STUDIES ON LACTIC FERMENTATION IN TWO MEAT SPECIES.

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INTRODUCTION

Contamination occurs in carcasses surfaces due to contact with floors, walls, tools and water at the slaughterhouse. The initial microbial load of a carcass surface is given by the hygiene of the abbatoir as well as by handling practices. In Mexico most meat animals are slaughtered in semirural or semiurban killing floors (88.5% of bovines and 95.5% of swine). Some of these slaughterhouses have refrigeration facilities but most of them do not, mainly due to the high refrigeration costs. As ambient temperatures are around 25 C or more microbial loads can be above 10E07/cm2 when the meat reaches the consumer. The use of lactic acid in chemical forms has been also reported as an effective decontaminant by some authors (Dezeure-Wallys and Van Hoof, 1980; Snijders et al., 1985; Smulders et al., 1986). However, reports on the use of lactic acid produced **in situ** by lactic acid bacteria as a means of decontamination are scattered. Most of the research work on lactic fermentation in meat is related to production of fermented sausages such as salami. The objective of this research was to study the influence of some extrinsec factors on lactic fermentation in meat cuts surfaces as a simple and unexpensive mean to reduce spoilage and pathoger microorganisms.

MATERIALS AND METHODS

In a first experiment pork and beef taken at random from carcasses were cut into 5 cm pieces and assigned to the various treatments. A split block design was applied. (main Oxygen availability block) was limited by wrapping the samples with a commercial saran semi-permeable film. (Split Incubation temperatures block 1) were 15 C was the maximum temperature at which meat treated with organic acids can be kept for 5 days in simulated rural conditions in Mexico and 27 C was the average room temperature of our laboratory when the experiments were carried out. Carbohydrate source was tested by adding 1% sucrose (Split block 2). Strains tested in Split block 3 were: **Lactobacillus bulgaricus, L. casei, L. acidophilus, L. plantarum, Pediococcus pentosaceus,** and 2 strains isolated from pork samples but not yet characterized. Inocula were prepared in Rogosa liquid medium, incubated at 37 C with continous agitation for 24 to 30 hours until O.D.=1 in the suspension. This was then diluted with sterile water (1:1) and the samples immersed in the diluted cell suspension. Response variables were: pH, titrable acidity (as lactic acid), Lactic acid bacteria and **Pseudomonas** counts. Samples were analyzed every 24 hours during a study period of three days. However the storage time effect was statistically confounded. Data were analyzed by a Daisy statistical package (Killion, 1981) run in an Apple

lle microcomputer. Tests performed were t-student between split blocks and, independently F-tests among strains, both at 5% significance level. In a second experiment a set of samples, in a similar Split block design as Experiment 1 were tested for three factors in Split-block 3 (inocula). These were: i. a commercial strain (LM-3 Starterkulturen, Vigusa, Mexico City) a mixed culture of **Micrococcus kristinae-varians** and **L. plantarum**); ii. a mixture of **Lactobacillus bulgaricus** and **Pediococcus pentosaceus** ; iii. uninoculated samples. Statistical analyses were performed in the same way as in Experiment 1. Lactic acid concentration was analyzed as total titrable acidity (AOAC, 1980). Lactic acid bacteria counts and **Pseudomonas** counts were determined by standard methods (APHA, 1976). All analyses were carried out in triplicate.

RESULTS

Experiment 1.: There was a significant difference in lactic acid concentrations in both species between wrapped and unwrapped samples. When this differences were compared with those for pH values, where there is a significant difference in pork but not in beef, it can be concluded that larger amounts of lactic acid are required to modify the pH in beef samples, due to the buffer capacity of this meat species. Pork samples also showed a significant difference between storage temperatures in wrapped samples with no sucrose added. Here, lactic acid production was considerably higher for wrapped samples (1.05%) as compared with unwrapped ones (0.74%).

No differences were observed in lactic acid concentration among pork samples inoculated with different strains. Oppositely, differences were observed in all cases among beef samples inoculated with the strains under study **L. bulgaricus** and **L. acidophilus** presented, on the average, the higher lactic acid production in beef although pork showed consistently higher values for titrable acidity as compared with beef.

Significant differences were found in pH among beef inoculated with different strains within incubation temperatures. pH reached the lowest value when beef was inoculated with **L. bulgaricus, L. acidophilus** and **L. plantarum.**

No significant difference was observed in pork for pH values among samples inoculated with different strains, although all sources of variation showed a significant difference. The lowest pH values were observed in samples inoculated with **L. acidophilus** and **P. pentosaceus** at 15 C, wrapped and added with sucrose.

There was also a significant difference between species in pH values with higher values for beef as compared with pork; the higher buffer capacity in beef requires larger acid production by microorganisms in order to decrease considerably its pH.

No significant differences were observed among pork or beef samples inoculated with the strains under study. Pork

showed a significant difference when samples were wrapped, increasing considerably the lactic acid bacteria numbers (log^{-1} 6.33 and log^{-1}5.52/cm2 for wrapped and unwrapped samples, respectively). Oppositely, beef did not show a significant difference between wrapped and unwrapped samples (log^{-1} 6.40 and log^{-1} 6.39/cm2, respectively). No other significant difference was observed for lactic acid bacteria counts in beef. Higher counts in pork samples were observed for those inoculated with **L. bulgaricus** and **P. pentosaceus** and in beef samples with **L. bulgaricus, L. casei** and "Strain 2". There was also a significant difference, as expected, between species with respect to lactic acid bacteria growth.

No significant differences were observed among pork and among beef samples inoculated with the strains under study. However, pork showed lower **Pseudomonas** numbers than beef, in both cases lowest values were observed in wrapped samples. This can be due to the accumulation of small amounts of carbon dioxide, inhibiting the growth of **Pseudomonas** and encouraging the growth of lactic acid bacteria (pork: log^{-1} 6.29 and log^{-1} 6.60/cm2; beef: log^{-1} 6.95 and log^{-1}6.92/cm2 for wrapped and unwrapped samples, respectively). The lowest **Pseudomonas** counts were observed in pork inoculated with **L. acidophilus** and **P. pentosaceus** and in beef inoculated with **L. bulgaricus** and **L. plantarum.**

There was a significant difference between species with respect to **Pseudomonas** counts, with lower values for pork samples (log^{-1} 5.945/cm2) as compared with beef samples (log^{-1} 7.150/cm2) due to factors such as buffer capacity of pork meat, lactic acid production by lactic acid bacteria, although these last bacteria counts were higher in beef samples (log^{-1} 7.251/cm2) than in pork samples (log^{-1}5.704/cm2).

Experiment 2.: There was no significant difference for any of the sources of variation for pork and beef regarding lactic acid concentration. Oppositely, significant differences were observed for all sources of variation in beef for the three treatments. As expected the commercial inoculum produced the largest lactic acid concentration in the samples. The only significant difference in the case of pork was observed for unwrapped samples added with sucrose and stored at 27 C. Samples inoculated with the commercial strain also showed the highest lactic acid concentration. This different behaviour between the two species could be due to the buffer capacity of beef, where a strain producing larger amounts of lactic acid will be more evident than in pork, i. e. suitable strains produce easier amounts of lactic acid to be detected than in beef. No significant difference was detected between species although concentrations were higher in pork as compared with beef samples.

No significant difference was observed in pH values in beef for any source of variation; significant differences were observed in pork for Oxygen availability and Carbon sources of variation in wrapped samples; pH values decreased

significantly when the atmosphere in the meat surface has CO_2, promoting growth of lactic acid bacteria, hence showing higher lactic acid concentrations in the substrate. Although there was a significant difference between wrapped and unwrapped pork samples regarding pH values, this difference does not exist regarding lactic acid concentration for the same source of variation, therefore a decrease in pH values does not mean necessarily higher lactic acid production, but probably the presence of other acidic compounds. The lack of correlation between lowering of pH and lactic acid production could be due to the buffer capacity of the meat. There were significant differences between temperatures for beef among samples inoculated with different strains. In pork , temperature demonstrated to be important only in unwrapped samples the difference was due to naturally contaminated samples, were pH values were considerably lower.

No significant differences were observed in lactic acid bacteria counts in beef for any source of variation; pork showed a significant difference between wrapped samples with no sucrose added probably due also to the large numbers of non-lactic acid producing organisms presesent in the uninoculated samples. Storage temperatures seem to be of a second importance regarding growth of the strains studied. Both, pork and in beef, had bacteria numbers higher in samples inoculated with **L. bulgaricus** and **P. pentosaceus.** However, lactic acid production was consistently higher when the commercial inoculum was applied in both species which reflects the higher lactic acid productivity of this starter. No significant difference was observed between species although the counts were higher in beef than in pork samples, the variance was also higher ($\bar{X} = \log^{-1} 6.751/cm2$, $\sigma^2 = 2.581$ and $\bar{X} = \log^{-1} 6.211/cm2$, $\sigma^2 = 1.446$, respectively).

No significant difference was observed in **Pseudomonas** counts for any source of variation in both species. No pattern was observed in cell numbers for the conditions studied. Samples inoculated with the commercial inoculum and with **L. bulgaricus** and **P. pentosaceus** did not show a reduction in **Pseudomonas** counts. This could be due to the aerobic conditions which prevent the growth of anaerobic lactic acid bacteria in unwrapped samples, or to microarophilic conditions in saran-wrapped samples (Nychas et al., 1988). No significant difference was observed between species, mean values and variances were similar ($\bar{X} = \log^{-1} 7.127$, $\sigma^2 = 2.723$ and $\bar{X} = \log^{-1} 6.754$, $\sigma^2 = 2.302$ for beef and pork respectively).

CONCLUSIONS.

Experiment 1: In general, the use of lactic acid bacteria would be of importance in reducing naturally occurring microbial contamination as well as spoilage organisms only when strict extrinsec factors favouring lactic acid bacteria growth are applied. The two strains which seems to favour a decrease in **Pseudomonas** were **L. bulgaricus** and **L. acidpohilus.** Although the uso of commercial inocula would provide some

evidence in the benefits of using lactic acid bacteria as decontaminants. All response variables showed a significant difference between species, probably due to the well known better lactic fermentation characteristics of pork as compared with beef.

Experiment 2: The use of two lactic acid bacteria strains, **L. bulgaricus** and **P. pentosaceus**, reported to have a synergistic effect which improved meat shelf life, showed to have some advantages upon non inoculated meat. However, the use of a commercial inoculum containing improved strains produced the best results, decreasing **Pseudomonas** counts and keeping a low pH value. Extrinsec factors such as Oxygen availability and storage temperature showed to be important in prometing growth of lactic acid bacteria, although the improved characteristics of the commercial inoculum made it to have good growth and production of lactic acid in both species. For this reason, no significant difference was observed for any response variable when the two species were compared.

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