

Inhibitory Action of Lactic Cultures on Gram-Negative Bacteria in Ground Meat

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INTRODUCTION

Lactic cultures are used extensively in the food industry for the manufacture of various cheeses and cultured milk. The cultures most commonly used consist of a mixture of *Streptococcus lactis* or *Streptococcus cremoris*, which produce lactic acid from lactose, and *Leuconostoc* species or *Streptococcus diacetylactis*, which produce diacetyl and other volatile compounds from citrates in milk. Marth (1962), Mather and Babel (1959) and Lukovnikova (1961) reported that the metabolism of lactic cultures is complex, resulting in not only the production of lactic acid, flavor and aroma substances, but also some antibiotic-like substances. These substances were proved to be responsible for the inhibition of certain types of spoilage in dairy products. Mather and Babel (1959) showed that inoculations of cream dressing with *Leuconostoc citrovorum* prevented spoilage in cottage cheese by certain gram-negative organisms.

Since milk products and meat are similar in regard to nutritive properties and types of organisms causing spoilage, it was presumed that lactic cultures could be used to prolong the shelf life of ground beef. Most of the rapid spoilage of milk and meat products is due to the growth of gram-negative, nonsporeforming, rod-shaped bacteria. The work herein reported was undertaken to determine the effects of inoculating with lactic cultures on the rate and extent of growth of gram-negative bacteria in ground beef.

EXPERIMENTAL PROCEDURE

Fresh ground beef was divided into aliquot portions, treated with various substances, and stored at 7 C, and gram-negative bacterial counts determined at intervals. The inhibitory effects of the various treatments on gram-negative bacteria were measured by comparing the counts on the treated samples with those on the untreated controls. The test materials were mixed with an approximately 5-lb. sample of meat, divided into 5 aliquots and then dispensed into plastic bags and stored in a cold room maintained at 7 C. The gram-negative bacterial counts were determined by plating on Standard Plate Count agar (Difco) with 1 ppm crystal violet and 50 ppm 2,3,5-triphenyltetrazolium chloride added (C V A agar) and incubating 2 days at 32 C.

Mixed cultures used in these trials were those used for making cottage cheese and cultured buttermilk. The pure cultures used were *S. lactis* and *Leuconostoc citrovorum* isolated from the mixed cultures.

RESULTS

Experiment 1—The influence of adding 5, 10, and 20% lactic cultures and of like amounts of skim milk without culture to ground beef was determined. The regular mixed culture was propagated in skim milk. Counts were determined after 2, 6, and 10 days storage.

The results indicated that the lactic cultures had a definite inhibitory effect on the gram-negative bacteria in ground beef and that the inhibitory effect became greater as the amount of culture used was increased. After 10 days storage, the count in the untreated samples was 2.3×10^8 , while those on the inoculated samples were 5.6×10^7 , 9.6×10^7 , and 2.4×10^8 respectively for 5, 10, and 20% culture. The addition of uninoculated skim milk had no inhibitory effect on the bacteria.

Experiment 2—In order to determine if the inhibitory effect of added cultures was due to reduction in pH or to the culture organisms, aliquots of fresh ground beef were inoculated with 20% milk cultures of pure and mixed-culture organisms. The counts after 5 days were compared to those of untreated samples and samples with lactic acid added to reduce the pH to 4.5. With *Leuconostoc citrovorum*, which produces little or no acid in milk, the pH of two lots of meat was reduced to 5.0 and 4.5 to simulate the pH of cottage cheese and of cultured buttermilk respectively.

The terminal counts after 5 days storage ranged from 4.9×10^7 for the sample inoculated with *L. citrovorum* at a pH of 4.5, to 5.4×10^8 for the untreated control sample. It appeared that pH was a major factor in inhibiting the gram-negative bacteria, as the count on the meat inoculated with *L. citrovorum* at pH 5.0 was much higher (1.5×10^8) than that on the similar sample with a pH of 4.5. Also, reduction of the pH to 4.5 with lactic acid definitely reduced development of gram-negative bacteria; however, at the same pH level, the sample with *L. citrovorum* added had a considerably lower count (4.9×10^7). The sample inoculated with a pure culture of *S. lactis* had a slightly higher count (1.4×10^8) than the one with the mixed culture (8.0×10^7), indicating that the *L. citrovorum* contributed to the inhibitory action.

In this trial, the initial count on the meat was rather high (9.6×10^8). After 5 days storage at 7 C, the counts on the treated samples were lower than the initial count, except for the one inoculated with 20% *L. citrovorum*, at a pH of 5.0, which increased slightly.

Experiment 3—In this experiment pure *S. lactis* and mixed cultures were grown in reconstituted milk with 20% solids content in order to provide a higher concentration of organisms in the medium and thereby reduce the amount needed for inhibition of growth of the gram-negative bacteria.

Counts were determined initially and after 2, 5, 7, and 9 days. The results show that counts on the untreated control sample increased steadily to a terminal count of 3.6×10^8 , while the counts on the inoculated samples decreased slightly during the first 2 days of storage and then remained constant during the next 5 days. The sample inoculated with *S. lactis* culture maintained the same level of gram-negative bacteria from the second to the ninth day (1.2×10^8) while the mixed culture (*S. lactis* plus *L. citrovorum*) had a lower terminal count (5.2×10^7). These results demonstrate the pronounced inhibitory action of added lactic culture and indicate that mixed cultures are more effective than pure *S. lactis*.

Experiment 4— Since the results of a preliminary study (Experiment 1) indicated that inhibitory action increased as the amount of culture used was increased, an experiment was conducted in which concentrations of 0, 5, 10, and 20% mixed cultures grown in reconstituted milk with 20% solids added to ground beef. Gram-negative counts were determined initially and after 2, 5, 7, and 9 days storage at 7 C.

The results show that 5% culture was effective in reducing the rate of gram-negative bacterial growth in the meat but 10 and 20% concentrations were apparently completely effective in preventing growth of these organisms. The terminal counts were 5.4×10^6 , 6.3×10^6 , 2.1×10^6 and 1.3×10^6 , respectively for the samples with 0, 5, 10, and 20% cultures added.

SUMMARY

Lactic cultures had a definite inhibitory action on gram-negative bacteria in ground beef. Lactic acid produced by *S. lactis* and *Leuconostoc* in the culture, both have an influence on the gram-negative bacteria, but *S. lactis* provided some other inhibitory factor. Additions of 10% lactic culture grown in 20% milk solids is sufficient to inhibit the growth of gram-negative bacteria in ground beef.

LITERATURE CITED

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