Meat and Muscle Biology™

Inactivation of *Salmonella enterica, Escherichia coli* O157:H7, and *Campylobacter jejuni* in a Restructured Beef Jerky Developed for Production in Ethiopia

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Abstract: Drying is one of the oldest methods of food preservation, involving the addition of salt and removal of water to generate a shelf-stable and nutrient-dense product. Pathogens have demonstrated the ability to adapt during a slow drying process, making them more resistant to dehydration and heat treatment. In Ethiopia, an inconsistent electrical grid makes refrigeration unreliable, making dried meat products desirable to consumers. The main dried beef product, qwanta, is very labor intensive to produce. It is therefore not well suited for commercial production and drying at ambient temperatures limits inactivation of pathogenic bacteria. This study quantitatively evaluated the inactivation kinetics of foodborne pathogens during the drying of a restructured beef jerky product produced by a heat treatment that could be applied in an Ethiopian butcher shop. A secondary objective was to evaluate effects of including 15% (w/w) pureed dates or raisins into the formulation. Challenge studies were performed on 5 serotypes of *Salmonella enterica*, 3 strains of *Escherichia coli* O157:H7, and 3 strains of *Campylobacter jejuni*. Meat formulations were inoculated with stationary phase cultures, formed into strips, and dehydrated for 6 h in a home-style dehydrator. Samples were weighed pre- and post-drying, plated for enumeration of challenge strains each hour, and water activity (a_w) was measured at each sampling interval. Nonlinear predictive models were fit to the inactivation data, revealing an inverse sigmoidal curve for *S. enterica* and concave downward curves for *E. coli* and *Campylobacter*. *Salmonella* and *E. coli* were reduced 4.56 ± 0.35 and 6.27 ± 0.69 log (CFU/g), respectively, after 6 h of drying, while *Campylobacter* was reduced below the limit of detection (>4.32 log [CFU/g] reduction) after 3 h of drying. Including dates (15% w/w) into formulation improved the reduction of *S. enterica* by 0.63 log (CFU/g) compared to the control ($P = 0.02$), whereas the addition of raisins showed a non-significant improvement (0.50 log CFU/g; $P = 0.07$). Modified processes to improve the safety and extend the shelf life of animal-sourced foods in Ethiopia can reduce the incidence of foodborne disease and provide consumers with a more convenient and accessible source of protein as well as reduce food waste. These predictive models and validation studies may be useful for Ethiopian and US jerky producers, giving them the ability to more accurately assess the microbial risk of their products.

Introduction

The World Health Organization (WHO) estimates that 600 million people worldwide become ill due to unsafe food each year, with 420,000 cases resulting in death (Havelaar et al., 2015). The global burden of foodborne diseases poses a direct threat to public health, but it has also been shown to impede the socioeconomic development of a country. Low- and middle-income countries, especially in Africa, are more affected by the burden of foodborne disease than high-income countries (Havelaar et al., 2015). Foodborne disease has a significant effect on Ethiopia's population, contributing to a high rate of mortality associated with diarrheal disease (Misganaw et al., 2017). Despite Ethiopia being home to the 10th largest livestock inventory in the world, the supply chain has many inefficiencies (older age of livestock at market, low market weights, disease, transportation, etc.) that limit the availability of animal-sourced foods to its rapidly growing population (United States Agency for International Development, 2013). This, along with other factors, has resulted in one of the lowest per capita red meat consumptions in the world, and it is likely a contributing factor to 27% of the population being undernourished and 38.4% of children under the age of 5 being stunted (Food and Agriculture Organization of the United Nations, 2019). When access to animal-sourced foods is limited, the safety and preservation of this valuable resource becomes a top priority (Ayana et al., 2015). The microbial safety of meat is particularly concerning for Ethiopian consumers due to the popularity of raw meat consumption (Reminick, 2020).

Causes of foodborne bacterial diarrheal illness commonly linked to red meat include non-typhoidal *Salmonella enterica* and Shiga-toxin–producing *Escherichia coli*. Foodborne non-typhoidal *Salmonella* is responsible for nearly 59,000 deaths globally each year, a staggering 32,000 of which occurred on the continent of Africa (Havelaar et al., 2015). Shiga-toxin–producing *Escherichia coli* is less common, but it is considered a major public health concern due to the severity of the diseases that they cause (Lee et al., 2016).

The leading bacterial cause of foodborne diarrheal disease is *Campylobacter* spp., causing nearly 96 million cases a year worldwide (World Health Organization, 2015). In the United States, *Campylobacter* is most commonly found in raw poultry products but is rarely associated with beef, pork, or lamb. The low frequency of *Campylobacter* on red meat products is partially due to their susceptibility to dehydration (Murphy et al., 2006). When processing red meat in high-income countries, carcasses are commonly placed into a cooler immediately after slaughter. During this cooling period, carcasses are subjected to low temperatures and continuous air movement. This combination of temperature and air flow causes the outer surface of the carcass to dry, mitigating the risk of *Campylobacter* contamination. In Ethiopia, the meat supply chain operates under a different set of processing conditions; the supply chain is generally not refrigerated. Instead, the time between slaughter and consumption is shortened, often to less than a day. In this type of processing environment, time is not afforded for the outer surface of the carcass to dry, potentially explaining the increased prevalence of *Campylobacter* associated with red meat products produced in this type of system (Dadi and Asrat, 2009). Studies have revealed that these 3 pathogens are prevalent on raw retail meats found in Ethiopian butcher shops and supermarkets.

Refrigerating meat is not a common practice in Ethiopian butcher shops. Instead, carcasses are typically displayed on metal or wood racks in an open-air environment at ambient temperatures until sold. It is not until end of day that leftover meat may be refrigerated overnight for sale the next day. This lack of refrigeration has led to the suggestion of alternative preservation methods, such as drying, to reduce food waste and make animal-source foods more accessible.

Drying is one of the oldest forms of food preservation, involving the addition of salt and removal of water to reduce water activity and create a shelf-stable, protein-rich, safe product. Qwanta is a traditional Ethiopian, whole-muscle, dried beef product made almost exclusively in the home. It is made using an intricate cutting technique to create elongated, thin strips of meat, which are then seasoned and air dried at ambient temperatures. This is a very labor-intensive process that is not conducive to commercial production. It allows for the preservation of meat, but likely offers limited pathogen control.

The objective of this research was to develop and evaluate the microbial safety of a jerky making process that could be performed under the constraints of an Ethiopian butcher shop. This process involves the use of a low-powered home-style dehydrator (600W) to heat and dehydrate a ground and reformed jerky while significantly reducing the bacterial load. The study was designed to simulate the less than ideal conditions that could be expected in actual butcher shops. An inconsistent electrical grid is a contributing factor to the lack of refrigeration, making it also a concern for an

electricity-dependent process such as drying. However, unlike refrigeration, drying is a finite process and the time of day for operation may be chosen to reduce impact of power outage. Regardless, understanding the dynamics of pathogen inactivation during drying may inform decisions that can be made in the event of a power failure and ensure the sale of a safe product.

Natural antimicrobial ingredients are a valuable tool in product development and risk mitigation. The inclusion of natural antimicrobials into a jerky formulation can be an inexpensive way to improve the safety of a product, as well as develop a variety of flavor profiles. Raisins and dates are 2 ingredients that are available in Ethiopia and have been shown to have antibacterial properties (Lemlem et al., 2018; Demelie, 2020). The benefits of including pureed raisins into jerky formulations have been previously demonstrated, but the benefits associated with dates are largely unexplored (Bower et al., 2003). Therefore, a secondary objective of this research was to evaluate potential antimicrobial benefits of including raisins and dates, separately, into the original jerky formulation. The validation of additional formulations broadens the creative license for Ethiopian producers to develop new flavors, encouraging competition and acceptance in the marketplace.

Materials and Methods

Preparation of inoculum. Five different serotypes of *Salmonella enterica* including Anatum (KRS 051404), Newport (ATCC 6962), Saintpaul (LJH1211), Typhimurium (ATCC13311; all kindly provided by Dr. Keith Schneider), and Dublin (Strain 1724, provided by Dr. Michelle Danyluk); 3 strains of *E. coli* O157:H7 (ATCC 700599, 43894, and 49834 respectively); and 3 strains of *Campylobacter jejuni* (ATCC 33291, 33560, and 43446) were used to prepare 3 cocktail cultures for inoculation of a ground meat and spice mixture.

Salmonella and *E. coli* isolates were maintained in tryptic soy broth (TSB) (Difco Laboratories, Sparks, MD, USA) containing 15% glycerol and stored at −80°C. Cultures were prepared by streaking each strain onto selective agar plates, XLT-4 (Difco Laboratories, Sparks, MD, USA) and cefixime-tellurite Sorbitol MacConkey (CT-SMAC) (Oxoid LTD, Basingstoke, Hampshire, England) for *Salmonella* and *E. coli*, respectively, and incubated at 35°C for 18 to 24 h. A single isolated colony was harvested from each plate, suspended in 5 mL of TSB, and incubated at 35°C for 18 to 24 h. After incubation, a 100 μL aliquot from each

culture was transferred to a flask containing 100 mL of TSB and grown overnight (18 to 24 h) in a shaking incubator at 35°C.

Campylobacter strains were maintained in Wang's freezing media and stored at −80°C (USDA FSIS, 2017). Cultures were prepared by streaking each strain onto a modified version of Campy-Line blood agar (mCLBA) (Neogen, Lansing, MI, USA) plates and incubated in a microaerobic chamber (BD GasPak jar with CampyGen™ sachet [Thermo Scientific, Waltham, MA]) at 42°C for 42 to 48 h. The mCLBA plates were prepared using the protocol previously developed by Line (2001) with slight modifications made to cycloheximide (220 ppm) and cefoperazone (32 ppm) concentrations. An isolated colony from each strain was harvested and suspended in 5 mL of Mueller Hinton (MH) broth (Difco Laboratories, Sparks, MD, USA) and incubated in a microaerobic chamber at 42°C for 42 to 48 h. After incubation, a 2 mL aliquot from each culture was transferred into the liquid portion (20 mL) of a MH biphasic culture and incubated microaerobically at 42°C for 42 to 48 h.

All cultures were centrifuged (Du-Pont Co., Newton, CT, USA) at 3000 x g for 10 min at 4°C. The supernatant was decanted, and pellets were resuspended with 0.1% sterile peptone water (Oxoid LTD, Basingstoke, Hants, UK) and combined to achieve the desired cocktail cultures. The cocktail cultures were recentrifuged at 3000 x g for 10 min at 4°C, the supernatant was decanted, and the pellets were resuspended in 0.1% sterile peptone water in preparation for inoculation. Jerky batter was inoculated at approximately 8.0-log CFU/g based on raw formulation weight.

Jerky preparation and formulation. Top round (98% lean, 2% fat) were purchased from local grocery stores. Excess fat and connective tissue were removed prior to being ground through a 1.0 cm grinding plate. Berbere, an Ethiopian spice mixture (Pinch Spice Market, Chicago, IL, USA; 10% salt), was added to the coarsely ground meat at a ratio of 18.9 g/kg and salt was added to achieve a final concentration of 2.5% salt in raw formulation. The seasoned meat mixture was then ground through a 0.5 cm grinding plate and portioned into 500 g batches for inoculation. Generic raisins and dates were pureed in a food processor and included at a ratio of 15% (w/w) to create the additional jerky formulations outlined in Table 1. The inoculum was distributed throughout the batch by pipette, targeting a final concentration of 8 log CFU/g for each pathogen, before hand-mixing and resting for 10 min to allow for adhesion. The inoculated meat mixtures were rolled out into 0.5 cm thick sheets and portioned into 20 g strips.

Table 1. Formulation of Ethiopian beef jerky treatment groups with the inclusion rates of spices, salt, and dried fruit

Treatment	Formulation
Control	1.9 % berbere + 2.5% salt
15% Dates	1.9 % berbere + 2.5% salt + 15% pureed dates (w/w)
15% Raisins	1.9 % berbere + 2.5% salt + 15% pureed raisins (w/w)

Strips were placed on sanitized stainless-steel drying racks (BioChef Arizona Sol, New South Wales, AU).

Preparation of dehydrator and jerky orientation. Two 8-tray, rectangular, home-style dehydrators (600W) (BioChef Arizona Sol, New South Wales, AU) were used with all time and treatment combinations equally distributed between units. Dehydrator doors were modified to allow for easy sample extraction with minimal heat loss. The front door was replaced with 2 layers of plexiglass, a base sheet with window cut outs and a second layer consisting of 3 sections acting as sliders. Thirty minutes prior to loading the strips, dehydrators were turned on and set to the maximum temperature (70°C) to preheat. A plastic dish with a surface area of approximately 567 cm^2 was filled with hot water and placed in the bottom of the dehydrator, prior to the preheating step, to increase humidity within the chamber. Wet bulb and dry bulb temperatures were recorded twice an hour using thermocouples and a 4-Channel Datalogging Thermometer (Extech Instruments Inc., Boston, MA, USA). Relative humidity was calculated using the Florida Department of Agriculture and Consumer Services "Relative Humidity Calculator" (Florida Department of Agriculture and Consumer Services, 2020). The time zero temperature measurements were taken immediately after trays were loaded into the dehydrators. Each treatment group consisted of 21 strips of jerky, 3 of which were sampled without drying, while the remaining 18 were placed on drying racks (6 strips/rack). The strips were positioned on the leeward side of the dehydrator, and each rack held one replicate per sampling interval. The racks were positioned in the dehydrator in descending order, the first replicate being at the top of the chamber and the third being closest to the bottom. Strip placement was designed to best account for pathogen lethality variability relative to sampling location (Minarsich et al., 2018). Samples were dried for 6 h with sampling intervals at times 0, 1, 2, 3, 4, 5, and 6 h of drying.

Moisture loss and water activity. Weights were collected for each strip pre- and post-drying to measure the moisture loss over time. Percent fat was determined on raw unseasoned meat using a fat analyzer (Univex,

Salem, NH, USA). Percent moisture was determined using an air-drying method (AOAC 950.46), and percent protein was calculated by subtracting percent moisture, fat, non-protein components of muscle, and non-meat ingredients from the post-drying strip weight (Aberle et al., 2001). Moisture and protein percentages were divided to determine the moisture-to-protein ratio (MPR) for each jerky formulation. Water activity was measured at each sampling interval on one non-inoculated strip per treatment. Duplicate readings were taken on 2 portions of the same strip using a water activity meter (Decagon Devices, Inc., Pullman, WA, USA).

Microbial analysis. Sampling was done in triplicates at times 0, 1, 2, 3, 4, 5, and 6 h of drying. For each treatment, 3 raw samples at time zero and 3 samples at each subsequent interval were plated for enumeration. Each strip was aseptically transferred into a stomacher bag containing 180 mL of 0.1% sterile peptone water. The strips were rehydrated for 2 min prior to homogenization in a stomacher (Paddle Lab Blender, IUL, Spain) and then serially diluted in 0.1% sterile peptone water. For analysis of *Salmonella* and *E. coli*, 1 mL of diluted homogenate was transferred to a test tube containing 4 mL of tempered tryptic soy agar (TSA) (Difco Laboratories, Sparks, MD, USA), vortexed, and overlayed onto either XLT-4 or CT-SMAC agar plates to best account for sub-lethally injured bacteria (Kang and Fung, 2000). Plates were incubated at 35°C, and colonies were manually counted after 18 to 24 h. The limit of detection for *Salmonella* and *E. coli* was 1×10^1 CFU/g. For analysis of *Campylobacter*, 100 µL of the diluted homogenate was directly transferred onto mCLBA plates and spread using a plastic cell spreader. An additional 30 ml of homogenate was combined with 30 ml of 2X-blood-free Bolton Broth (Oxoid LTD, Basingstoke, Hampshire, England) and incubated for 48 h at 42°C under microaerobic conditions for enrichment and subsequently plated if no enumerable colonies were detected on initial plating. Plates were incubated under microaerobic conditions at 42°C, and colonies were manually counted after 40 to 48 h. The limit of detection for *Campylobacter* was 1×10^2 CFU/g.

Consumer taste panel to determine product acceptability. Two jerky formulations, with and without 15% raisins, were selected to determine preliminary acceptability by Ethiopians in a consumer taste panel approved by the University of Florida Institutional Review Board (IRB 201803020). Fifteen native Ethiopians and one Eritrean (10 male, 6 female) living in the greater Gainesville area were recruited to

participate in the panel. The 2 jerky products were dehydrated for 7 h in a 6-tray dehydrator (BioChef Arizona Sol, New South Wales, AU) to less than 0.70 a_w and stored at room temperature in sealed plastic bags before the panel. Participants were asked to answer study-related questions and evaluate jerky products on visual appeal, texture, and overall liking on a 10-point hedonic scale. Sensory attributes with averages below 5.0 for any attribute will be deemed unacceptable.

Statistical analysis. Microbial data were $\log_{10}$ transformed and reported on a log CFU per gram of raw strip weight basis (log CFU/g). Log reductions were calculated separately for each trial by subtracting the enumerated population of each sample at each sampling interval from the average initial population at 0 h. Data were initially analyzed for treatment effects using a one-way ANOVA with trial as blocking variables, and when appropriate, pairwise comparisons were performed using Tukey's Protected HSD. Studies were independently replicated 3 times (trial) with 3 replicates per treatment per time (replicate). Results were considered statistically significant at or below a P-value of 0.05, with marginally significant reported at a 0.05 to 0.10 level of significance. Microbial data analyses were conducted using R (Version 3.6.2; R Core Team, Vienna, Austria).

Sensory panel data were analyzed using the GLIMMIX model of SAS 9.4 (Cary, NC). Participant was included as a random variable. No interaction of sample and participant gender was detected, and it was therefore excluded from the model. Results were considered statistically significant at or below a P-value of 0.05.

Nonlinear inactivation kinetics. The Geeraerd biphasic model as reparameterized by Abee et al. (2016; Geeraerd et al., 2005, 2006; R Core Team, 2019) with a shoulder and tail was fitted to the *Salmonella* inactivation data as shown in Equation 1.

$$\log_{10}N(t) = \log_{10}N(0) + \log_{10}\Big\{(1-f)\cdot\exp(-k_{sens}\cdot t)$$
$$\cdot\frac{\exp(k_{sens}\cdot t_s)}{1 + [\exp(k_{sens}\cdot t_s)-1]\cdot\exp(-k_{sens}\cdot t)}$$
$$+f\cdot\exp(-k_{res}\cdot t)$$
$$\cdot\left(\frac{\exp(k_{sens}\cdot t_s)}{1 + [\exp(k_{sens}\cdot t_s)-1]\cdot\exp(-k_{sens}\cdot t)}\right)^{\frac{k_{res}}{k_{sens}}}\Big\}\quad (1)$$

The Geeraerd model assumes the population to consist of a sensitive and a resistant subpopulation causing a tail in the inactivation curve. It also assumes an extended lag period causing a shoulder. The 5 parameters included in the Geeraerd model are $\log_{10}N(0)$, f, k_{sens}, k_{res}, and t_s. $\log_{10}N(0)$ represents the initial

population, f is the resistant fraction of the initial population, k_{sens} and k_{res} are the inactivation rates of the 2 populations, and t_s defines the length of the shoulder. The Geeraerd model was used to generate predicted values with 95% confidence intervals.

A nonlinear Weibull model can be used to fit data with either a downward or upward concavity, depending on the shape parameter of the fitted curve as shown in Equation 2 (Mafart et al., 2001; Abee et al., 2016; R Core Team, 2019).

$$\log\frac{N}{N_0} = -\left(\frac{t}{\delta}\right)^p \quad (2)$$

The 3 parameters defined in the Weibull model are $\log_{10}N(0)$, δ, and p representing the initial population, the first decimal reduction time, and the shape of the curve, respectively. For $p < 1$, a concave upward curve is obtained, and for a $p > 1$, a concave downward curve is obtained. The Weibull model was used to generate predicted values with 95% confidence intervals. Nonlinear models were fitted using the *nlstools* package in R. Goodness of fit was determined by calculating the R^2 value, indicating the percentage of the variance in the dependent variable ($\log(N(t))$) that is explained by the independent variable (t). The 95% confidence interval of the log-inactivation after 6 h of drying was calculated from the fitted concentrations at t = 0 and 6 hours as $\log(\frac{N_0}{N_6}) = \log(N_0) - \log(N_6) \pm 1.96(\sqrt{\sigma^2(N_0) + \sigma^2(N_6)})$ where the variances of the log concentrations were computed from the fitted normally distributed 95% confidence intervals.

Results

Temperature and relative humidity. The modified dehydrator doors were successful in minimizing heat loss during sample extraction. In preliminary studies, temperature was measured immediately before and after sampling, and in the vast majority of cases less than a degree of difference was observed. The dry-bulb temperature steadily increased for the first 3 h of drying before stabilizing at $65.9 \pm 0.5°C$. The wet-bulb temperature remained between 26.9°C and 37°C for the entirety of the drying process (Figure 1). The average dry-bulb temperature exceeded 60°C for the last 4 h of drying, and the relative humidity was maintained around 10%. Dry-bulb, wet-bulb, and relative humidity measurement from all trials were combined and are displayed in Figure 1. Internal temperature was not
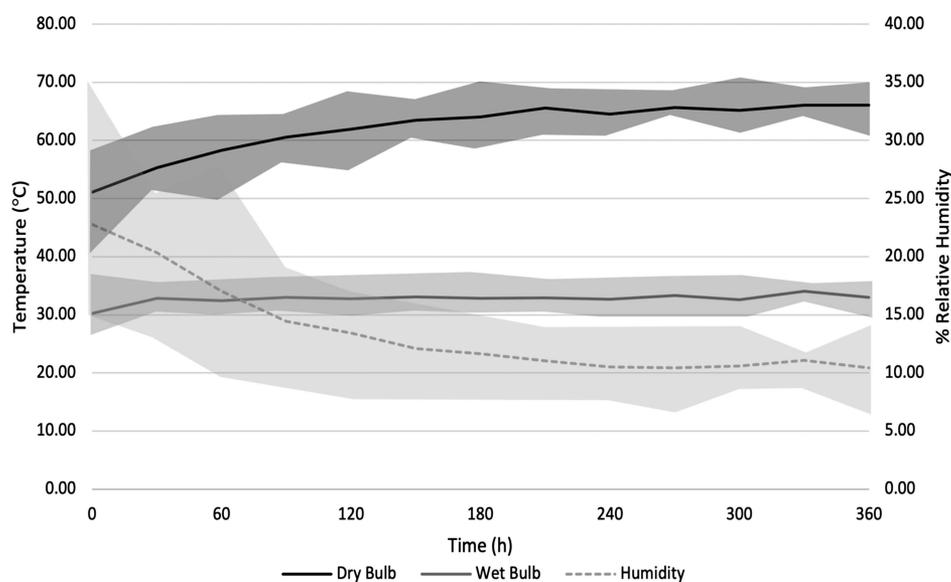
Figure 1. Temperature and relative humidity profile during dehydration. Lines represent averages, and shaded areas show the range between maximum and minimum values. Summarized data from all trials and the two dehydrators.

measured, but it is unlikely that the USDA Food Safety Inspection Service (FSIS) recommended internal temperature of 71.1°C was achieved due to the low air temperature in the dehydrators (USDA FSIS, 2014).
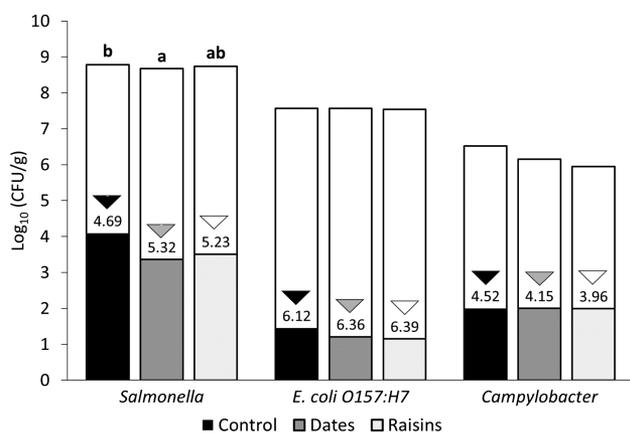
Moisture loss and water activity. Strips lost an average of $55.3 \pm 0.03\%$ of their initial weight due to moisture loss during drying. The inclusion of dates and raisins did not affect ($P = 0.18$) the final moisture loss. The MPR was <0.75:1, which meets the FSIS requirement for products to be labeled as jerky (≤0.75:1) (USDA FSIS, 2005). Evidence has shown that MPR is not an accurate indicator of shelf stability; therefore, facilities are encouraged to measure the a_w of their product to ensure it meets the critical limit of ≤0.85 (USDA FSIS, 2011, 2014). The observed decrease in a_w was similar in all treatment groups until 4 h of drying, when the dates and raisins treatment groups decreased significantly compared to the control group ($P = 0.007$, $P = 0.02$) (Figure 2). Nevertheless, after 6 h there were no significant differences between treatment groups and all treatments achieved an a_w of <0.70.

Contribution of fruit purees to microbial inactivation. Without the inclusion of purred raisins or dates, a fraction of the *Salmonella* and *E. coli* populations remained viable after 6 h of drying. *Salmonella* decreased 4.69 ± 0.15 CFU/g, whereas *E. coli* O157:H7 decreased 6.12 ± 0.16 CFU/g. In most cases, the inclusion of dates and raisins improved the overall lethality of *Salmonella* as shown in Figure 3. Jerky formulated with 15% dates achieved the highest overall *Salmonella* inactivation (5.32 ± 0.15 log CFU/g), a significantly greater reduction than the control group ($P = 0.02$). The inclusion of 15% raisins marginally improved *Salmonella* lethality compared to the control group (5.23 ± 0.16 log CFU/g; $P = 0.07$). There were no observed differences in the lethality of *E. coli* between treatments ($P = 0.42$; Figure 3). All treatments, including the control group, achieved a greater than 6.0-log reduction of *E. coli*. *Campylobacter* became undetectable by direct plating after 4 h of drying and undetectable after enrichment at 5 and 6 h of drying. In all trials, *Campylobacter* displayed a more rapid rate of decline than *Salmonella* and *E. coli*. The highest measurable log reduction (4.52 ± 0.02-log CFU/g) was observed in the control treatment. This value reflects the maximum lethality that could be observed relative to the low initial population (6.52 ± 0.12, 6.15 ± 0.12, and 5.95 ± 0.12-log CFU/g of control, dates, and raisin treatments, respectively) and higher limit of detection (1.99-log CFU/g). The lack of recovery after enrichment suggests that the observed reduction of 4.52 ± 0.02-log CFU/g is an underestimate of the true lethality achieved. It is logical to infer that *Campylobacter* was reduced beyond what our measurable reduction suggests, likely exceeding 5-log CFU/g. *Campylobacter* reached the limit of detection by 4 h of drying for all treatment groups (Figure 3). As a result, the total log reduction for control, dates, and raisins exceeded 4.52 ± 0.02, 4.15 ± 0.02, and 3.96 ± 0.02-log CFU/g, respectively.
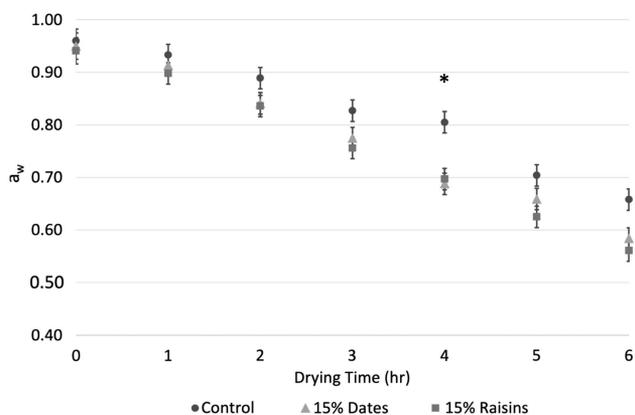
Figure 2. Water activity (a_w) by treatment during a 6 h dehydration process. Data points are represented as LSmeans ± standard error. *Means differ within time point ($P < 0.05$).

Table 2. Inactivation parameters calculated by the reparameterized Geeraerd model (Abee et al., 2016) and goodness of fit for *Salmonella enterica*

$Log_{10}N(0)$	f	k_{sens}	k_{res}	t_s (h)	Corr
9.0 ± 0.11	$8.6 \times 10^{-5} \pm 1.8 \times 10^{-4}$	4.1 ± 0.43	0.27 ± 0.57	1.9 ± 0.16	0.97

Log10N(0), initial population.

f, resistant fraction of the initial population.

k_{sens}, inactivation rate of sensitive subpopulation.

k_{res}, inactivation rate of resistant subpopulation.

t_s, length of shoulder.

Corr, Correlation of parameter estimates.

Figure 3. Summary of Log CFU/g reduction of *Salmonella enterica*, *E. coli* O157:H7, and *Campylobacter jejuni* separated by jerky formulation. Full bars represent the initial population at 0 h, the change in shading indicates the remaining population after 6 h, and the inset represents the log reduction measured between the two sampling intervals. The limit of detection for *Salmonella* and *E. coli* was 1×10^1 CFU/g, and the limit of detection for *Campylobacter* was 1×10^2 CFU/g. Data are LSmeans. Significance is denoted with an "a" or "b" ($P = 0.018$).

Inactivation kinetics. The 3 bacterial species exhibited different inactivation kinetics. *Salmonella* inactivation curves were characterized by both a shoulder and a tail, and the Geeraerd model was chosen to fit the inactivation curve. *E. coli* O157:H7 and *Campylobacter* exhibited only increasing inactivation rates, and the Weibull model was chosen to fit the inactivation. Parameter estimates and R^2 values for all inactivation curves are reported in Tables 2 and 3.

Salmonella displayed an inverse sigmoidal inactivation curve, exhibiting both a shoulder and a tail (Figure 4). The t_s value, defining the length of the shoulder or lag period, was reported at 1.85 ± 0.16 h. According to the predictive model, a significant log reduction (0.57-log CFU/g) will not be achieved until the strips have been in the dehydrator for 2.1 h. A large majority (71.2%) of the overall population reduction occurred between 2 and 4 h of drying (3.24-log CFU/g). The remaining population (a fraction, 8.6×10^{-5}, of the original inoculum) displayed a high degree of resistance to the drying process, and no significant reduction was observed between 4.1 and 6 h of drying.

The inactivation curve of *E. coli* (Figure 5) displayed a downward concavity, as indicated by a P-value of 1.57 ± 0.19. The first decimal reduction, or 1-log reduction, was observed after 1.86 ± 0.27 h of drying, indicating a more rapid decline than *Salmonella*. Inactivation rates steadily increased after the first hour, reaching rates as high as 1.56-log CFU/g/h during the last hour of processing.

Campylobacter inactivation was highly variable between trials; for this reason, each trial was modeled separately (Figure 6). All inactivation curves displayed a downward concaving curvature, some with a more pronounced curvature than others. The modeling parameters with the most variability between trials was the P-value, which defines the shape of each inactivation curve. Trial 2 displayed the highest degree of curvature with a P-value of 2.76 ± 0.36, followed by trial 3 with a P-value of 1.82 ± 0.29, and finally trial 1 displaying the most linear shape of all with a P-value of 1.39 ± 0.24. Trials 1 and 3 displayed similar δ values (1.05 ± 0.19 and 1.02 ± 0.12), predicting that a 1-log reduction will be achieved in just over 1 h of drying. Trial 2 displayed a much larger δ value (1.94 ± 0.12), meaning that a 1-log reduction was not achieved until nearly 2 h of drying. Some variation was observed in the initial populations of each trial at time zero, but because this parameter was not related to the inactivation dynamics during the dehydration process, it was not explored further.

Table 3. Inactivation parameters calculated by the Weibull model ([Mafart et al. 2001](#)) and goodness of fit (R^2) for *E. coli* O157:H7 and *Campylobacter jejuni*
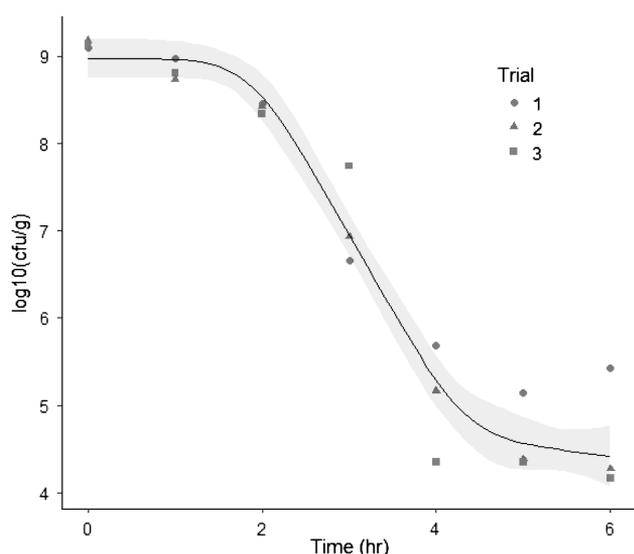
Pathogen	Log10N(0)	δ (h)	*p*	Corr
E. coli O157:H7	8.3 ± 0.22	1.9 ± 0.27	1.6 ± 0.19	0.93
Campylobacter jejuni (Trial 1)	7.2 ± 0.20	1.0 ± 0.19	1.4 ± 0.24	0.98
Campylobacter jejuni (Trial 2)	7.1 ± 0.11	1.9 ± 0.12	2.8 ± 0.36	0.99
Campylobacter jejuni (Trial 3)	6.6 ± 0.15	1.0 ± 0.12	1.8 ± 0.29	0.99

Log10N(0), initial population.
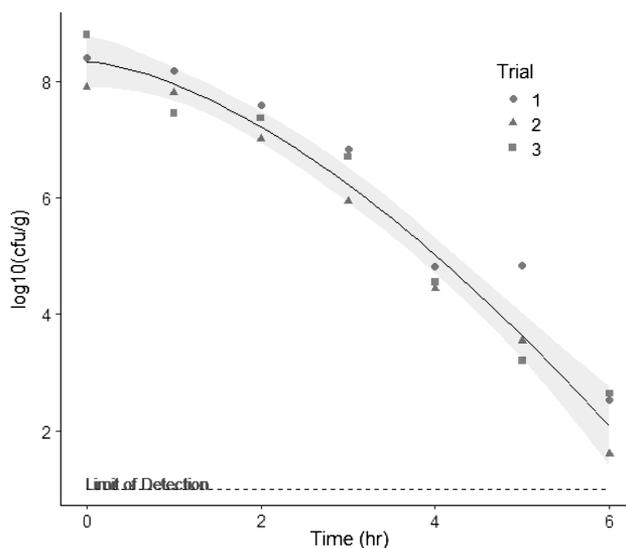
δ, required time for first decimal reduction.

p, shape parameter.

Corr, Correlation of parameter estimates.

Figure 4. Reduction in *Salmonella enterica* during production of an Ethiopian jerky. Data points are represented as LSmeans for each of the three trials. The line represents the values predicted by the Geeraerd model with a shaded 95% confidence interval.

Figure 5. Reduction in *E. coli* O157:H7 during production of an Ethiopian jerky. Data points are represented as LSmeans for each of the three trials. The line represents the values predicted by the Weibull model with a shaded 95% confidence interval.

After 6 h of drying, the predicted log reductions were 4.56 ± 0.35 and 6.27 ± 0.69 CFU/g for *Salmonella* and *E. coli*, respectively, and after 3 h of drying a log reduction of 4.32 ± 0.80 CFU/g for *Campylobacter* was predicted. Although we are unable to observe inactivation after *Campylobacter* becomes undetectable, the predictive models suggest high confidence that the inactivation after 6 h of drying exceeds 5 logs.

Consumer taste panel. Sixteen Ethiopians and one Eritrean national (10 men and 6 women) participated in the jerky consumer sensory panel to compare jerky made with and without raisins. Both products were deemed acceptable (scores >5) by 14 of the 16 participants. The product without raisins was rated higher for both texture and overall liking ($P < 0.03$; [Table 4](#)).

Discussion

To ensure a safe, ready-to-eat, shelf-stable jerky product in the United States, FSIS requires establishments to achieve a 5-log reduction of *Salmonella enterica* and Shiga-toxin–producing *E. coli* ([USDA FSIS, 2014](#)). The use of low-powered home-style dehydrator in this study perhaps provided a worst-case scenario in terms of temperature come-up time and emphasizes the importance of temperature and humidity, particularly early during the drying process. Although not all treatments achieved the performance standard required for shelf-stable products in the United States, this method of processing still has the potential to significantly reduce the pathogen load on Ethiopian raw beef. In general, the highest level of risk reduction is achieved by the first 2 or 3 logs of inactivation, and these translate to a 99% or 99.9% reduction in bacterial counts. Each

Table 4. Ethiopian consumer taste panel results of two restructured jerky formulations containing 0% and 15% pureed raisins[1]

Sensory Attribute	0% Raisins	15% Raisins	SEM	*P* value
Visual Appeal[2]	7.1	6.8	0.53	0.36
Texture[2]	7.7	7.0	0.55	0.03
Off-flavor[2,3]	8.1	8.0	0.51	0.79
Overall Liking[3]	8.5	7.7	0.46	0.02

[1]Fifteen native Ethiopians and one Eritrean living in the greater Gainesville, Florida area participated in the panel.

[2]Results on 10-point hedonic scale with 10 being more favorable and 1 being unfavorable.

[3]Higher value indicates less off-flavor.
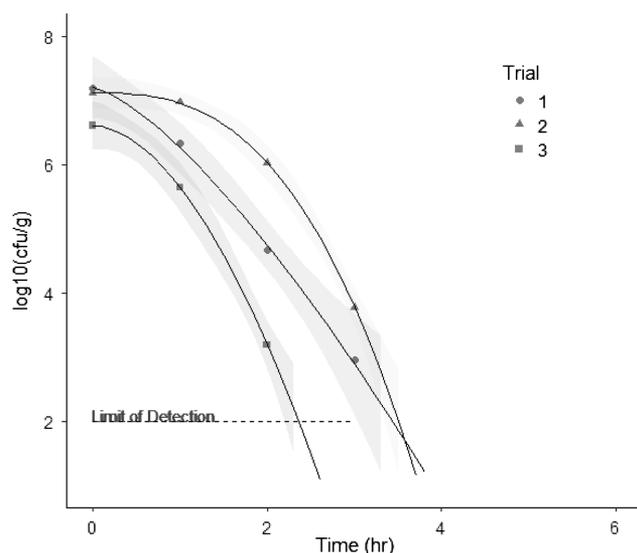
Figure 6. Reduction in Campylobacter jejuni during production of an Ethiopian jerky. Data points are represented as LSmeans for each of the three trials. The three lines represent the values predicted by the Weibull model with a shaded 95% confidence interval.

additional log reduction only marginally increases the reduction on the arithmetic scale.

Salmonella is commonly used as an indicator of lethality, because it is known to be more heat resistant than other pathogens (USDA FSIS, 2014). The resistance is clearly represented by the predicted inactivation curve and the overall predicted lethality after 6 h of drying (4.6 [95% CI 4.1–5.0] log CFU/g). Three distinct phases are represented by the Geeraerd model, including a prominent shoulder, an exponential death phase, and a leveled-off tail. This curvature suggests that there are 2 subpopulations present: a resistant population and a susceptible population. There are several reasons for the presence of a shoulder in death kinetics, often times related to physical orientation of the microorganisms, survival mechanisms, and the protective effects of the

food matrix (Geeraerd et al., 2000). It is likely that temperature played a major role in the low level of inactivation during the early portion of the drying process. According to the model, very little inactivation was observed before 1.85 h, as indicated by the t_s value. During this period of drying, the average dry bulb temperature ranged from $52.0 \pm 1.2°C$ to $62.5 \pm 0.8°C$, which, for that length of exposure, was insufficient to cause a significant reduction in viable cells. Despite pre-heating the dehydrators, this slow rise in temperature is primarily due to the low wattage capabilities of the home-style dehydrator (600W). Previous work by Borowski et al. (2009) identified a strong association between longer come-up times and lower pathogen lethality. Similar to trends observed in other studies using home-style dehydrators, a majority of the lethality occurred between 2 and 4 h of drying (Borowski et al., 2009).

The exponential death phase for *Salmonella* was associated with a reduction in water activity and temperature stabilization above 60°C after 2 h of drying. The rapid decline in viable cells is most likely due to the synergistic effects of desiccation and thermal treatment on the susceptible population. The tail implies the presence of a resistant sub-population. There are 3 approaches that describe this phenomenon: (1) the vitalistic approach where the sub-population is assumed to be very resistant, (2) the mechanistic approach that hypothesizes the tail is a "normal" trait of inactivation kinetics, and (3) the mechanistic approach that hypothesizes the tail is an artifact of the residual sub-population that either was genetically more resistant or did not receive the same lethal dose (Bevilacqua et al., 2015). Another explanation is that the *Salmonella* developed stress cross-tolerance from desiccation that may have occurred during the temperature come-up time. Gruzdev et al. (2011) found that desiccated *Salmonella* cells remained viable after being exposed to temperatures of 100°C for 60 min while non-desiccated and rehydrated cells were completely inactivated. This cross-tolerance, resulting from desiccation, may explain the increased resistance in the last hour of drying.

The inactivation curves of *E. coli* and *Campylobacter* displayed a downward concaving curvature created by the presence of a shoulder. Similar to the *Salmonella* curve, these shoulders are likely due to the slow come-up time caused by the low wattage dehydrator. A study using an American Harvest home-style dehydrator with a target temperature of 63°C observed similarly slow come-up times and did not achieve a significant reduction in *E. coli* O157:H7 until 6 h of drying

(Faith et al., 1998). The predicted *E. coli* lethality after 6 h of drying (6.30 [95% CI 6.08–6.43]-log CFU/g) was similar to the findings from other studies using similar processing conditions (Borowski et al., 2009).

Some variation was observed in the literature associated with rate of reduction of *E. coli* O157:H7. The results from this study were most similar to those found by Faith et al. (1998), achieving a 6- to 7-log reduction of *E. coli* O157:H7 after 6 h of drying at a target temperature of 68°C. However, other studies with the same target temperature did not achieve a 6-log reduction until 8 h of drying. Our results indicate the *E. coli* does not possess the same thermal tolerant characteristics observed in *Salmonella*, making *Salmonella* the more appropriate indicator organism for beef jerky.

The rapid die-off of *Campylobacter* is most likely due to desiccation, which *Campylobacter* spp. are especially susceptible to (Murphy et al., 2006). A discrepancy was observed between the inoculum concentration and the time zero population (8 vs. 6.96-log CFU/g). The time period between inoculation and time zero sampling was approximately 1 h, during which inoculated meat was exposed to normal atmospheric conditions at ambient temperatures. Many factors may have contributed to this difference in initial population, including exposure to osmotic or oxidative stress. There are very little data available on the survival of *Campylobacter* on dried products. In a quantitative microbial risk assessment of *Campylobacter* in Korean jerky, 275 commercial jerky samples were tested for *Campylobacter jejuni* with zero positive (Ha et al., 2019). This lack of prevalence, in addition to the lack of recoverable cells after enrichment, supports our assertion that *Campylobacter* was reduced to a further extent than was measurable using a direct plating method (4.52 ± 0.17-log CFU/g). The variability observed between replicate trials prevents the generation of a single predictive model representing the inactivation of *Campylobacter* during a 6 h drying process. More investigation and replication will be required to accurately assess the inactivation dynamics of this fastidious organism.

The inclusion of dates and raisins seemed to slightly lower the a_w compared to the control group. This decrease in a_w may be partially responsible for the improved pathogen lethality, as the microorganisms did not have enough available water for survival. The treatments containing purred fruit ingredients achieved shelf stability by 2 h, while the control group did not achieve this a_w until 3 h. Numerous studies have shown that the a_w in ground, restructured jerky is reduced faster, and to a further extent, than in whole muscle jerky (Buege et al., 2006; Borowski et al., 2009). In most cases, the inclusion of dates and raisins either maintained or exceeded the pathogen lethality achieved in the control group. The dates treatment achieved a significantly greater final reduction of *Salmonella* than the control group ($P = 0.018$), but no differences were observed in the gross lethality of *E. coli* or *Campylobacter*. The benefits of including raisins into jerky formulations has been explored previously. Bower et al. (2003) found the inclusion of 15% raisins in jerky created an environment that was inhibitory to growth of pathogenic bacteria after being dried. While focused on shelf life, the results of the Bower et al. (2003) study, in conjunction with our results, suggest that the inclusion of ingredients, such as raisins and dates, has the potential to further reduce the pathogen load and inhibit post-drying pathogen growth.

A potential barrier to introduction of this product is acceptability by the Ethiopian consumer. To gauge how this product may be viewed, we recruited Ethiopians and Eritreans living in and around Gainesville, Florida to participate in a pilot consumer sensory panel. Positive comments were included for both jerky formulations regarding flavor, although a common complaint was that chewier would be preferable. It was also indicated by participants that children may prefer the sweeter flavor of the raisin-containing jerky but both products would be demanded by consumers in Ethiopia if introduced by butchers. Certainly, a larger panel conducted in-country would provide stronger evidence of how this product may be accepted, but this preliminary panel suggests that this product would have a market if it were introduced by Ethiopian butchers.

Conclusions

Commercial production of restructured jerky by Ethiopian butchers may be a possible option to increase availability and reduce waste of meat while managing risk associated with common foodborne pathogens. More work needs to be done to assess the financial aspects of investment in a dehydrator and profitability for the butcher versus affordability of the product for consumers.

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