

# Analysis of Spoilage Bacteria Present in Vacuum Packaged Chilled Beef Treated with Organic Acids

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## Summary with Implications

*Preventing the spoilage of fresh, chilled beef is crucial for maintaining market value. Since organic acids are regularly used in the beef industry for pathogen control, their ability to improve the shelf life of fresh, chilled beef was evaluated. Fresh, chilled beef was individually treated with 4.5% lactic acid, 2.5% Beefxide®, or 380 ppm peroxyacetic acid. After storage, Lactococcus, a genus of lactic acid bacteria, became the most common spoilage organism across all treatment and control samples. Organic acid treatments were not able to slow the growth of this genus and may not be an effective method to control spoilage when lactic acid bacteria are the dominant spoilage organism present.*

## Introduction

In 2020, the U.S. beef industry exported 1.2 million tons of beef valued at over \$7 billion. Fresh, chilled beef from the U.S. is considered a premium product, and ensuring that these products arrive at their export destination without the negative effects of bacterial spoilage is crucial for maintaining their value. Spoilage bacteria can create a variety of off-aromas, textures, and colors that make the meat undesirable for the consumer. Often, organic acids like lactic acid are used in beef processing facilities to decrease the presence of *E. coli* O157:H7, but these compounds are also able to slow the growth of spoilage bacteria that may be present on the meat. An experiment was conducted to determine the impact of different organic acids on the prevalence of

spoilage bacteria during extended storage of vacuum packaged, raw beef.

## Procedure

Beef chuck rolls (N = 24) from two processing facilities were obtained on two separate days of production. Chuck rolls from each facility and day of production were processed separately seven days post mortem. Each chuck roll was cut in half and each half was assigned to a treatment (4.5% lactic acid, 2.5% Beefxide®, 380 ppm peroxyacetic acid, or a no-treatment control), and then halves were cut into at least six smaller pieces each weighing approximately two pounds. The pieces were submerged into the assigned organic acid treatment (73 °F) for 15 seconds, drip-dried for two minutes, and individually vacuum sealed. Samples were stored at 37 °F for 112 days in a dark cooler. Every 28 days starting on the day of organic acid treatment (day 0) and ending on day 112, plate counts, 16S sequencing, surface color, and surface pH were evaluated. For plate counts and sequencing, 100 g was cut from the surface of each piece and homogenized with 100 mL of buffered peptone water. Homogenate was plated in duplicate onto brain heart infusion agar for aerobic, anaerobic, and psychrotrophic plate counts; deMan Rogosa Sharpe agar for lactic acid bacteria; and cephaloridine fucidin cetrimide agar for *Pseudomonas*. DNA was also extracted from homogenates and then amplified targeting the V4 region of the 16S rRNA bacterial gene with polymerase chain reaction. Purified V4 16S gene segments were sequenced with the Illumina MiSeq and analyzed using R. L\*, a\*, b\*, and pH were measured on the surfaces of each two-pound piece after removing it from the vacuum package. The experiment was conducted in two independent replications with one day of production from each facility included in each replication. Data for microbial plate counts, sequencing alpha diversity, color, and pH were analyzed as an incomplete block design with 2 loca-

tions, 4 treatments, and 5 sampling days in SAS 9.4. Sequencing beta diversity was analyzed by conducting a principal coordinate analysis and a PERMANOVA in R with treatment, location, sampling day, block, and a treatment: day interaction included in the model. Samples stored for 28 days from the second location were not evaluated due to a COVID related laboratory closure that prevented sampling.

## Results

Treatment of raw beef with Beefxide®, lactic acid, and peroxyacetic acid resulted in spoilage by lactic acid bacteria during vacuum packaged, refrigerated storage. Meat spoilage is typically indicated by bacterial counts greater than 7 log<sub>10</sub> and a decrease in alpha diversity (diversity of bacteria within a sample). Concentrations of anaerobic and lactic acid bacteria plate counts approached 7 log<sub>10</sub> after 56 days of storage (Figure 1), and alpha diversity decreased from day 0 to day 28. The dominance of lactic acid bacteria is also shown by the relative abundance of 16S sequences. *Lactococcus* are present in a relatively small proportion on day 0 and then become the most abundant genus throughout the remainder of storage across all treatment and control samples. A trend in the *Pseudomonas* plate counts (P = 0.07) was seen where lactic acid and Beefxide® treatments had lower *Pseudomonas* concentrations than the control group (Figure 1). This pattern was also observed in the 16S abundances on days 56, 84, and 112 where the control and peroxyacetic acid treatment groups had slightly higher abundance of *Pseudomonas* compared to the lactic acid and Beefxide® treatments. This suggests that organic acid treatments containing lactic acid may aid in slowing the growth of *Pseudomonas*. However, clustering in the principal coordinate analysis showed that even though treatment was significant (P < 0.01), the primary grouping of samples is attributed to length of storage. The difference in alpha diversity between treatments was

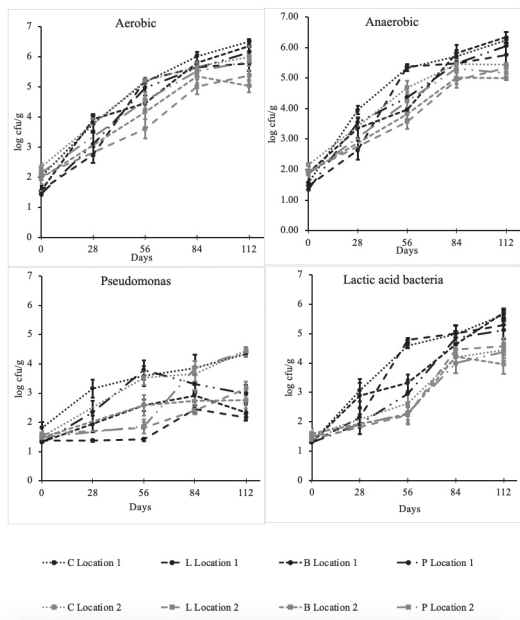


Fig. 1. Concentration of aerobic, anaerobic, *Pseudomonas*, and lactic acid bacteria ( $\log_{10}$  cfu/g) during cold storage. Error bars represent standard error. C = control. L = Lactic acid B = Beefixide. P = Peroxyacetic acid.

also not significant (Observed  $P = 0.30$ , Chao1  $P = 0.39$ ) further indicating that the bacterial composition of treated samples was not different than the untreated control samples. Both the principal coordinate analysis and alpha diversity measures indicate that the increase of lactic acid bacteria regardless of treatment was the most relevant biological change in these samples. Surface pH was not different between treatments ( $P = 0.16$ ) and decreased during storage ( $P < 0.01$ ), and the overall color of the samples followed a decrease in lightness and redness that is often seen in beef stored for an extended period of time. In con-

clusion, *Lactococcus* became the dominant genus in all samples by day 56. Because organic acids are less effective at slowing the growth of these organisms, there was minimal distinction between treatment and control groups. When addressing spoilage of raw meat, interventions should be targeted to slow the growth of the bacteria known to cause spoilage in a specific product so that ineffective interventions are not used.

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