Use of Lactic Acid Bacteria as Pathogen Surrogates to Validate Commercial Whole-Muscle Beef Jerky Process Lethality against *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus*

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Introduction:

Beef jerky is often processed at low temperature and/or low humidity to attain desired quality. The low-humidity heating reduces process lethality due to enhanced heat-resistance in pathogens such as *Salmonella.* Additionally, evaporative cooling occurs on the surface of the strips, reducing the temperature to which pathogens are exposed.

The USDA/FSIS has identified *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* as pathogens of concern in beef jerky *(5)*. Because *Salmonella* is considered the most heat resistant, destruction of *Salmonella* is believed to ensure the destruction of the other pathogens *(5)*. In response to several illness outbreaks linked back to beef jerky over the last 40 years, the USDA/FSIS requires processors to validate a 5-log reduction in *Salmonella* during the manufacture of jerky in order to ensure the safety of the finished product *(5)*. Validation can be costly and time consuming for small processors. Most available research validates specific processes or additional chemical treatments *(2, 3, 4,)*; thereby limiting process options and making it difficult for processors to continue using their unique processes *(2, 3, 4)*.

Lactic acid bacteria (LAB), particularly *Pediococcus* spp., have been shown to be effective pathogen surrogates in the validation of ground-and-formed beef jerky processes *(1).* Using LAB as surrogates is a safe way to perform challenge studies specific to unique processes.

Objective:

Our objective was to develop a novel in-plant validation method which uses a GRAS LAB starter culture as a surrogate for pathogens in whole muscle beef jerky processing.

Materials and Methods:

Jerky Strip Preparation:

•Beef rounds, knuckles, and shoulder roasts sliced and cut into identical strips (5.08 cm by 15.24 cm by 0.60 cm).
•Average strip weight was 22.9g (n=2754).

Inoculum Preparation:

•Inocula consisted of approximately 10^9 CFU/ml of either *Listeria monocytogenes* (5 strains), *Staphylococcus aureus* (5 strains), Saga 200 (LAB – *Pediococcus acidilactici;* Kerry Bioscience, Rochester, MN), or a combined inoculum of *E. coli* O157:H7 and *Salmonella* spp. (5 and 8 strains, respectively).
•Pathogen inocula were prepared from stationary-phase cells that were re-suspended in Butterfield's phosphate diluent (BPD).
•LAB inoculum was prepared from 0.5g of Saga 200 re-suspended in 9ml BPD.

Inoculation of Jerky Strips:

•Either a pathogen- or LAB-inoculum (0.4 ml) was pipetted onto the surface of each meat strip at 21°C in a biosafety hood and evenly spread (Figure 1).
•After a 30 min attachment period, the strips were turned over and the inoculation repeated on the other side (Figure 2). Initial inoculum level was ~10^7 CFU/cm^2.
•Inoculated strips were transferred to 1-gal Ziploc ® bags for marination using one of three spice blends: Colorado, Terikyaki or Original (Excalibur Seasoning Company). Inoculated meat/spice mix was hand-tumbled for 5 min and stored for 22-24 h at 4ºC.

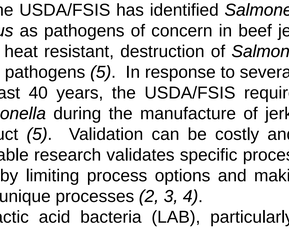

Figure 1.

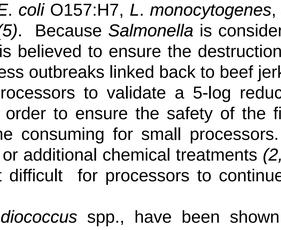

Figure 2.

Jerky Processing Conditions:

•Marinated strips were dried in a small-scale commercial dehydrator (Pragotrade model TS160, Cabela's Inc., Sidney, NE) in the laboratory, or in a commercial smokehouse/oven (Model 2000, Alkar-RapidPak, Lodi, WI) at the Alkar-RapidPak Research and Technology Center (ARPRTC, Lodi, WI) (Figure 3).
•Six processes were evaluated (Table 1).
•Strips were laid out in groups of four, consisting of one strip inoculated with *E. coli* O157:H7 and *Salmonella* spp., and one strip each inoculated with *L. monocytogenes, S. aureus,* or LAB (Figure 4).

Table 1. Representative Commercial Processing Schedules Used in the Preparation of Whole-Muscle Beef Jerky

Process Number	Step Time (min)	Cumulative Time (min)	Smoke* (Y/N)	Set Dry Bulb Temp. ºC (ºF)	Set Wet Bulb Temp. ºC (ºF)
1[a]	420	420	N	68.3 (155)	NC[b]
2-A[c]	30	30	N	76.7 (170)	60.0 (140)
	120	150	N	54.5 (130)	NC
	90	240	N	76.7 (170)	NC
3-A	90	90	N	57.2 (135)	51.7 (125)
	150	240	N	85.0 (185)	NC
4-A	90	90	N	54.5 (130)	NC
	60	150	N	60.0 (140)	21.1 (70)
	60	210	N	65.6 (150)	26.7 (80)
	60	270	N	70.1 (160)	32.2 (90)
	60	330	N	76.7 (170)	NC
5-B	30	30	N	54.5 (130)	NC
	60	90	Y	54.5 (130)	NC
	60	150	Y	60.0 (140)	21.1 (70)
	60	210	Y	65.6 (150)	26.7 (80)
	60	270	Y	70.1 (160)	32.2 (90)
	60	330	N	76.7 (170)	NC
6-B	30	30	N	46.1 (115)	NC
	30	60	Y	46.1 (115)	NC
	60	120	N	54.5 (130)	NC
	60	180	N	62.8 (145)	NC
	60	240	N	70.1 (160)	NC
	30	270	N	76.7 (170)	NC

*Smoke added during the step (yes/no). When added, smoke was added during the entire step unless noted.
[a]Process 1 – small commercial dehydrator (Cabela's); Processes 2-7 – commercial oven/smokehouse (Alkar)
[b]NC, not controlled
[c]A= processes without smoke, B= processes with smoke

Figure 3.

Figure 4.

Enumeration of Surviving Cells:

•Samples were taken post-inoculation, post-marination, and at intervals throughout the drying process.
•At designated intervals, four jerky strips per spice (one per inoculum) were removed from the dehydrator/smokehouse, placed separately in Whirl-pak filter bags with 99 ml BPD and stomached for 2 min at medium speed. Further serial dilutions were made in BPD.
• *E. coli* O157:H7 and *Salmonella* were enumerated using modified eosin methylene blue (M-EMB) agar prepared from lactose-free EMB (Difco) with the addition of 10 g/l D-sorbitol and 5 g/l NaCl. This medium has been shown to be a superior for enumerating these pathogens in jerky *(1)*.
•For enumeration of *L. monocytogenes*, *S. aureus*, and Saga 200, samples were spread on brain heart infusion agar (BHIA, Difco; Figure 5), followed by 1 h at 35ºC (injury-repair) and selective-medium overlays: *Listeria* Selective agar (LSA, Difco) with *Listeria* selective supplement (Difco), Baird-Parker (BP) agar (Difco) with egg yolk-tellurite supplement (Difco), and lactobacilli deMan Rogosa Sharpe (MRS) agar (Difco), respectively (Figure 6).
•Samples were incubated at 35ºC for 24 h (MEMB) or 48 h (LSA, BP, and MRS overlay plates). The count (log CFU/cm^2) for each organism was calculated, and mean counts were calculated for each organism-spice combination at each sampling time.

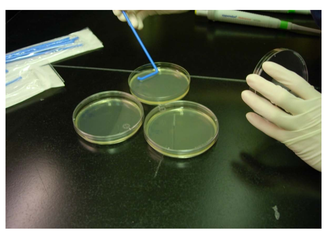

Figure 5.

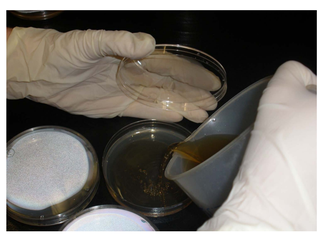

Figure 6.

Statistical analyses:

•Mean log reduction for each process-spice-organism combination was calculated.
•Mean log reduction values were put into a three way Anova to determine significant differences between processes, spice marinades, and organisms, with interactions between these factors also evaluated for significance (version 9.1; SAS, Institute, Inc., Cary, NC).

Results and Discussion:

Across all processes, spices, and organisms, Saga 200 survived significantly ($p<0.0001$) better than the four pathogens of concern. With a criterion of ≥5.0 log CFU reduction, Saga 200 is a suitable surrogate for evaluating adequate process lethality against these pathogens. (See Figures 7-8 as examples).

•Processes 3 and 5 did not differ in average lethality, but processes 1, 2, and 4 were significantly different in lethality ($p<0.0001$). Processes 2, 3, and 5 averaged ≥5 log CFU reduction across all inocula.
•Processes 2 and 3 were successful in reaching an average ≥5 log CFU reduction because of early-process steps with high dry-bulb temperature and high relative humidity (high wet-bulb temperature). Process 5 likely achieved high lethality because of the high dry-bulb temperature associated with added smoke.
•Spice mix did not have a significant effect on pathogen lethality.
•Across processes and spices, the average decrease in *Salmonella* was not significantly different ($P > 0.05$) than the average values for the other pathogens of concern.

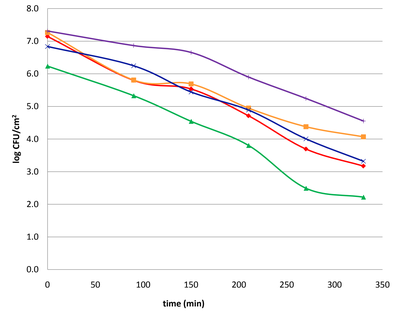

Figure 7. Lethality throughout process 4 Key: + Saga 200, ■ *E. coli* O157:H7, x *S. aureus*, ◆ *Salmonella*, and ▲ *L. monocytogenes*.

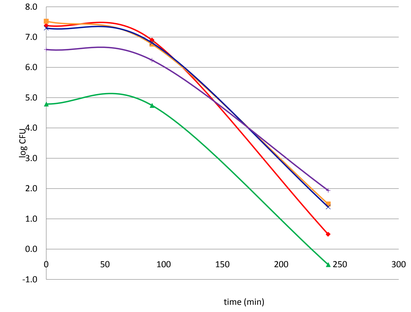

Figure 8. Lethality throughout process 3 Key: + Saga 200, ■ *E. coli* O157:H7, x *S. aureus*, ◆ *Salmonella*, and ▲ *L. monocytogenes*.

Conclusions:

Saga 200 is a suitable pathogen-surrogate that can be used to create an in-plant validation tool. This validation tool will be cost-effective and easy for the processor to use. It will also provide the processor with a scientifically supported method to meet FSIS regulations while maintaining their original process and product. The validation tool will help processors produce safe jerky and identify processes that could result in unsafe jerky.

References:

1. Borowski, A., S.C. Ingham and B.H. Ingham. 2009. Validation of ground-and-formed beef jerky processes using commercial lactic acid bacteria starter cultures as pathogen surrogates. *J. Food Prot.* 72: 1234-1247.
2. Buege, D.R., G. Searls, and S.C. Ingham. 2006. Lethality of commercial whole-muscle beef jerky manufacturing processes against *Salmonella* serovars and *Escherichia coli* O157:H7. *J. Food Prot.* 69:2091-2099.
3. Calicioglu, M., J.N. Sofos, J. Samuels, P. A. Kendall, and G.C. Smith. 2002. Inactivation of acid-adapted and nonadapated *Escherichia coli* O157:H7 druing drying and storage of beef jerky treated with different marinades. *J. Food Prot.* 65:1394-1405.
4. Harrison, J.A., and M. A. Harrison. 1996. Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* during preparation and storage of beef jerky. *J. Food Prot.* 59:1336-1338.
5. United States Department of Agriculture, Food Safety and Inspection Service. 2001. Performance Standards for the Production of Processed Meat and Poultry Products. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97-013P.htm. Accessed 5 May 2009.

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