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DEGRADATION OF BOVINE LEAN MEAT UNDER THE CHILLED STORAGE CONDITION

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SUMMARY

The effects of *Pseudomonas fragi* and *Proteus vulgaris* on deep, block bovine lean meat samples after 0, 3, 7 and 14 days of storage in comparison with uninoculated control samples were studied in five replicate samplings. Results of the microbial analysis had shown more rapid growth of *Pseudomonas fragi* than *Proteus vulgaris* on meat samples while minimal counts were enumerated on the control samples at the end of storage time.

Visual examination of the SDS polyacrylamide gel electrophoretic patterns showed that *Pseudomonas fragi* exhibited slightly greater proteolytic activity than *Proteus vulgaris* with minor changes observed after the 7th day.

Scanning electron microscopy results have shown that the muscle fibers from *Pseudomonas fragi*-inoculated samples exhibited more breakages than the *Proteus vulgaris* inoculated samples as a probable result of spoilage. On the myofibrillar level, results have shown that no major proteolysis occurred, thus supporting the results of the previous protein analysis by SDS gel electrophoresis.

INTRODUCTION

Rapid deterioration of foods is mainly the consequence of bacterial action specifically in meat whose moisture content with its rich load of nutrients in solution provides a favorable medium for bacteria. A very important factor which affects the growth of these bacteria is temperatture and since it is a common practice to store meat at refrigeration temperature (5℃ or less), most of the spoilage organisms involved are psychrotrophic groups such as *Achromobacter*, *Lactobacillus*, *Micrococcus* and *Pseudomonas* (Hawthorn, 1981). Among these spoilage flora of meat, the most widely existing type under aerobic condition are *Pseudomonas* species (Tarrant et al., 1973, Gill & Newton, 1978). *Pseudomonas fragi(Ps. fragi)* is the most popular strain which has gained attention among various workers (Dutson et al. 1971, Tarrant et al., 1971, Bala et al., 1979, Wing et al., 1983) in their spoilage studies of meat. *Proteus vulgaris(Pr. vulgaris)* is also known to produce a strong putrefactive odor in a protein-containing media and for that reason it was selected in this study. *Pr. vulgaris* can bring about a 51% breakdown of partially purified beef actomyosin at 5℃ for 14 days (Jay & Shelef, 1976) whereas *Ps. fragi* in the same investigation brought about a 76% destruction, which hereby indicates that the former is a weaker strain. The purpose of this research therefore, is to characterize the effects of *Ps. fragi* and *Pr. vulgaris* on the microbial, physical, chemical qualities and fiber ultrastructures of deep, block bovine lean meat samples.

MATERIALS AND METHODS

Preparation of bovine lean meat samples;

Big muscle blocks (apporoximately 4 Kg) were washed with 70% ethanol to eliminate surface bacterial contamination and then finally sprayed with 95% ethanol prior to actual sample trimming. Aseptic techniques were carefully adopted in all stages sample preparation and handling. Block samples used in the experiments were trimmed from the deepest middle portion of the original whole muscles. These represent the sterile deep muscle tissues indicated in the theme of this experiment.

Each piece weighed approximately 50g and measured 8×5×2 cm. Five replicate samplings were conducted throughout the study. In each sampling trial twelve pieces of muscle block samples were trimmed. Four pieces were inoculated with *Ps. fragi*, four pieces with *Pr. vulgaris* and the remaining one's were left uninoculated representing the control samples.

Preparation of the inoculum and storage of muscle block samples;

Stock pure cultures of *Ps. fragi* and *Pr. vulgaris* were inoculated separately in 10 ml of nutrient broth and incubated for 48 hrs. at 27℃. Into sterilized 500 ml distilled water, the whole nutrient broth culture of each of the organisms was poured and mixed to ensure uniform distribution of the microorganisms in the entire solution. Initial bacterial counts were taken and recorded.

To the prepared inoculum, muscle blocks were carefully dipped for about 30 seconds and then layed in a sterile rectangular plastic container equipped with a cover and stored in a refrigerator specially intended for the purpose so that the samples were not disturbed while kept for storage at 2℃.

Sample analysis;

A surface area in the middle portion of the muscle block samples representing 20 cm^2 was swabbed following the standard of Harrigan and McCance (1970) with some modifications. Samples were plated in duplicate using standard agar and incubated for 48 hrs. at 27℃.

pH of the homogenized sample was determined using Hitachi-Horiba pH meter.

Determination of the total volatile basic nitrogen quantities followed the method of Kawamura (1977).

Amino nitrogen quantities were determined by the method of Kawamura (1977) with some modifications.

Protein extracts were analyzed by SDS gel electrophoresis using the method of Porzio & Pearson (1977).

Fixing of the samples was done by the modified tannin-osmium method for non-coated scanning electron microscope speciment of Murakami (1977) with slight modifications. Samples were freeze dried and stored in a dessicator until actual observation using Hitachi scanning electron microscope (Model S-510) at an accelerating voltage of 15 kV.

RESULTS AND DISCUSSION

Sample evalution and microbial analysis;

Initial counts of the microbial inocula range from 10^6 to

10^7/ml. Dipping of the fresh sterile deep, block bovine lean meat samples into this inocula determined their initial bacterial load. Bacterial attachment may be considered as a preliminary step in the microbial spoilage of meat. The surface and total bacterial counts of the muscle samples at 0 day of storage therefore, play a major role as a measure of the attached organisms after inoculation. Attached organisms after inoculation range from 10^3 to 10^4/cm^2 (Fig. 1). It can be observed from the graph that the control samples were found to be free from microbial growth at 0 day. Non-detectable levels of initial contamination was neither observed in any of the control samples. Until the 7th day of storage no visible growth were observed, however after the 14th day microbial counts were enumerated. The graph likewise illustrates that surface growth of both organisms, but the especially that of *Ps. fragi* was faster up to the 7 th day of storage and moderate there after until the 14th day. Yada & Sukura (1981) also reported a rapid growth of *Ps. fragi* on intact beef muscles during the initial six days period and followed by a more modest growth rate during the next six days of storage. Changes in the total bacterial counts of the control samples were likewise determined as a measure of the penetration of microorganisms into the inner portion of the muscle samples as storage time lengthens. Growth tendency of both organisms followed the same pattern as the previously reported surface counts. Total bacterial counts of the samples were lower in values between the 3rd and 7th day when growth tends to be more concentrated or is faster in the surface. This is not a surprising fact because both are aerobic organisms.

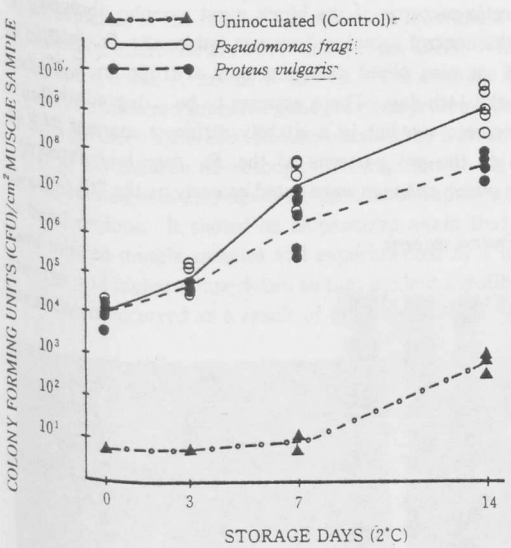

Fig. 1. Changes in the surface counts.

pH;

Changes in the pH effects of both organisms on deep, block bovine lean meat samples are presented in Fig. 2. The change, however, is quite negligible to note that a change has really occurred. *Ps. fragi* has increased the pH of the block muscle samples significantly more than *Pr. vulgaris*. The rise in pH is attributed to the liberation of ammonia and amines (Tarrant et al., 1971, Wing et al., 1983). *Ps. fragi* is known to produce an increase in the pH of the meat muscles (Ockerman et al., 1969, Tarrant et al., 1971). *Pr. vulgaris*, as

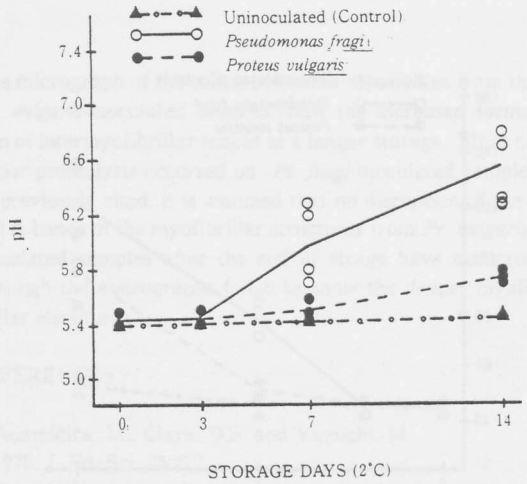

Fig. 2. Changes in the pH values.

a weaker strain has caused a fair increase in the pH values of the muscle samples. It can be observed from the graph that there is a distinct difference between the two organisms in affecting the pH of the muscle samples.

Total volatile basic nitrogen;

An increase in the total volatile basic nitrogen quantities was noted for all the samples in the course of storage (Fig. 3). *Ps. fragi*-inoculated samples produced greater quantities of total volatile basic nitrogen than *Pr. vulgaris*-inoculated samples. Significant differences in the total volatile basic nitrogen values of the two inoculated samples

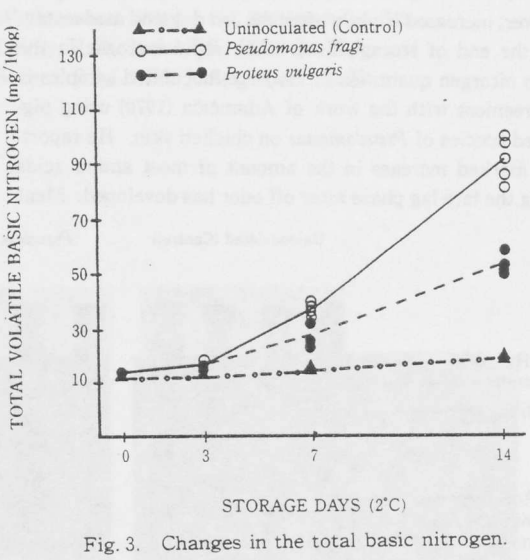

Fig. 3. Changes in the total basic nitrogen.

were noted from the 7th day until the 14th day of storage. The graph illustrates the strong activity of *Ps. fragi* in producing ammonia and amines with *Pr. vulgaris* exhibiting fairly.

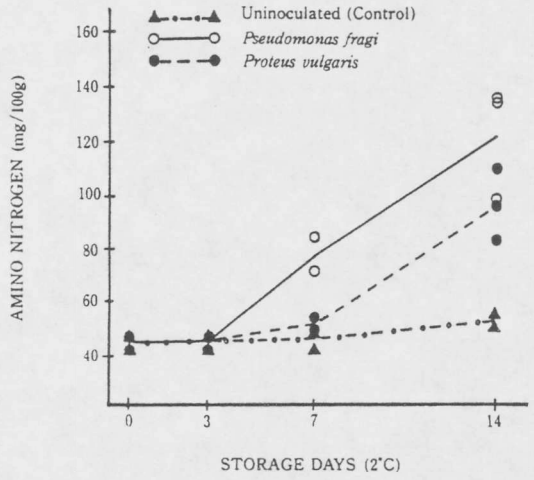

Fig. 4. Changes in the amino nitrogen ibiting fairly.

Amino nitrogen;

Amino nitrogen quantities (Fig. 4) of the control samples remained relatively stable as noted after the 7th day of storage. A slight increase was noted, however, in all the uninoculated control samples at the end of storage time. *Ps. fragi*-inoculated samples were noted for higher quantities of amino acids than *Pr. vulgaris*-inoculated samples. The graph shows that until the 3rd day of storage amino nitrogen values were relatively constant. For samples inoculated with *Ps. fragi*, a rapid increase was noted after the 3rd day until the 14th day of storage. Samples inoculated with *Pr. vulgaris* however, increased slightly after the 3rd day and moderately unitl the end of storage time. The rapid increase in the amino nitorgen quantities of *Ps. fragi*-inoculated samples is in agreement with the work of Adamčiča (1970) using pigmented species of *Pseudomonas* on chicken skin. He reported a marked increase in the amount of most amino acids during the late lag phase after off odor has developed. Meat has a generally low level of carbohydrate so that the spoilage bacteria affect the deamination of amino acids and utilize the remaining molecules as energy sources with a consequent increase in ammonia and hydrogen sulfide in the spoiling meats (Jay, 1976).

SDS gel electrophoresis;

The gel patterns of the representative control samples (Fig. 5) show the thick filament proteins such as myosin and C-protein, and the thin filament proteins such as α-actinin, actin, troponins, trpomyosins and some unidentified protein. Through visual observation of the gels, it can be noted that all the bands remained intact even after the 14th day of storage except for the decreasing intensity of troponin T which is presumably due to some autolytic changes in the samples. There seem to occur very slight changes on the gel patterns of *Ps. fragi*-inoculated samples, but were not noted as very significant upon quick observation. Through a closer appraisal of the gels, however, C-proteins seem to have slightly decreased in intensity.

There was also the occurrence of a band in between the C-protein and α-actinin which was not identified. Unlike the control samples, troponin T was degraded on the 7th day and completely disappeard on the 14th day. Dainty et al. (1975) as reported by Jay & Shelef (1976) using species of *Pseudomonas* on intact bovine lean meat samples found that α-actinin, troponin T and tropomyosin were destroyed within nine days of storage at 5℃. Results of this study as characterized by minimal changes in the gel patterns can be attributed to the nature of the samples used and to lower storage temperature. Changes caused by *Pr. vulgaris* on the elecrophoretic patterns of the block meat samples showed that like the control samples, however unlike the *Ps. fragi*-inoculated samples, slight disappearance of troponin T occurred on the 14th day. There appears to be a degradation of the C-protein too but in a slightly different manner as compared to the gel patterns of the *Ps. fragi*-inoculated samples in which changes were noted as early as the 7th day

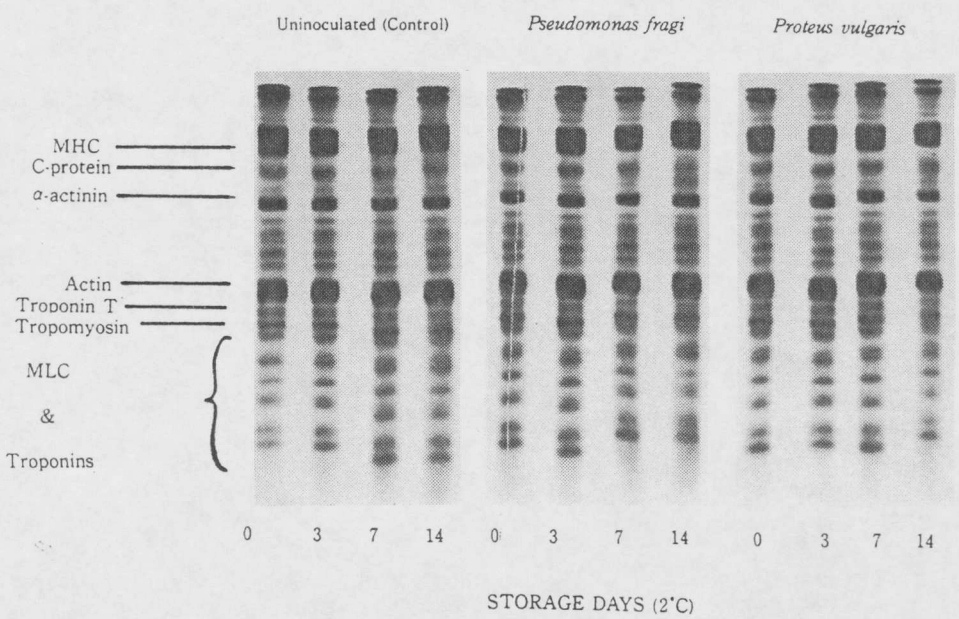

Fig. 5. SDS polyacrylamide gel electrophoresis of meat protein from deep, block bovine lean meat samples uninoculated and inoculated with *Ps. fragi* and *Pr. vulgaris*.

of storage.

Scanning electron microscopy;

Surface ultrastructures of the muscle fibers and longitudinal view of myofibrils from the uninoculated control and inoculated samples were examined after each storage time. On the 3rd day of storage there were not significant changes observed which can point to differences between the control samples and the inoculated samples. After the 7th day of storage, undamaged muscle fibers were observed from the uninoculated control samples. It is probable that fiber breakages increase as a result of spoilage since the muscle samples inoculated with *Ps. fragi* were already judged spoiled after the 7th day of storage. Muscle fiber surface structures observed from *Pr. vulgaris*-inoculated samples exposed the myofibrils underneath with the evident formation of intermyofibrillar spaces. Myofibrillar structures form the *Ps. fragi*-inoculated samples seem to be still intact and displaying some slight separation or loosening. The *Pr. vulgaris* inoculated samples showed evident formation of intermyofibrillar spaces.

Loosening of the myofibrils is displayed in Fig. 6 as a result of the formation of intermyofibrillar spaces. Katsaras et al. (1984) also pointed out that as aging proceeds, the transverse breaks in the myofibrils and the formation of interfilamentary and intermyofibrillar spaces increase. Fortunately, the micrograph in Fig. 6 gave evident results which can support the general purpose of this electron microscopic investigation. No disruption of the I and A bands, and the Z line were evidently observed except for slight damage observed within the I band region. This was rather believed to be an evidence of the removal of materials from the Z lines as caused by microbial growth (Dutson et al., 1971). With the undisrupted I and A bands, it can be noted that no major proteolysis occurred supporting the previous protein analysis by SDS gel electrophoresis. Results obtained by Dutson et al. (1971) in his electron microscopic investigation of pig muscles inoculated with *Ps. fragi* noted specific disruptions of the A band regions. It should be emphasized again that they used minced muscle samples and experimented at a longer atorage and higher temperature so that evident myofibrillar breakdown occurred as a result of proteolysis.

The micrograph of the subsarcolemmal myofibliles from the *Pr. vulgaris*-inoculated samples show the increased formation of intermyofibrillar spaces at a longer storage. Since no major proteolysis occurred on *Ps. fragi*-inoculated samples as previously cited, it is assumed that no disruption of the I and A bands of the myofibrillar structures from *Pr. vulgaris*-inoculated samples after the end of strage have occurred although the micrographs failed to show the deeper myofibrillar structures.

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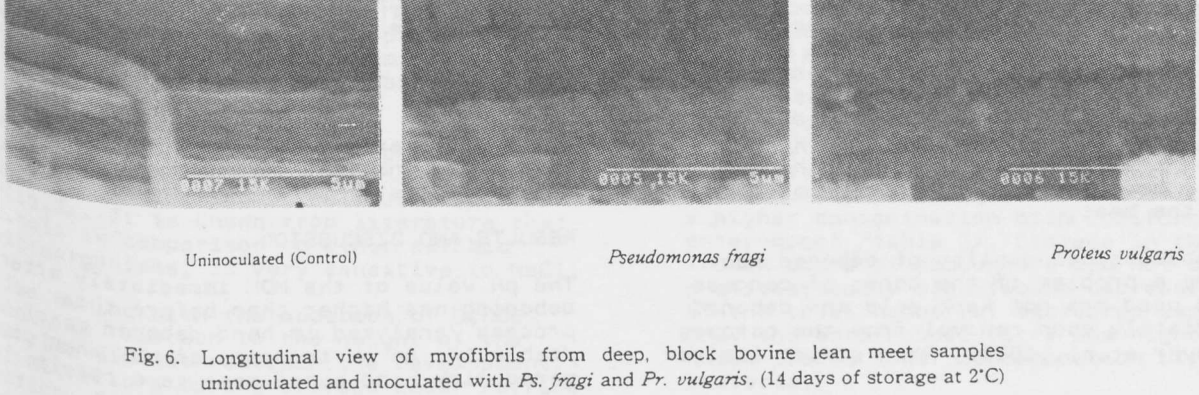

Uninoculated (Control) *Pseudomonas fragi* *Proteus vulgaris*

Fig. 6. Longitudinal view of myofibrils from deep, block bovine lean meet samples uninoculated and inoculated with *Ps. fragi* and *Pr. vulgaris*. (14 days of storage at 2°C)