Death of *Salmonella* serovars, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes* during the drying of meat: a case study using Biltong and Droëwors

G.M. Burnham[1], D.J. Hanson[2*], C.M. Koshick[1] & S.C. Ingham[1]

[1]University of Wisconsin-Madison, Department of Food Science, 1605 Linden Drive, Madison, WI, USA 53706-1565.

[2]North Carolina State University, Department of Food, Bioprocessing & Nutrition Sciences, 129 Schaub Hall – Campus Box 7624, Raleigh, NC, USA 27695-7624.

*E-mail: dana_hanson@ncsu.edu

Abstract

Biltong and droëwors are ready-to-eat dried seasoned meat strips and sausages. Procedures to meet USDA-FSIS lethality requirements for these products have not been validated. Three lots each of seasoned surface-inoculated beef strips or homogenously inoculated ground meat and seasoning were dried at 20-22°C and 38-64% RH to a_w of ca. 0.60. Acid-adapted pathogens were used as inocula (ca. 7 log CFU per sample of *Salmonella* serovars and *Escherichia coli* O157:H7, *Staphylococcus aureus*, or *Listeria monocytogenes).* Products were then vacuum-packaged and stored 7 d at 20 - 22°C. The manufacturing processes for biltong and droëwors achieved significant lethality. The biltong process reduced pathogen levels from 1.2 to 3.8 log CFU (*S. aureus* and *L. monocytogenes*, respectively). Less lethality was achieved in making droëwors, probably because of the higher fat content. Combined with additional intervention steps and/or raw material testing, the processes may achieve mandated levels of pathogen destruction. These results may be applied to commercial dried meat products with water activity, MPR, pH, and % water-phase salt at least as restrictive as our trial products. These data suggest that drying of seasoned beef strips or sausage would result in significant reduction in numbers for all four pathogens tested.

Application

The results of our experimental trials can be applied to commercial dried meat products with water activity, MPR, pH, and % water-phase salt at least as restrictive as our trial products. It was clear from our results that drying of seasoned beef strips or sausage would result in significant reduction in numbers for all four pathogens tested. Drying is used in making a variety of specialty processed meats. Drying procedures vary in terms of temperature, relative humidity, air movement, and final product characteristics. Biltong and droëwors are two shelf-stable ready-to-eat dried beef products developed in South Africa. Traditionally these products were made by drying under ambient conditions. Both biltong and droëwors are currently made in the USA. The United States Department of Agriculture (USDA) currently requires processors to provide validation that supports the safety of their meat products. The objective of this study was to determine the extent to which *Salmonella* serovars, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogene* died during the manufacturing process.

Materials and methods

Five strains each of *Salmonella* serovars, *E. coli 0157:H7*, *Staphylococcus aureus*, and *Listeria monocytogenes* were used for inoculation. In each of three trials, fresh vacuum-packaged, beef bottom round (flat), were obtained from a local processor and stored at 5°C until used ($\leq$ 7 days). The meat was trimmed of external fat and the ends squared off to produce a uniform shape. Prior to further fabrication the trimmed sub-primal cuts and all of the trimmings were liberally sprayed with a 0.13% (v/v) peracetic acid solution (Enviro Tech Chemical Services, Modesto, CA) and allowed to sit for 2 minutes.

Biltong was made by slicing the beef using a commercial deli slicer (model 919E, Berkel Co., Denver, CO) to approximately 2.5 cm thickness. To inoculate each of 12 biltong strips with a given inoculum, a 0.3 mL volume of the undiluted inoculum (ca. log 9 CFU/mL) was pipetted onto one side of each strip surface and distributed evenly over the entire side using a sterile bent plastic spreader. Strips for each inoculum were then placed into a double-layer of vacuum-packaging bags and proprietary amounts of vinegar and spice blend added;

the bags were then heat-sealed and tumbled in a small table-top tumbler (Flavor Maker F15, Doug Care Equipment, Inc., Springville, CA) for 30 minutes.

Droëwors were made by grinding beef trimmings using a commercial meat grinder (Model 5323, Toledo Co., Toledo, OH), first through a plate with ~10 mm size holes and then through a plate with ~4 mm size holes. Four vacuum-packaging bags (one control and one for each of the three inoculum types) were each filled with about 3.6 kg of the ground meat and proprietary amounts of vinegar and spice blend were added. To inoculate the droëwors mix, a 10 mL volume of the undiluted inoculum (ca. log 9 CFU/mL) was added to the mixture and manually massaged for ten minutes. Each bag of inoculated prepared droëwors mix was then stuffed into natural lamb casings.

Both the biltong and droëwors were then hung on racks in an environmental chamber set 22.2ºC (actual range 20 – 22°C) with a target of 50% relative humidity (RH; actual range of 38-64%) for a period ranging from 12 – 21 days for droëwors and 17 – 26 days for biltong. Periodic measurements of water activity (a_w) were made on uninoculated biltong and droëwors. When a product's a_w had fallen to approximately 0.60, three strips or sausages per inoculum were separately vacuum-packaged and stored at 20 - 22°C for 7d.

Samples were obtained after inoculation (day 0), when the product a_w had fallen to approximately 0.85, when the product a_w had fallen to approximately 0.60, and after 7-d of 20-22°C vacuum-packaged storage. At each sampling time, three strips or sausages were sampled, with three sub-samples taken from each strip or sausage. The individual sub-sample sites per strip or sausage were consistent throughout the study and designated as end, between and middle.

For analysis, three sub-samples of either end, between, or middle were placed in a filter bag (15 x 23 cm, Nasco), along with 99 ml BPD, and pummeled in a stomacher for 2 minutes at medium speed (Stomacher 400 Circulator lab blender; Fisher). This initial dilution was arbitrarily denoted as 10^{-1}. From the initial dilution, 1.0 mL was distributed (0.3, 0.3, and 0.4 ml) for spread-plating among three plates of Nutrient Agar (NA; Difco), and from the original dilution and each subsequent dilution, 0.1 mL was spread on one NA plate per dilution. Plates were incubated at 35°C for 1 h to allow for repair of injured cells, and then overlaid with XLD agar (Difco), MacConkey Sorbitol agar (SMAC; Difco), Baird-Parker agar base (B-P; Difco) with added egg yolk tellurite supplement (Difco), or Listeria Selective Agar base (LSA; Oxoid, Ogdensburg, NY) with added Listeria Selective Supplements (Oxford formulation; Oxoid). Plates were incubated at 35°C (24 h for SMAC and XLD; 48 h for B-P and LSA) and then examined for typical colonies of *Salmonella* serovars (black on XLD), *E. coli* O157:H7 (white-colorless on SMAC), *S. aureus* (black surrounded by clear to opaque clearing zone on B-P), and *L. monocytogenes* (grayish, small, surrounded by black precipitate on LSA). For simplicity, results were expressed as log CFU per sample for a given sub-sample location (end, middle, between).

Data analysis. Three samples of each product were analyzed for each inoculum at each sampling time. No difference in numbers of surviving pathogens was noted between the sample locations (end, between, or middle), so the mean log CFU and standard deviation for the three locations combined were calculated for each sampling time. A value of 1 CFU less than the detection limit was assigned when no colonies were present for the least dilute plating. The two-sample t-test (Snedecor and Cochran, 1980) was used to compare the level of a given pathogen at a given sampling time to that at the preceding sampling time within a single trial. A significance level of 5% ($P < 0.05$) was used.

Results and discussion

There was a general reduction in pathogen numbers throughout the drying and vacuum-storage of biltong. *Salmonella* serovars populations fell 2.0 – 3.3 log CFU by the time the a_w was approximately 0.85, with overall population decreases of 3.0 – 3.3 log CFU when the a_w was approximately 0.60, and 3.1 – 4.2 log CFU at the end of vacuum-storage. For *E. coli* O157:H7, the corresponding population decreases were 2.0 – 2.8 log CFU, 2.8 log CFU, and 2.8 – 4.4 log CFU. For both of these pathogens, populations decreased significantly ($P < 0.05$) relative to the preceding step during the first stage of drying (all trials) and occasionally thereafter.

The decreases in populations for *S. aureus* were generally smaller than for *Salmonella* serovars and *E. coli* O157:H7. After drying, populations had fallen 1.2 – 1.7 log CFU and after vacuum-storage, the decreases were 1.7 – 2.6 log CFU. Step-to-step decreases in *S. aureus* population were significant for the first stage of drying (all trials) and for one trial each in the second stage of drying and in vacuum-storage.

Decreases in *L. monocytogenes* numbers were initially somewhat less than for *Salmonella* serovars and *E. coli* O157:H7, but by the end of drying and vacuum-storage, *L. monocytogenes* die-off was very similar to

that of these other two pathogens. Significant step-to-step decreases in population were observed after the first drying stage (all trials), the second drying stage (two trials) and after vacuum-storage (one trial).

For droëwors, steady population decreases were obtained with significant step-to-step decreases frequently occurring during drying. Compared to vacuum-storage of biltong, vacuum-storage of droëwors was much less likely to cause a significant step-to-step decrease in pathogen numbers. Overall, the pathogen decreases associated with the manufacture of biltong were greater than those occurring during the manufacture of droëwors.

It was clear from our results that drying of seasoned beef strips or sausage would result in significant reduction in numbers for all four pathogens tested. It also appears that higher fat content in the droëwors product exerted a protective effect that more than compensated for the slightly higher % water-phase salt level in that product. Thus, processors should not apply our results to droëwors with a fat content higher than the 35.6% level.

The lethality achieved in making biltong and droëwors is not sufficient to meet USDA regulatory standards of either $\geq$ 6.5-log or $\geq$ 5-log reductions for beef products as stated in guidance for cooked products or fermented shelf-stable products, respectively (USDA 1999 and 2005).

In summary, the drying process used in making biltong and droëwors causes significant decreases in numbers of *Salmonella* serovars, *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes*. In order to meet USDA requirements for process lethality, processors of these products should incorporate additional intervention treatments and/or raw material pathogen testing into their process.

References

United States Department of Agriculture, Food Safety and Inspection Service, 1999. FSIS Directive 7111.1, Performance standards for the production of certain meat and poultry products. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7111-1.pdf. Accessed 28 September 2006.

United States Department of Agriculture, Food Safety and Inspection Service, 2005. Microbiology – Shelf-stable dried meats. http://www.fsis.usda.gov/PDF/FSRE_SS_5MicrobiologyDried.pdf. Accessed 28 September 2006.