CONTROL OF *ESCHERICHIA COLI* O157:H7 IN LARGE-DIAMETER, LEBANON-STYLE BOLOGNA

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Summary

Lebanon bologna raw batter was mixed with a five-strain mixture of *Escherichia coli* O157:H7 to achieve average inoculum levels of 7.79, 7.77, and 7.92 log CFU/g as determined on MSA, 202, and PRSA media, respectively. Treatment 1 consisted of a fermentation cycle of 8 hrs at an internal temperature (I.T.) of 80°F then 24 hrs at 100°F I.T., followed by 24 hrs at 110°F I.T. Treatments 2, 3, and 4 included additional heating at 115°F I.T. for 1, 2, and 5 hrs, respectively. All heat treatments resulted in product that was negative (<1.9 log CFU/g detection limit) on all culture media and negative after enrichment on mEC selective medium. This study validates that a five-log reduction of *E. coli* O157:H7 can be achieved using the described protocol, thus meeting USDA/FSIS requirements.

(Key Words: *E. coli* O157:H7, Food Safety, Fermented Beef, Sausage.)

Introduction

In December of 1994, an outbreak of *Escherichia coli* O157:H7 was linked to the consumption of dry cured salami. This outbreak caused USDA/FSIS to require that fermented sausage processes achieve a five-log reduction of *E. coli* O157:H7 in a test situation when starting with at least 7.3 CFU.

Lebanon bologna is a fermented beef sausage that utilizes a low-temperature, long-time fermentation process, but is susceptible to *E. coli* O157:H7 contamination. An available medium is sensitive to recovering heat-injured cells. Therefore, the objectives of this study were: 1) to determine the effects of typical thermal processing temperatures and times for Lebanon bologna on reducing *E. coli* O157:H7 and 2) to evaluate the effectiveness of MacConkey Sorbitol Agar (MSA), 202 agar, and Phenol Red Agar with 1% sorbitol (PRSA) for detecting *E. coli* O157:H7.

Experimental Procedures

Five different isolates of *E. coli* O157:H7 were used. Two were human isolates, and the others were of meat origin, one being implicated in the 1995 salami outbreak. Isolates were incubated on tryptic soy agar slants at 98°F for 20 ± 2 hrs and maintained at 40°F until needed. After further inoculation, cells were harvested by centrifugation, resuspended, centrifuged again, and then held at 40°F until needed (less than 2 hrs).

Commercially prepared beef meat batter (90% lean) containing salt; sucrose; dextrose; spices; potassium nitrate; sodium nitrite; and starter culture (*Pediococcus*, *Lactobacillus*, and *Micrococcus* spp.) was received overnight from the manufacturer. Upon receipt, the raw batter was at 45 ± 4°F.

For the inoculated treatments, 55 lb of meat batter was spread evenly (1 to 1.5 in. thick) onto a flat surface to allow for even distribution. The inoculum was intermittently pipetted drop-wise over the meat surface and thoroughly mixed.

The meat batters (control and inoculated) were transferred to a hand stuffer and stuffed into prestuck, presoaked, 4½ in.-diameter casings. Each chub weighed approximately 6.6 lb and was about 10 in. long.

Chubs were hung vertically on racks. Inoculated and control chubs were placed randomly in a commercial smoke house (Alkar, Lodi, WI). Fermentation included 8 hrs at an internal temperature (I.T.) of 80°F, then 24 hrs at 100°F I.T., followed by 24 hrs at 110°F I.T. Natural smoke was applied during the last 2 hrs of the 110°F cycle. Heat treatments 2, 3, and 4 included additional heating at 115°F I.T. for 1, 2, and 5 hrs, respectively. For each internal temperature, an appropirate time was allowed for that temperature to be reached. The relative humidity (RH) for the 80°F stage was 90%. For the 110, 110, and 115°F stages, the relative humidity was 60 to 65%. In the commercial Lebanon bologna process, the RH is maintained at 90% throughout the process, and the specified moisture to protein ratio is 3.1:1. Because of the lower relative humidity, our product is referred to as "Lebanon-style bologna."

MacConkey Sorbitol Agar, 202 agar, and PRSA were used for enumerating *E. coli* O157:H7. All plates were spiral plated and incubated at 107°F for 24 hrs. Modified *E. coli* broth was used for enrichment of *E. coli* O157:H7. Identification was confirmed with API 20# and RIM *E. coli* O157:H7 latex agglutination test.

A special medium (APT) was used for lactic acid bacteria (LAB) enumeration. All LAB plates were incubated at 95°F for 24 hrs in a CO_2 chamber with 20% CO_2.

Both raw and heat-treated samples were analyzed for moisture, fat, salt, protein, ash, water activity, pH, and titratable acidity.

Results and Discussion

For all heat treatments, the log (CFU/g) reduction values were 5.89, 5.87, and 6.07 on MSA, 202, PRSA media, respectively. A 6 log reduction means a kill of 99.9999% of original *E. coli* O157:H7 organisms. All heat treatment samples were also negative after enrichment on mEC selective medium. The LAB counts were between 7.2 and 7.4 log CFU/g for the raw batter and 6.8 to 6.9 log CFU/g for all of the heat treatments. A 7 log population is 10 million organisms.

Minimal variation was found for all product characteristics both within and between treatments. Overall pH was 4.4 after fermentation. Moisture was 60.8%; protein, 22.5%; fat, 10.6%; and salt, 4.8%. The moisture to protein ratio was 2.7, with water activity at 0.94. All heat treatments on all media resulted in a product that was negative (<1.9 log CFU/g detection limit) for *E. coli* O157:H7 and negative after enrichment on mEC selective medium. This study validates that a five-log reduction of *E. coli* O157:H7 can be achieved using the described heating protocol, thus meeting USDA/FSIS requirements.