



Microbial Validation of Biltong Processing to Achieve 5-Log Reduction of *L. monocytogenes* and *E. coli* O157:H7



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Biltong:

- A popular ready to eat salted and dried meat product from South Africa.
- Uses lean strips with excess fat trimmed that may cause rancidity and extend the time for the meat to absorb the salt.

Production of Biltong

Salting

Spicing

Marination

Drying

- Dried at ambient humidity and temperature
- Recently, increase in interest to manufacture biltong in US
 - Requires USDA-FSIS validation

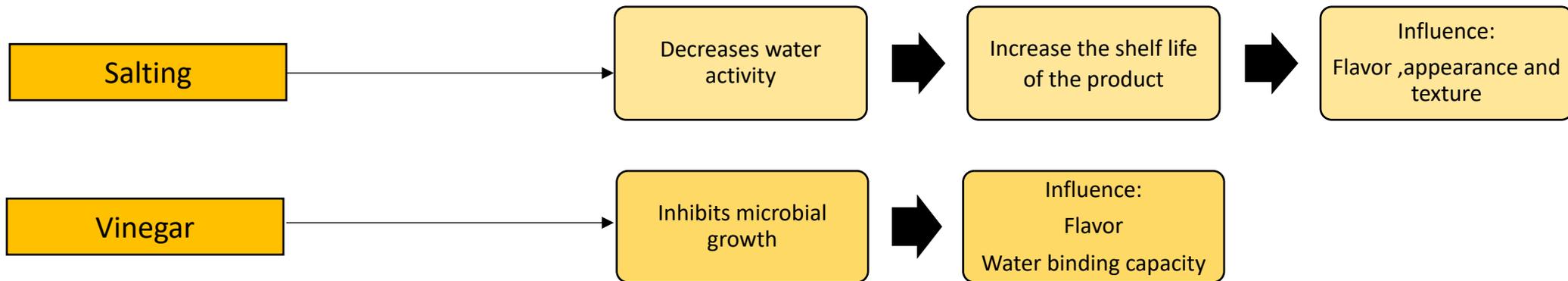
Popular choice for consumers



Favorable go to snack food

Healthy



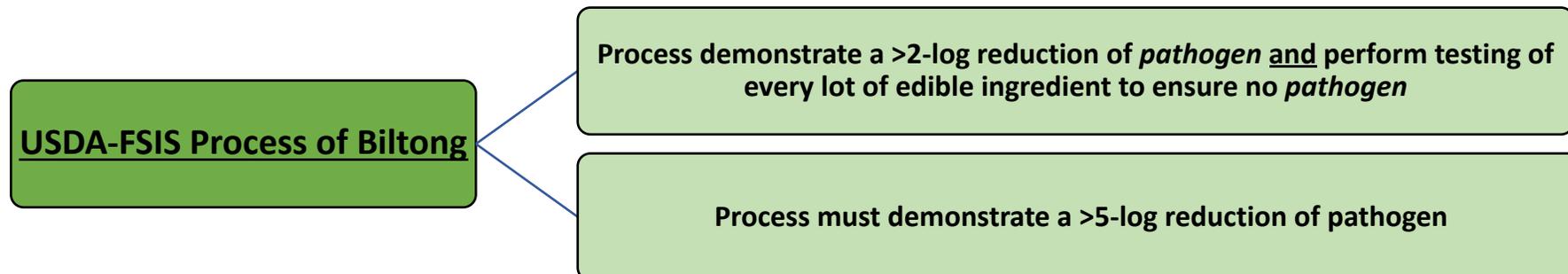


- Biltong is produced without a heat lethality step but still must be determined to be safe for consumer consumption with a sufficient (5-log) reduction of pathogenic bacteria as required by the USDA-FSIS.

USDA-FSIS Biltong Requirements

In the US, beef jerky is manufactured under USDA-FSIS compliance guidelines to achieve >5-log reduction for pathogen of concern (*Salmonella*, *L.mono*, *E. coli* O157:H7, *Staphylococcus aureus*)

- Relative humidity : >90% throughout cooking or thermal heating process
- Cooked at >175F using sealed oven or steam injector



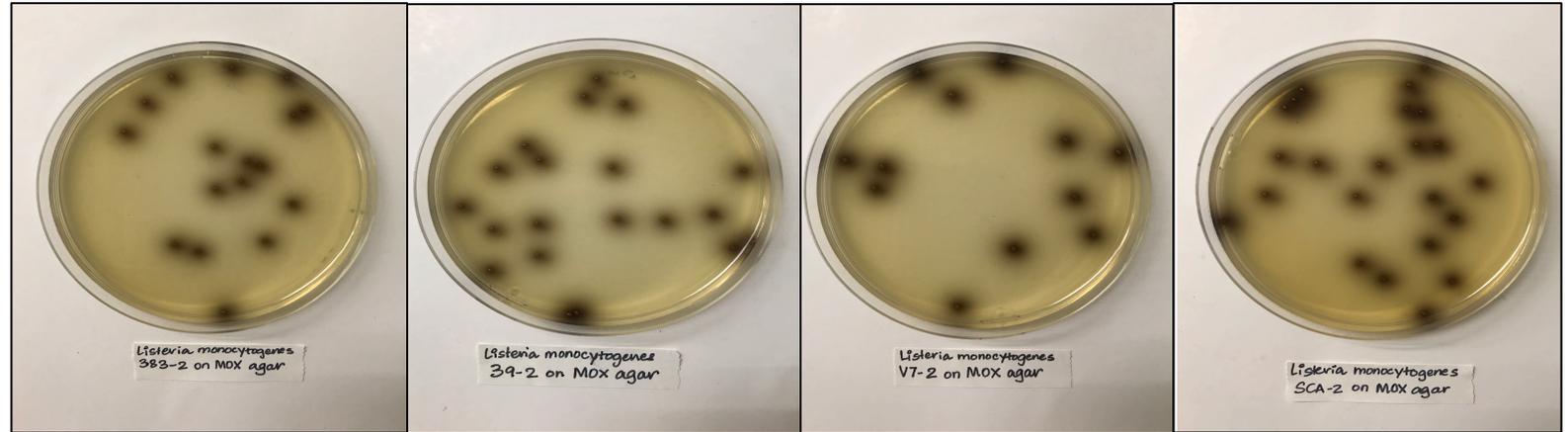
Preliminary confirmation test for *Listeria monocytogenes* using MOX agar

On modified MOX agar *Listeria monocytogenes* strains are :

- Round but the surface is concave and depressed into the media
- Approximately 1mm in diameter
- Black in color and surrounded by a black zone

Growth conditions: TSB broth ; 30 °C for 24 hours

Incubation: 30 °C for 24 hours



383-2

39-2

V7-2

SCA-2

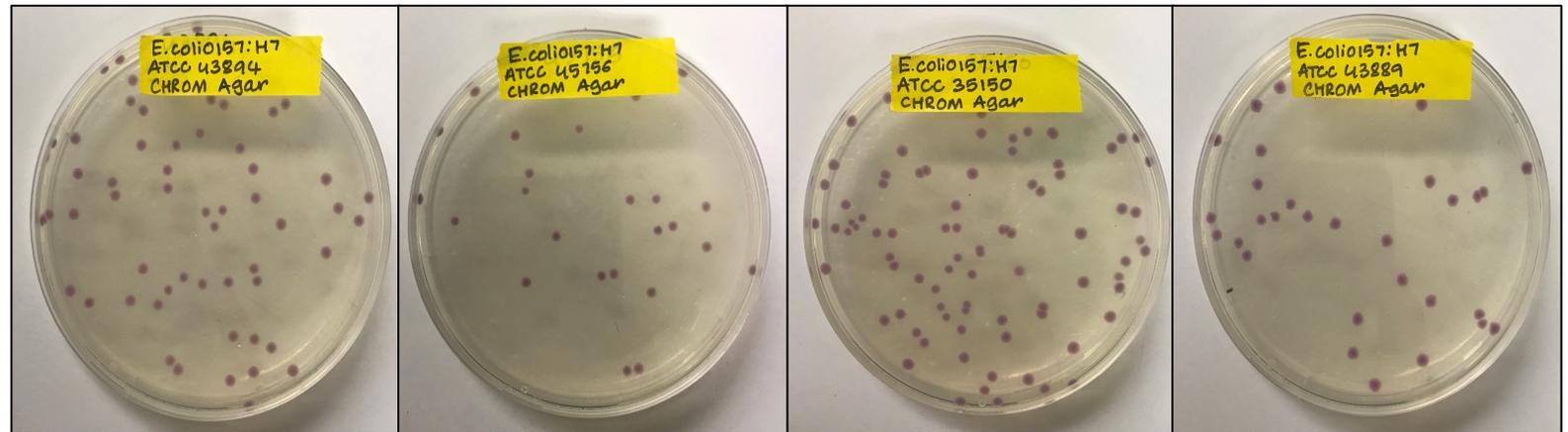
Preliminary confirmation test for *E. coli* O157:H7 using CHROMagar O157

On CHROMagar O157 *E. coli* O157:H7 strains are:

- Mauve colored due to the utilization of chromogenic substrates in the medium.
- Non-*E. coli* O157:H7 bacteria may utilize other chromogenic substrates resulting in blue to blue-green colored colonies .

Growth conditions: TSB broth ; 37 °C for 24 hours

Incubation: 37 °C for 24 hours



ATCC 43894

ATCC 45756

ATCC 35150

ATCC 43889

Microbial Validation of Biltong Processing to Achieve 5-Log Reduction of *L. monocytogenes* and *E. coli* O157:H7

1. Bacterial strains and growth conditions

- Strains are acid adapted by growing overnight at suitable temperature in TSB and re-inoculated into pre-warmed TSB(1% glucose)
- Centrifugation (8000 RPM, 5 °C, 20 min)
- Preparation of culture cocktail



Cultures

Antibiotic resistance:

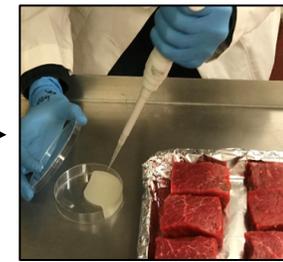
- Listeria monocytogenes*: 100ug/ml streptomycin and 10 ug/ml Rifamycin.
- E. coli* O157:H7 : 5ug/ml of Novobiocin and 2.5ug/ml Rifamycin.

2. Inoculation of culture cocktail on beef pieces.

- Trimming of beef pieces and cutting into similar size of (~1.9cm thick X ~5.1cm wide X ~7.6 cm long)
- Inoculation of beef pieces with 150ul of culture cocktail on each side
- Refrigeration at 5 °C for 30 minutes



Biltong beef pieces



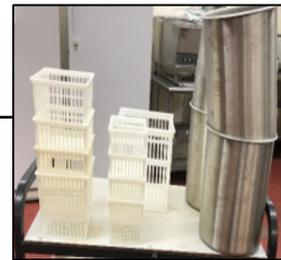
Inoculation



Gloved finger technique

3. Processing Of Biltong

- Water dip for 30 seconds
- Rotated manually and excess liquid is drained



Dip tanks and baskets



Water dip

4. Marination and Drying

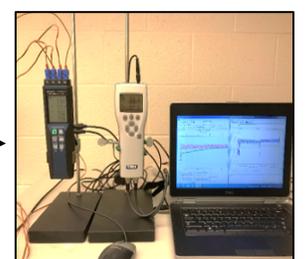
- Short time marination-30 minutes
- Vacuum tumbling (15 inches Hg) of beef pieces with either mixture of spice, salt and Vinegar or with individual components of marinade
- Spice: (Coriander 1.1 % and black pepper 0.8%), Salt (2.2 %) and Vinegar -100 grain (4%) of total formulation
- Stomaching → Dilutions → Plating
- Drying for 10 days: Temperature- controlled humidity oven (75°F/23.9°C; 55% RH)



Vacuum tumbler marination



Treatments hanging in oven



Temperature and humidity probe

Water Activity (A_w) and 'Non-Intact Beef'

- Non-intact beef: ground beef, blade-tenderized beef, vacuum-tumbled beef
- Safety of ground beef (hamburger) & bladed-tenderized beef (steak) can be obtained by cooking to a certain internal temperature
- Biltong is not 'cooked', so safety depends on maintaining low water activity
- USDA-FSIS requires biltong A_w to be < 0.85 (to prevent enterotoxin formation should *Staphylococcus aureus* be present since *S.a.* can tolerate low A_w).

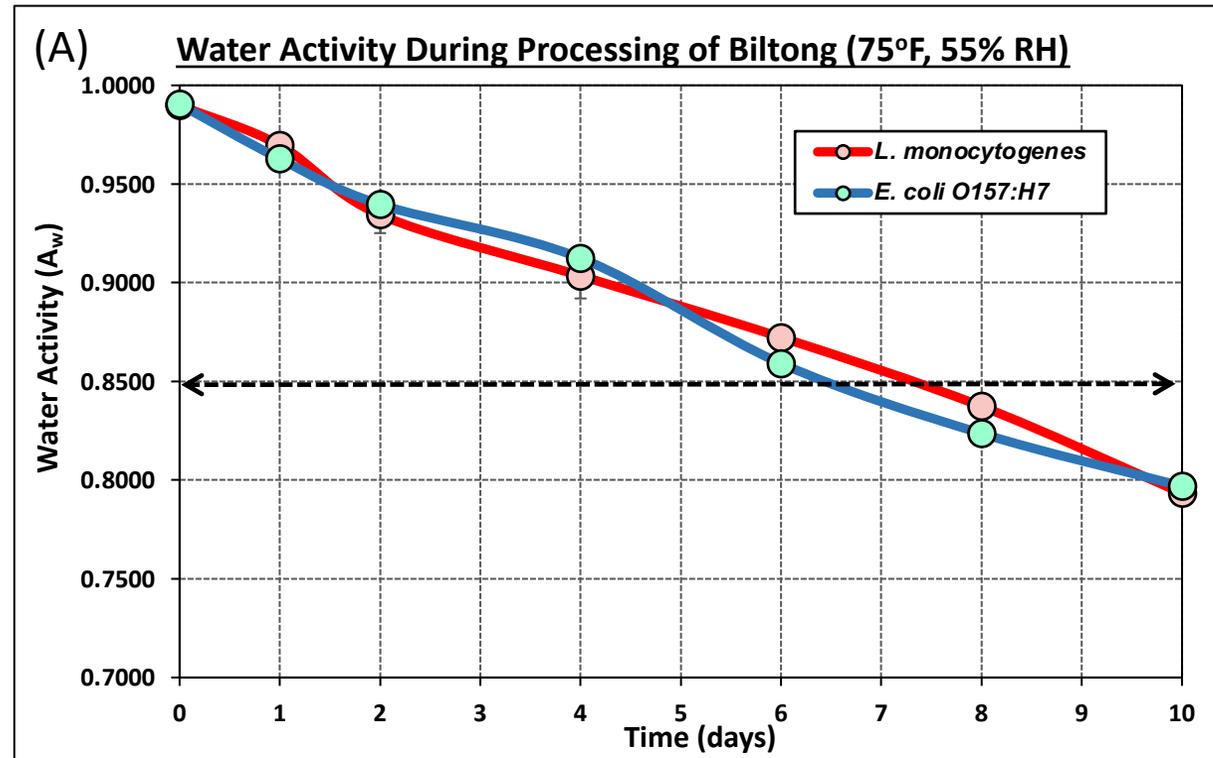
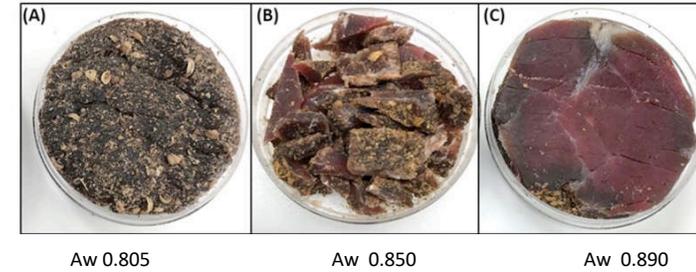


Figure A: Water activity during processing of Biltong for the reduction of *Listeria monocytogenes* and *E. coli* O157:H7 (75°F, 55% RH)

Biltong Processing to Achieve 5-Log Reduction of *L. monocytogenes*

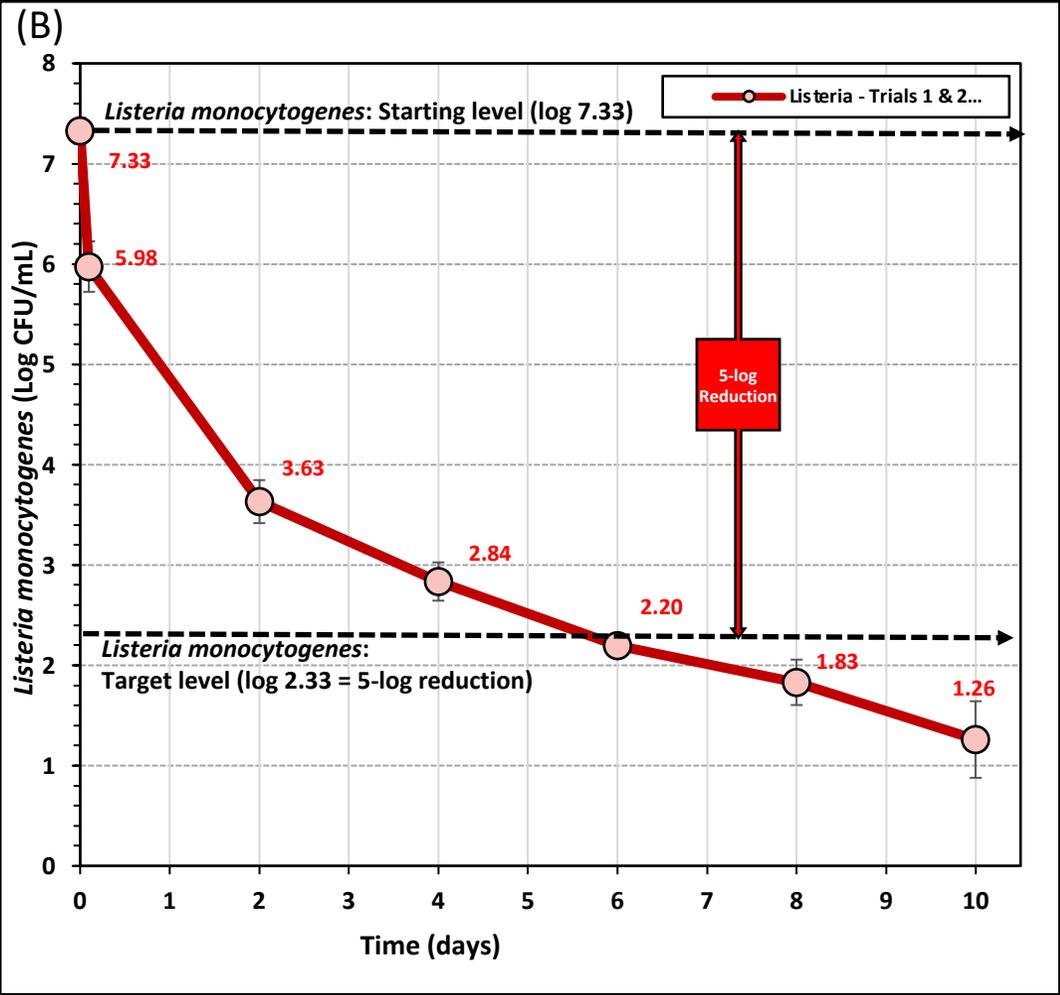


Figure B : Processing of biltong to achieve 5 log reduction of *Listeria monocytogenes* for the combined components of the marinade

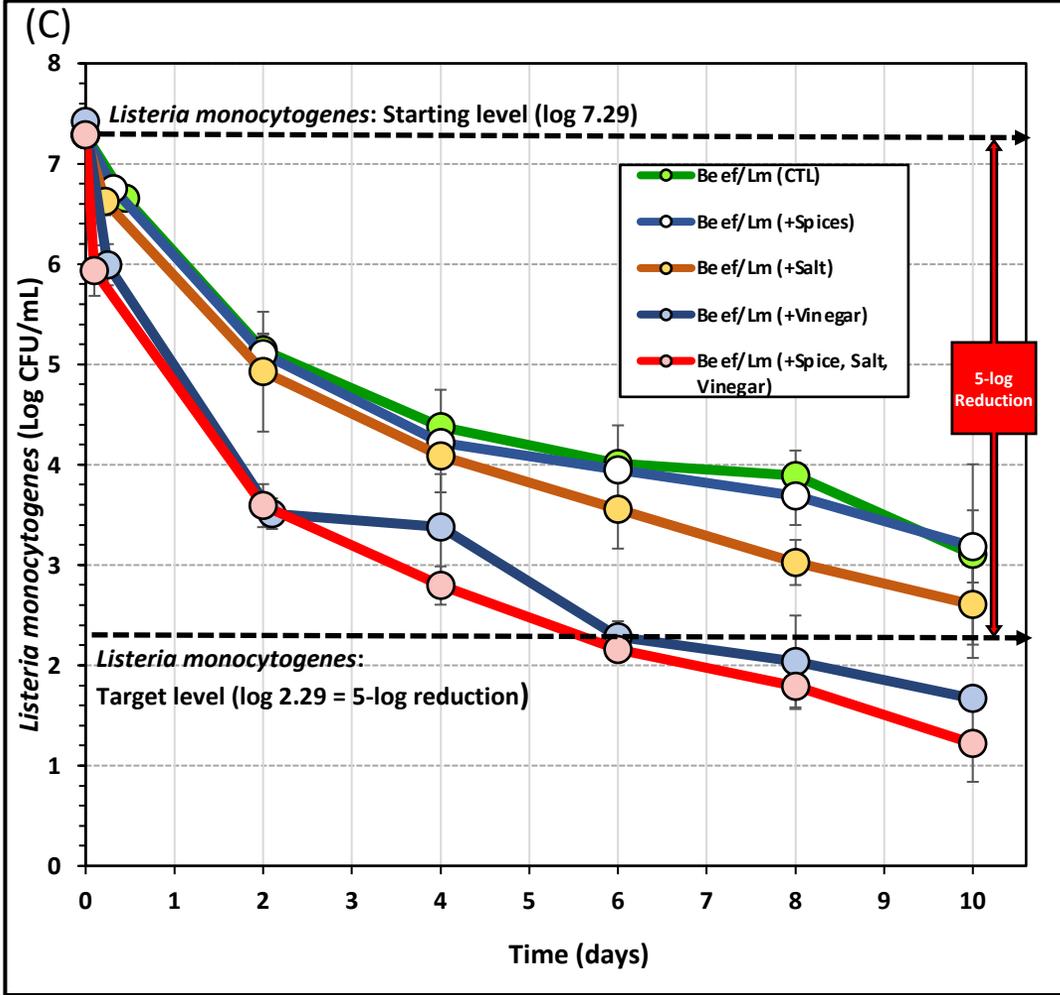


Figure C: Processing of biltong to achieve 5 log reduction of *Listeria monocytogenes* for the individual components of the marinade

Biltong Processing to Achieve 5-Log Reduction of *E.coli* O157:H7

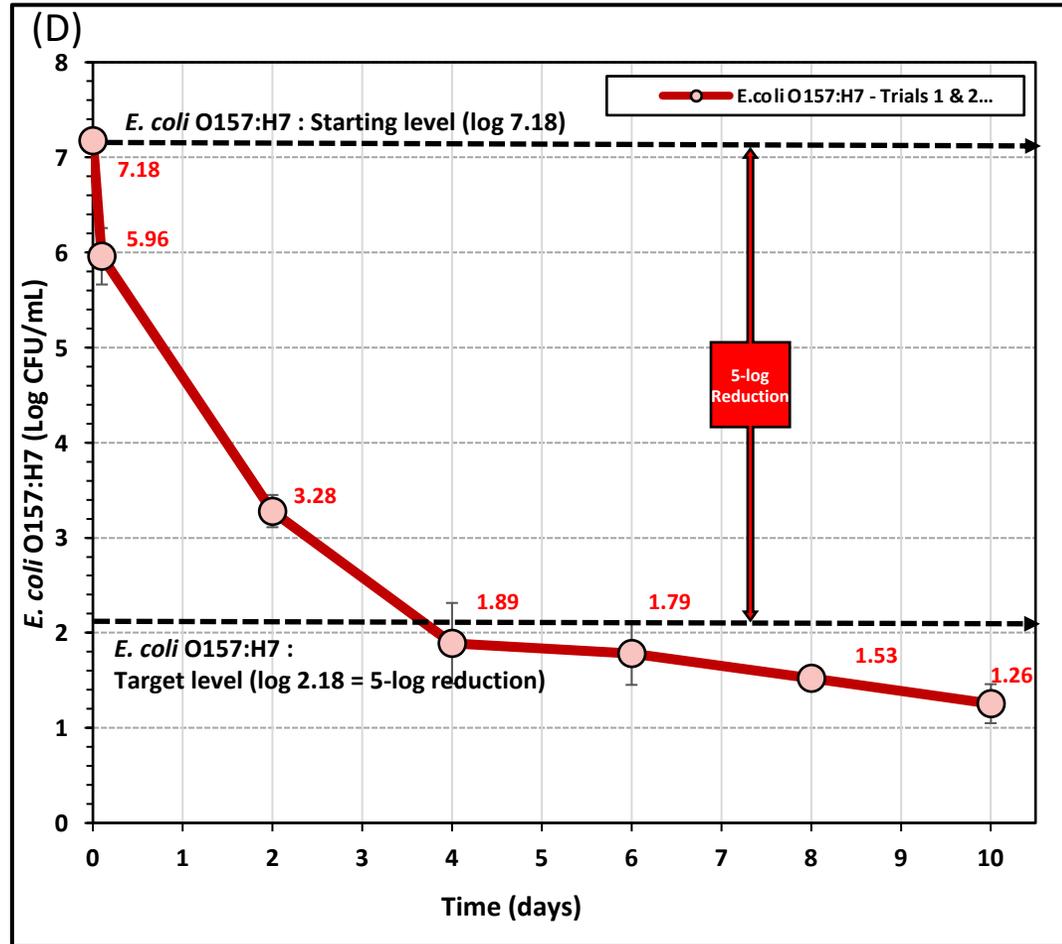


Figure D : Processing of biltong to achieve 5-Log the reduction of *E.coli* O157:H7 for the combined components of the marinade

Temperature and Relative Humidity Measurements for Biltong

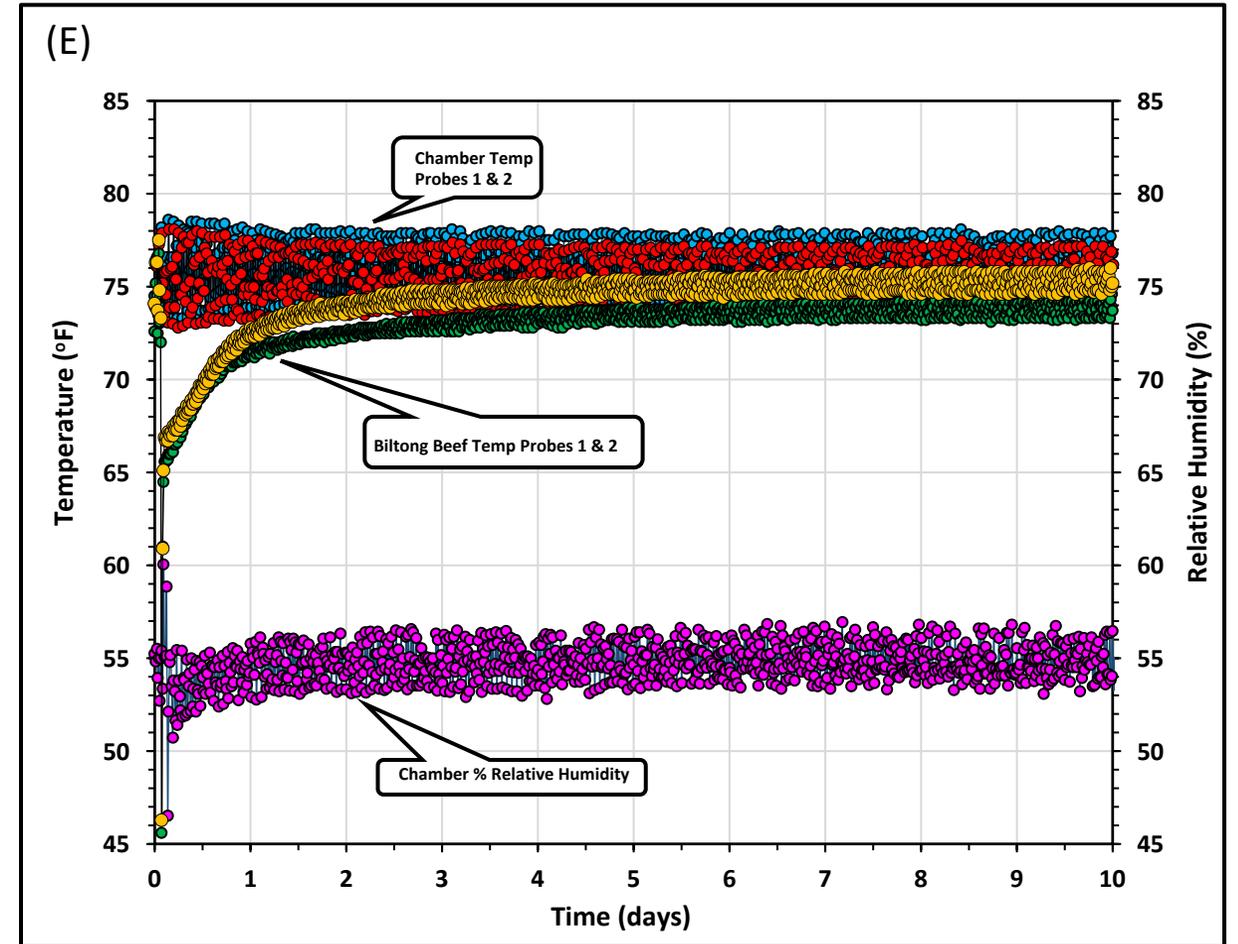


Figure E: Temperature (°C) and relative humidity (% RH) during biltong processes maintained at 75°F, 55% RH

Conclusion

- The combination of spice, salt and vinegar in the marinade resulted in a reduction of > 5 log and >6 log with subsequent drying period of 10 days, for *E. coli* O157:H7 and *Listeria monocytogenes* respectively.
- The greatest reduction of *Listeria monocytogenes* from the individual components of the marinade was exhibited from the treatment with vinegar(>5 log) followed by salt-only (4.68 log), spice-only (4.1 log) and conditions of drying, without any added ingredients, rendered a 4.2-log reduction over a period of 10 days and water activity levels of 0.79.
- No one previously was able to achieve 5-log reduction:
 - Meat loses 60% of moisture at the end of the process
 - Method of enumeration
 - a)CFU/gm : residual counts are increased which goes against the log reduction
 - b) CFU/ml: fixed volume diluent which eliminates the issue regard loss of moisture

