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

Production of Biltong:
- Salting
- Spicing
- Marination
- Drying

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

**USDA-FSIS Process of Biltong**

- Process demonstrate a >2-log reduction of pathogen and perform testing of every lot of edible ingredient to ensure no pathogen
- Process must demonstrate a >5-log reduction of pathogen
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

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
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

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

3. **Processing Of Biltong**
   - Water dip for 30 seconds
   - Rotated manually and excess liquid is drained

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)

**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.
**Water Activity (Aw) 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 $A_w$.

![Water Activity During Processing of Biltong (75°F, 55% RH)](image)

*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.

**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 (>5log) 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