidenced protein solubility patterns similar to those of the control samples.

The samples inoculated with *L. mesenteroides* and stored at 10°C did have different protein solubilities from those of the controls, whereas those stored at 2°C were not significantly different from the controls (Fig. 6). The amount of water-soluble protein nitrogen was significantly lower ($P \leqslant .05$) at days 12, 16 and 20 (14.47, 14.98 and 15.27 mg/100 mg total nitrogen) than the amount found in the control samples (all $\geqslant$ 17.0 mg/100 mg total nitrogen), which can be seen by comparing Figures 6c and 6g. There was little difference in the amount of salt-soluble protein nitrogen of the *L. mesenteroides*-inoculated samples when compared to controls, except after 20 days of storage at 10°C, at which time a considerable decrease was noted (Fig. 6e). There was no difference between the NPN content of the *L. mesenteroides*-inoculated samples and controls. The insoluble fraction increased greatly at day 20,

Table 1—Emulsion stability as measured by oil and water separation of control and inoculated samples at day 0 and after 12 days of storage at 2 and 10°C.

Sample/ time/hr emulsion held	Control		*Pediococcus cerevisiae*		*Leuconostoc mesenteroides*		*Micrococcus luteus*		*Pseudomonas fragi*	
	Oil[a]	Water[a]	Oil[a]	Water[a]	Oil[a]	Water[a]	Oil[a]	Water[a]	Oil[a]	Water[a]
Day 0										
0	0	0	0	0	0	0	0	0	0	0
.25	0	0	0	0	0	0	0	0	0	0
.50	0	0	0	0	T	0	0	0	0	0
.75	0	0	0	0	T	0	0	0	0	0
1	T	0	T	0	1	0	0	0	0	0
2	T	0	T	0	1	0	0	0	T	0
24	T	0	T	0	1	0	T	T	T	0
48	1	T	1	0	2	0	T	T	T	T
Day 12—2°C										
0	0	0	0	0	0	0	0	0	0	0
.25	0	0	0	0	0	0	0	0	0	0
.50	0	0	0	0	0	0	0	0	0	0
.75	0	0	0	0	T	0	0	0	0	0
1	T	0	0	0	T	0	0	0	T	T
2	T	0	T	0	1	0	0	0	T	2
24	T	1	1	0	1	0	T	T	T	4
48	T	1	1	0	1	0	T	T	1	5
Day 12—10°C										
0	0	0	0	0	0	0	0	0	0	0
.25	0	0	0	0	0	0	0	0	0	0
.50	0	0	0	0	0	0	0	0	0	0
.75	T	0	0	0	T	0	0	0	T	T
1	T	0	0	0	T	0	T	0	T	T
2	T	0	T	0	1	0	T	0	T	1
24	1	0	1	0	1	0	T	0	1	4
48	1	0	1	0	1	0	T	0	1	5

[a]Ml of separation—oil at top of graduated cylinder, water at bottom. T = Trace amount (definite separation but not 1 ml).

the result of the loss of solubility of the water and salt-soluble proteins. The loss of solubility of these fractions was significantly related ($P \leq .01$) to the decreased pH, with correlation (r) values of 0.50 and 0.39 for water and salt-soluble fractions, respectively.

The amount of nitrogen found in the various fractions of the *P. fragi*-inoculated samples is compared to the controls in Figure 7. Inoculation of porcine muscle samples with this organism and storing at 2 and 10°C for 20 days caused the greatest change in the amounts of the various nitrogen fractions when compared to control samples. The *P. fragi* over-all water-soluble protein nitrogen mean of 22.14 mg/100 mg total nitrogen was significantly higher ($P \leq .01$) than the 20.28 mean of the controls. The water-soluble protein of the *P. fragi*-inoculated samples decreased until 4 days at 10°C and until 8 days at 2°C and then increased throughout the remainder of the storage period (Fig. 7g). The salt-soluble protein nitrogen extracted from the *P. fragi*-inoculated samples stored at 10°C was the highest at day 4, whereas it was highest at day 8 for those stored at 2°C (Fig. 7e). After this time, there was a marked decrease in the amount of salt-soluble protein nitrogen, especially in the samples stored at 10°C. There was a decrease in the amount of insoluble protein nitrogen (Fig. 7f) from the *P. fragi*-inoculated samples, the result of the increasing solubility of the water and salt-soluble protein nitrogen fractions and the NPN fraction. The NPN fraction of samples inoculated with this organism increased considerably during the 20-day storage period, with a greater increase in the samples stored at 10°C (Fig. 7h). The increase in NPN content indicates proteolysis had occurred, which also could explain the decrease in salt-soluble fraction. The increase in the water-soluble fraction was correlated ($P \leq .01$) with increased pH ($r = 0.37$) and partial proteolysis of the salt-soluble protein ($r = -.37$). Results of the protein solubility study of the *P. fragi*-inoculated samples are in general agreement with those reported by Ockerman et al. (1969).

Emulsifying capacity

The emulsifying capacity did not seem to be influenced by storage temperature or time, as with protein extractability. The samples inoculated with *M. luteus* and *P. cerevisiae* had almost the same emulsifying capacity as related controls. The emulsifying capacities of control and *L. mesenteroides*-inoculated porcine samples stored at 2 and 10°C are shown in Figure 8. Disregarding the result of the inoculated sample stored at 2°C for 12 days, the control samples stored at 2 and 10°C and the inoculated samples stored at 2°C had emulsifying capacities in the same general range. The inoculated sample stored at 10°C exhibited a decrease in emulsifying capacity from day 8 to days 16 and 20 similar to the decrease in pH noted in Figure 5. These results are similar to the results reported by Borton et al. (1968a). The relationship of the emulsifying capacities of control and *P. fragi*-inoculated samples is shown in Figure 9. The mean over-all emulsifying capacity of 103.9 ml oil/10 mg total nitrogen for the *P. fragi*-inoculated samples was significantly higher ($P \leq .01$) than the over-all mean of 98.5 ml oil/10 mg total nitrogen for the control samples. This type of relationship can be seen for the latter days of storage in Figure 9.

The higher emulsifying capacity was related ($P \leq .05$) to increased pH ($r = 0.33$), which may increase protein extractability and thereby affect emulsion formation (Hansen, 1960). Results obtained in this study with *P. fragi*-inoculated samples were in general agreement with those reported by Ockerman et al. (1969).

Emulsion stability

The stability results are shown in Table 1. It should be noted that even though samples inoculated with *P. fragi* had a greater emulsifying capacity, such emulsions were very unstable and separated easily when they were allowed to set at room temperature. The other inoculated samples produced emulsions as stable as the control samples.

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