PATHOGEN INACTIVATION ON BEEF JERKY IN A COMMERCIAL-TYPE DEHYDRATOR UNDER VARIOUS MOISTURE CONDITIONS

by

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(Under the Direction of MARK A. HARRISON)

ABSTRACT

A small commercial-type dehydrator was modified to add steam during the processing of whole muscle beef jerky. The incoming air's relative humidity was decreased to15 %- 20% by using a home dehumidifier. Two processing temperatures 62.5°C and 71°C were used to compare the addition of steam for thirty minutes during processing to adding a pan of water for the duration of processing and to a control of adding no moisture. Although steam caused the relative humidity to reach 100%, when the dehydrator's fan was turned on, the relative humidity fell to approximately 30-35%. Effects on the inoculated five strain mixture each of *Escherichia coli* O157:H7 or *Salmonella* serovars were examined by direct plating enumeration and enrichment. No significant difference was found between the treatments when organisms were recovered on nonselective media ($P > 0.05$). A five log reduction was not realized at either temperature. Pathogenic bacteria could be recovered either on direct plating or enrichment after 8 or 6 hours, and water activities were approximately 0.7 and below. Dehydrators used for beef jerky processing should be designed to produce a high relative humidity during heating and prevent condensation from dripping on the product before the drying stage.

INDEX WORDS: Beef jerky, food dehydrator, E. coli O157:H7, Salmonella

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DEDICATION

To Persistence

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To Mr. Daniel Morris &

Mrs. Ruth Ann Morrow, M.S.

For your support without which this work could not have been done.

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CHAPTER 1

Introduction and Literature Review

Home-prepared meat jerky and commercially manufactured dried-cured salami have caused illness outbreaks due to enterohemorrhagic *Escherichia coli* O157:O7 (EHEC) and *Salmonella* serovars (*3, 12, 26*). An EHEC outbreak was linked to venison jerky produced at home (*26*). After exposure to drying in a home-style dehydrator at 62.5˚C for ten hours, the isolate was recovered from the finished product after enrichment.

The USDA Food Inspection and Safety Service (FSIS) has developed guidelines for jerky processing and states in them that the processing must be done at humidity above 90% 'throughout cooking or thermal heating process (*4*).' Added humidity has been used to produce rare roasts free of *Salmonella* serovars under a low processing temperature of 54.4˚C where temperatures to 107˚C could not eliminate *Salmonella* under dry conditions (*20*). However, dehydrators, especially home-style ones, are not equipped to produce elevated moisture levels within the drying chamber. The FSIS guidelines recommend using a pan of water to raise the humidity. There are questions as to whether or not this would be sufficient to raise the humidity sufficiently to be of any benefit.

Keen et al. (*26*) linked an enterohemorrhagic *Escherichia coli* O157:O7 (EHEC) outbreak to venison jerky produced at home. Subsequently, the isolate was inoculated on meat to evaluate the process. After exposure to drying in a home-style dehydrator at 62.5˚C for ten hours, the isolate was recovered from the finished product after

enrichment. The final water activity was not reported. Harrison and Harrison (*21*) studied *E. coli* O157:H7 populations inoculated on marinated whole muscle beef strips treated by heating to 71˚C prior to drying at 60˚C for ten hours to an unspecified water activity in a home-style food dehydrator. After enrichment, the organism was not recovered from either the treated meat or the control jerky which was not preheated. Harrison et al. (*24*) repeated the earlier study with a five strain inoculum of *E. coli* O157:H7. They reported over a five $\log_{10}$ reduction on the marinated samples and approximately a 4.25 $\log_{10}$ reduction on the unmarinated samples. The marinade consisted of soy sauce and flavoring without nitrite. No record was published of the final water activity.

Processing under naturally low humidity conditions (19-24% relative humidity) in Colorado, Albright et al. (1) reported that neither marinated nor unmarinated whole muscle beef jerky achieved a five $\log_{10}$ reduction. The marinade was basically the same as used in the Harrison and Harrison study. The jerky was produced in a home-style dehydrator (from two different manufacturers) at 62.5 or 68.5˚C for ten hours reaching a water activity of 0.65 to 0.64. For the four strain study of *E. coli* O157:H7, reductions ranged from 2.2 to 4.6 log cfu/cm^2 while unmarinated sample reductions were 3.2 to 3.4 log cfu/cm^2. During processing at each temperature, the rate of population attrition reached a plateau at four hours. The population numbers on the jerky strips held steady at 2.8 to 4.4 log cfu/cm^2. Also, they noted the 'tailing' phenomenon of surviving bacteria may mean that not all individuals in a population are uniformly susceptible to treatment. The unmarinated samples were subjected to the lower temperature, 62.5˚C, only and reached a water activity of 0.83. An infrared heat measuring device read the surface

temperature during processing. It took between four and five hours to reach the desired temperature. The authors hypothesized that the disparity in results with the southeastern U. S. based Harrison and Harrison (*21*) studies may be due the low ambient relative humidity (19-24%) causing a low processing relative humidity (4-10%). The low relative humidity causes case hardening of the meat surface especially in the beginning of processing. The change could protect bacteria on the meat surface from the destructive effects of heat and dehydration. Under USDA Food Inspection and Safety Service guidelines, jerky processing must be at humidity above 90% 'throughout cooking or thermal heating process (4).' Dehydrators, especially home-style ones, are not able to produce steam.

Smith et al., (*33*) using two strains of *Salmonella*, found bringing the internal temperature of a nonfermented snack sausage to 53.9-55˚C for 3.5 h and drying for four days at 21˚C at 50-55% humidity was effective. Using a six $\log_{10}$ initial *Salmonella* inoculum, populations fell below the detection limit after heating and could not be recovered after drying (water activity not given). If a lower temperature, 52˚C, was used which failed to eliminate the pathogen, drying did little to decrease the remaining populations. Post-drying reductions were less than 0.5 $\log_{10}$.

Decreasing the water activity increases heat resistance. Goodfellow and Brown (*19*) investigated the survival of *Salmonella* on the surface of dry (oven-roasted) roast beef. The study's purpose was to establish a method that eliminated *Salmonella*, but preserved the roast's rare state (internal temperature of less than 57.2˚C) for commercial distribution to restaurants. Dry oven temperatures of 93.2 and 107.1˚C could not eliminate a 7 $\log_{10}$ surface inoculum. At an oven temperature of 121.0˚C, it could be

eliminated if the internal temperature reached 54.4˚C. In a smokehouse at 79.4˚C, dry roasting was combined with a thirty minute steam treatment. Roast internal temperatures reached 54.4˚C. The method, regardless of when the steam was injected even at the end of cooking, eliminated *Salmonella.* In another type of oven which maintains high humidity, *Salmonella* was eliminated from the surface reaching an internal temperature of only 48.8˚C.

The effect of dehydration and increased heat resistance was investigated by Goepfert et al. (*19*). Using seven *Salmonella* and one *E. coli* strains, the water activity was increased using a sucrose solution (pH 6.9) while the organisms were exposed to a water bath of 57.2˚C. Depending on the *Salmonella* strain, the D value average increased by 26 to 95 times when the water activity decreased from 0.99 to 0.87. The single *E. coli's* resistance increased over 40 times over the same treatments.

Hiramatsu et al. (*25*) found that bacteria previously desiccated on paper discs to a water activity of 0.56 survived subsequent exposure to 70˚C for five hours in a forced-air oven with a one $\log_{10}$ decrease. The strains studied were five each of *Salmonella*, *E. coli* O157:H7 and O26. Each was associated with a foodborne illness outbreak.

Over time the storage temperature affects survival of dried pathogenic strains in dried media. Albright et al. (1) found that beef jerky stored in Ziploc™ bags at 21˚C under 19-24% relative humidity did not yield *E. coli* O157:H7 after thirty days. However, these samples were not enriched, and injured cells may not have been given opportunity to recover sufficiently by direct plating. Hiramatsu et al. *(25*) found high survival rates by desiccated bacteria on paper discs after 22-24 months of 4˚C. All four *Salmonella* and 12 of 15 *E. coli* pathogenic strains had survivors. Faith et al. (*14*) found

greater reduction of *E. coli* O157:H7 in salami stored at 21˚C than 4˚C. In sixty days, the population stored at the higher temperature declined four $\log_{10,}$ while the population in the refrigerated temperature declined by two $\log_{10}$. Harrison and Harrison (*21*) could not detect *E. coli* O157:H7 and *Salmonella* Typhimurium single strain inoculums after eight weeks of storage on enriching the samples. The storage temperature is not given.

Ryu et al. (*32)* found the lower of two storage temperatures, 5˚C, produced less decrease in *E. coli* O157:H7 populations than 25˚C. Placed in dried beef powder at water activity 0.68 and 0.28 to 0.41, samples were tested weekly for eight weeks. At water activity 0.68 and salt concentration 0.5%, nonacid adapted control populations were reduced by 1.78 $\log_{10}$ at 5˚C; but were detectable after enrichment only at 25˚C, which was approximately a four fold difference in that study.

Some characteristics of food effect pathogen survival in low water activity. Fat is protective. Faith et al. (*13*) examined the effect of 5 or 20% fat content on the survival of a five strain mixture of *E. coli* O157:H7 added into the batter of ground beef jerky and dried in a home-style dehydrator at 63˚C. The water activity was not given. Time to reduce the population by four $\log_{10}$ was reached at six hours for both the 5 and 20% fat product. The D value was greater for the 20% fat jerky. In another study by Faith et al. (*16)*, D values of the same pathogen were found to differ in pepperoni sliced and placed on frozen pizza heated in an oven at 135˚C. Organisms in salami containing 32% fat had a D value of 8.14 minutes compared to the ones in the 15% fat content salami which had a D value of 6.57 minutes.

Plant derived fat is protective. Hiramatsu et al. (*25)* inoculated EHEC and pathogenic *Salmonella* into a cocoa drink without sucrose. They dried the inoculated

media for 24 hours at 35˚C. Afterwards populations in the cocoa were 51 to 370 times higher than the control in sterile physiologic saline. In chocolate with 30% to 40% sucrose, Hiramatsu et al. (*25)* found better survival than in 36% sucrose alone.

Sucrose is protective. Hiramatsu et al. (*25*) inoculated dried squid chips, water activity 0.52 to 0.56, with EHEC and *Salmonella* then dried the chips again at 25˚C for 24 h. Populations on sucrose containing chips were significantly higher than those on chips without sucrose. *Salmonella* decreased to1.95 $\log_{10}$ versus 0.08 $\log_{10}$ on sweetened. chips. *E. coli* O157:H7 was reduced to1.36 $\log_{10}$ on sucrose containing chips as compared to 0.18 on plain chips. *E. coli* O26 was reduced to 1.89 $\log_{10}$ versus 0.18 $\log_{10}$ on plain chips.

Goepfert et al. *(19*), working with eight strains of *Salmonella*, found that reducing the water activity increases the heat resistance of the organisms. More importantly, the type of humectant had the greatest effect on the heat resistance. To illustrate, one particular isolate, *S.* Montevideo, had a D value of 16.5 minutes in a sucrose solution at water activity 0.96 compared to 5.5 minutes in sorbitol, 1.3 in fructose and 1.2 in glycerol when challenged at 57.2˚C.

Mattick et al. (*28)* investigated the response of a strain of *Salmonella enterica* serovar Typhimurium DT 104 to over 54 conditions of water activity and temperature. Sucrose was more protective than a combination of glucose and fructose. For example, at water activity 0.90 and 55˚C it took almost five times as long in sucrose as in glucose-fructose combination solution to reduce the starting population by three $\log_{10}$.

Sodium chloride is detrimental to *Salmonella* and *E. coli* O157:H7. Ryu et al. (*32)* found that the population of desiccated *E. coli* O157:H7 in beef powder was

significantly lowered at the 20% sodium chloride level when compared to the 3%. Hiramatsu et al. (25) added 5, 10, and 20% sodium chloride to a combination of fifteen pathogenic *Salmonella* and EHEC strains desiccated on paper discs. The discs were redried at 25˚C for 24 hours. At 10 and 20% levels, survivors were not detected. At the 5% level, the populations fell to 1.5 $\log_{10}$ less than the controls treated with sterile physiologic saline.

Clavero and Beuchat (*11*) found that numbers of a seven strain mixture of *E. coli* O157:H7 in a tryptic soy broth, pH 6.0, with the water activity adjusted from 0.99 to 0.95 by sodium chloride addition were significantly reduced after 30 minutes at 52˚C. A 1.5 $\log_{10}$ CFU/ml reduction was noted at water activity 0.95, but the reduction was less than one log when heated at water activity 0.99. The reduction by 1.5 $\log_{10}$ CFU/ml was uniform across a pH range of 6.0, 5.4 to 4.8.

Acid adaptation by E. coli O157:H7 gives mixed responses to challenge**.** Calicioglu et al. (*9*) acid adapted *E. coli* O157:H7 by adding 1% glucose to the culture in tryptic soy broth. After 22 to 24 h the pH of the adapted cultures was 5.5 while the control culture medium was pH 7.1. Inoculated whole muscle beef strips were treated as follows: marinated with a soy sauce-base recipe (pH 4.3): dipped in 5% acetic acid (pH 2.5) then marinated: dipped in Tween 20 (pH 6.6) for 15 min followed by acetic acid dip, or treating with a second marinade (pH 3.0) acidified with 1.2% lactic acid, 9% acetic acid and 5% ethanol. The beef was dried at 60˚C for 7 h to a water activity of 0.73 to 0.58. The results of the experiment were mixed with regards to the reductions in acid adapted and nonadapted populations. The acid adapted population decreased significantly faster after the acetic acid and Tween 20 treatments than nonadatped

populations. However, there were not great differences in the populations after drying. Ending populations after drying were 1.9 and 1.1 $\log_{10}$ CFU/cm^2 for acid adapted populations and 1.6 and 1.4 $\log_{10}$ CFU/cm^2 for the nonadapted populations after acetic acid or Tween 20 dips. These reductions were not statistically significant. While the more acidic marinade caused more inactivation in the nonadapted than the adapted, there was not a statistically significant difference after drying with the traditional marinade recipe. Ending populations were 1.7 $\log_{10}$ CFU/cm^2 for nonadapted and 2.9 $\log_{10}$ CFU/cm^2 for adapted treated cultures.

Brudzinski and Harrison (*7*) examined the effect of an acid tolerance response (ATR) and acid shock on three strains of *E. coli* O157:H7. Cultures were grown in E medium, a minimal medium, and incubated at either 25˚C or 32˚C while under static or agitated conditions. To acid adapt the organisms, glucose was added to the media then pH was adjusted to five with glacial acetic acid. Adapted populations were challenged by lowering the pH to 2.5. The controls were not given time to adapt to acid conditions after glucose was added before the acid treatment. Survivor counts were made by direct plating on to plate count agar for 24 hours. For one isolate, acid adapted populations survived 150 times higher than nonadapted especially during the first hour. By 24 h the difference had narrowed to 10 times higher at 25˚C, pH 4 and with agitation. The narrowing of the population survival gap between initial and 24 hours time between acid adapted and nonadapted was also noted in a generic *E. coli* strain. Adapted population counts were 50 times that of the nonadapted population during the first three hours, but narrowed to below 10 times by 24 h at 25˚C, pH 3.5 and with agitation. No statistical tests of significance were given. The study was reported as purely observational.

Does acid tolerance give cross-protection to other environmental stressors? Two isolates of *E. coli* O157:H7 were acid tolerance induced by Garren et al. (*17*) similarly to the preceding study by Brudzinski and Harrison *(7*). However, lactic acid was used instead of acetic acid to challenge the organisms to a pH of 4.0. An automated Bactometer counted levels grown in coliform medium. Sodium chloride was added to the medium for final concentrations of either 5, 10, or 15%. The two strains gave mixed results one acid adapted isolate tolerated all concentrations of sodium chloride without decreasing in population for 24 h although acid shock population levels fell by 7 $\log_{10}$ when exposed to the two higher salt levels. There may be an adaptive cross mechanism in some isolates, but it is not uniform. The acid-adapted population of the second isolate fell equal to that of acid shock levels. All concentrations of sodium chloride reduced the acid adapted and the acid shocked populations 6.5 $\log_{10}$ in 24 h. One generic *E. coli* strain tolerated 15% sodium chloride with a one $\log_{10}$ decrease in both the acid adapted and acid shocked populations. The lower salt concentrations caused less than 0.5 $\log_{10}$ reduction in the generic *E. coli* populations whether adapted or shocked. There were no statistical analyses provided.

Ryu et al. (*32*) used acid adapted *E. coli* O157:H7. The cultures were grown in 1% glucose supplemented tryptic soy broth. Lactic acid (2M) was used to reduce the pH to 4.9 in the acid shocked population. To test the rate of inactivation over time in various sodium chloride concentrations at two storage temperatures, commercially processed beef powder (pH 6.7) was prepared at two water activity levels, 0.28 which increased to 0.41 over the study and 0.69. Organisms were recovered regardless of prior treatment water activity or storage temperature, although the lower storage temperature produced less of a

decrease than the higher one. There was no cross protection from acid adaptation to osmotic stress or dehydration. Controls unexposed to acid, acid adapted and acid shocked populations showed significantly similar response (P>0.05).

Treatments before dehydration will decrease pathogen levels. Albright et al. (2) evaluated predrying treatments on a four strain mixture of *E. coli* O157:H7 inoculated on whole muscle beef strips. Strips (8.7cm x 4.0cm x 0.6cm) were inoculated with 5.3 and 7.6 $\log_{10}$ organisms then dried in a home-style dehydrator for 10 hours at 62.5˚C. Data presented was from the counts on the sorbitol MacConkey agar. The least effective treatment was immersion into boiling water for 15 sec followed by 24 h marination in a predominately soy sauce based product without nitrite. Pretreatment reduced populations by 1.3 $\log_{10.}$ The most effective pretreatment reduced the initial inoculum amount by 3.1 $\log_{10.}$ With this treatment, samples were first seasoned with pickling spice for 24 h at 4˚C then immersed in hot pickling brine at 78˚C for 90 sec. The pickling brine consisted of 27 g iodized sodium chloride, 12 g granulated sugar and 1.9 g black pepper per kg of sliced meat. The reductions during drying, 3.2 CFU/cm^2 for boiling in water and 2.6 CFU/cm^2 for hot pickling brine, were not statistically significant (P<0.05). The boiling water treated meat reached a water activity of 0.75 and the brine treated meat reached 0.59. A plateau in population reduction was noted at four hours although the water activity was 0.89 to 0.84 at that time. The surface meat temperature took between 4.5 and 5 hours to reach 62.5˚C.

Faith et al. (*14*) inoculated a five strain mixture of acid adapted *E. coli* O157:H7 in salami batter which included a pedioccocal starter culture. Freeze-thaw insult and its relationship to population reduction during storage of the finished product were

examined. The batter was either refrigerated at 4˚C for 6 to 8 h, or frozen in -20˚C for three days and thawed at 4˚C. The starter culture was allowed to ferment at 24˚C to approximately pH 4.8. The salami was dried at 13˚C for 21 days. The ending water activity is 0.90. The salt content after drying was 4.41%. The fat content was approximately 27%. Fermentation and drying reduced pathogen populations by 1.1 to 2.1 $\log_{10}$/unit. Immediately after freezing and thawing pathogen populations were similar between the treatment groups. After seven days of storage at either 21˚C or 4˚C, the effect is realized. Greater decrease in pathogen populations occurred with the frozen and thawed treatments where the reduction ranged from 2.3 to 4.5 $\log_{10}$/unit. Refrigerated pathogen populations were reduced 1.6 to 3.1 $\log_{10}$/unit. The difference in the treatment results was statistically significant.

Pretreatment with organic acids will decrease bacterial levels during drying. Yoon et al. (*36*) treated unpeeled, unblanched Roma tomatoes halves by dipping them into ascorbic (pH 2.48) or citric acid (pH 2.51) for 10 minutes. The halves were dehydrated at 60˚C in a home-style dehydrator for 14 h to a water activity of 0.237 to 0.218. Populations of a five strain *Salmonella* mixture decreased below the detection limit for ascorbic acid and by 5.1 log CFU/g for citric acid treated samples. The control sample populations were reduced by 3.2 log CFU/g as recovered on tryptic soy agar with 0.1% sodium pyruvate. Directly after dipping, there were no significant differences between acid and water control numbers.

Albright et al. (*2*) treated beef slices inoculated with a four strain mixture of *E. coli* O157:H7 in 2.5% vinegar solution (pH not published) at 57.5˚C for 20 sec. The initial reduction was less than one $\log_{10}$ on sorbitol MacConkey agar. The slices were

then marinated for 24 h and dried at 62.5˚C for 10 h. The population declined by 4.9 $\log_{10}$, and the product reached a water activity of 0.50. There were no controls with which to compare the reduction in population, but another paper by the same authors showed that populations on meat which was not treated but only marinated were reduced by 2.2 $\log_{10}$ at the end of identical processing conditions (*1).*

Rakiti et al. (*29*) evaluated acidic calcium sulfate (pH 1.4-1.7) and acidified sodium chlorite (pH 2.7-2.9) during dehydration in a commercial-style dehydrator on populations of *Salmonella* and *E. coli* O157:H7 at 62°C. Beef strips were immersed for 30 s then marinated with a recipe which included sodium nitrite. Acidified sodium chlorite reduced *Salmonella* numbers by 0.3 $\log_{10}$ and *E. coli* O157:H7 by 0.2 $\log_{10}$ over the controls as counted on nonselective media. The water activity averaged 0.67. Acidic calcium sulfate reduced *Salmonella* by $\log_{10}$ 2.7, and *E. coli* O157:H7 by no difference when compared to the controls.

Hiramatsu et al. (*25*) found that none of the 15 strains of EHEC and *Salmonella* inoculated in acidic (pH less than 4) dry foods, pickled sour plums and dried apple slices (water activity not given) survived 24 h of drying at 25˚C. On paper discs, populations treated with a lactic acid solution did not survive at pH 3 after 24 h of drying at 35˚C. If the pH was in the range of 4.0-9.0, all strains survived at 3 to 4 $\log_{10}$ CFU/disc.

Can survival be predicted at different water activities? Mattick et al. (*28*) modeled the response of a single *Salmonella enterica* serovar Typhimurium DT 104 strain in 54 combinations of low water activity, adjusted by sucrose, and temperature in tryptic soy broth pH 6.5. Response curves were fitted using a Weibull model.

Response prediction was tested using six different *Salmonella* types giving mixed results. The populations were tested at temperatures of 60, 65 and 72˚C and water activities of 0.90, 0.80 and 0.65. For example, the time until inactivation of 3 $\log_{10}$ of the model strain is 3.5 min at water activity of 0.65 and a temperature of 72˚C, but for another strain it takes 2.5 min. Conversely, at 65˚C and water activity 0.90, one strain takes four minutes longer to reach the desired reduction that the model's strain reached. The author did not give any tests of statistical significance.

The single strain on which the model was based was inoculated in various commercially prepared foods and challenged at different temperatures. Lower pH foods (pH 3.0 to 5.6) caused five to ten times the expected rate decrease at 55˚C. However, reduction rates were not well forecasted in some foods. Coconut cake, water activity 0.86 and pH 6.2, gave slower reduction rates at all three temperatures. There was no explanation for the observation, as the physical parameters are within those modeled. The model was not able to accurately forecast the reduction rate outside the range of tested parameters. Reduction rates in peanut butter, water activity 0.5, pH 6.1, were faster at 55˚C but much slower at 65 and 74˚C. Food type and *Salmonella* strain, as well as, physical parameters cause different rates of population change.

Bacterial responses to environmental stress are varied. Using a strain of *E. coli* O157:H7 and with simulated stressors commonly found in the food processing environment, Garvande and Griffiths (*18*) investigated two heat shock protein promoters, *usp*A and *grp*E, and RNA polymerase sigma factor, *rpo*S. The promoters were fused individually to a green fluorescence reporter and inserted into an *E. coli* O157:H7 isolated from meat. Osmotic stress was made using sodium chloride solutions of 4, 6, 8,

10 and 12 percent. The signals from the three promoters increased in a dose dependent pattern. The expression rate was higher at room temperature than at 37˚C. At 5˚C, there was no significant difference (P>0.01) in signal strength between the control and the osmotically challenged cells. By itself, cold shock (5˚C) up regulated *usp*A nine fold and *grp*E six fold, but not *rpo*S. Starvation induced different expression depending on the temperature. At 5˚C, *rpo*S was not induced. At 37˚C, *usp*A increased by the greatest amount, but at 5˚C *grp*E increased more. Oxidative stress was induced by adding 1 or 5M hydrogen peroxide. Temperature exerted an effect on expression during oxidative stress. At 37˚C, the three promoters were greater in 45 min than at 5˚C after one hour. The treatments were administered individually; only the effect of different temperatures was added, no interaction effect was studied.

Nonreducing sugar accumulated in the cell works to stabilize the cell membrane during desiccation. It does not make a difference if the sugar is made by the bacteria or exogenous. Billi et al. *(6*) transformed an *E. coli* with sucrose-6-phosphate synthase, *sps*A, gene from a cyanobacterium. Using continuous spectrophotometric assay, sucrose-6-P was found to be 2.4 x 10^7/cell, and sucrose was 7.7 x 10^7 molecules/cell. Using exponential phase populations, they studied survival under three conditions of desiccation: freeze drying, air dried using air at a water activity 0.25 for 72 h or in a nitrogen atmosphere over phosphorus pentoxide at water activity 0 for 72 hours. Desiccated cells were rehydrated in sterile water and plated onto Laurie-Bertani medium. Five $\log_{10}$ of the transformed cells survived. One log of the non*sps*A containing cells survived of the seven log initial inoculum.

In a separate experiment, cells inoculated on trehalose treated nylon membranes, survived desiccation better than those without trehalose. Using Fournier transform infrared spectroscopy, they documented that cell membrane phospholipid phosphate oxygen bonds were not as stretched in transformed cells. The authors explained that bonding between the sucrose hydroxyl groups and the phosphate stabilized the phospholipids.

White et al. (*34*) found a naturally occurring colony morphotype called *rdar* (red, dry and rough when grown on media containing Congo red) survives starvation and drying significantly better than *rdar* negative ones. When not on Congo red media, the *rdar* morphotype is visualized as a coordinated colony event that over time produces a colony with concentric rings and a lacy appearance. Thin fimbriae and exopolysaccaride production is responsible for this appearance. If the colony lacks both thin aggregate fimbriae and cellulose, it is smaller and mucoid. If it lacks either, it is smooth, but not as mucoid. This widely occurring adaptation aids *Salmonella* survive between hosts.

The *rdar* morphotype is controlled by operons encoding thin aggregate fimbriae and cellulose. *Agf*D activates *agf*B which codes for thin aggregate fimbriae. *Agf*D also regulates biofilm formation, and activates *adr*A which causes cellulose production. Extracellular matrix of cellulose and exopolysaccharides is produced when *Salmonella* is under conditions that are nutrient limiting, contain low osmolarity and temperature below 30˚C.

White et al. (*34*) transformed a *Salmonella* Typhimurium (ATCC 14028) strain with luciferase linked to the each of the promoters, *agf*D, *agf*B, *adr*A and *rpo*S. In liquid media with a mineral oil overlay, the promoters gave a coordinated response. After 8 to

12 h, *rpo*S activated first, followed immediately by *agf*D, and after five to six more hours *agf*B activated. *Adr*A signaled 90 to 120 min after *agf*B. Fimbrial synthesis precedes or may initiate the extracellular matrix production. The peak in *agf*B and *adr*A corresponded to visual expression of the concentric ring formation at 40 to 42 hours on solid media.

Colonies were grown for six days at room temperature on an agar medium. The colonies were collected and placed in tissue culture plates to be stored for three or nine months (relative humidity not given). After three months, the naturally occurring microbial types were at 68% survival; and after nine months, 9.7% survived. Survival was compared to mutants deficient in thin aggregate fimbriae and cellulose or both. The cellulose deficient population survival was the same as for wild-type. Fimbriae deficient survivors were only 1.2% of their original numbers by nine months. Combined cellulose and fimbriae deficient populations were 0.27% of their initial numbers.

E. coli strains tested for biofilm response by Reisner et al. (*30*) showed a great deal of diversity. The study's objective was to correlate pathogenic *E. coli* strains with increase biofilm formation *in vitro.* The strains came from 105 healthy humans, 68 children under the age of five years with diarrhea, 90 bacteremic patients and 68 urinary tract infections where the patient was febrile. Pathogenic strains did not make any better biofilms than nonpathogenic strains *in vitro*. F-like conjugative pili, aggregative adherence fimbriae, and curli did not account for increased biofilm formation. Media made the most difference in biofilm formation with porcine mucous being the best biofilm media and Luria-Bertani medium the worse. The presence of F-like plasmid, *ara*A and *fim*O, genes did not predict increased biofilm formation under any growth

media. Enteroaggregative adherence genes, *aap* and *agg*R, were found in twenty-two strains. They did not consistently give good biofilm results. Although strains positive for the adherence genes were better biofilm formers in Lurie-Bertani media, they were not in minimal media. There was no difference in porcine mucous.

CHAPTER 2

Purpose

The purpose of this study is twofold: 1) Pathogen levels on beef jerky produced under artificially created low humidity conditions are compared to earlier studies under similar naturally occurring conditions, and 2) two methods of adding humidity to the process are used to determine if the humidity can be raised in order to increase pathogen lethality. A small commercial style dehydrator unit was used. The reasonable costs of this type of unit combined with the increased production capacity over the smaller home-style models are attractive to very small commercial producers. Also, the small commercial-type unit is robust enough to accommodate modification necessary to produce higher humidity.

CHAPTER 3

Materials and Methods.

Study design. A small commercial-type horizontal air flow dehydrator (Excalibur Commercial Dehydrator, 240V with six trays, Sacramento, CA) was modified for steam production by adding a steam nozzle just after the heating element before the trays. Intake air humidity was lowered by use of a home dehumidifier (ultra low temperature home dehumidifier 70, 500ml extra wet area, Whirlpool Home Appliances, Benton Harbor, MI). Intake humidity was measured by a temperature and humidity chart recorder (TH6, Dickman Co. Addison, IL), by suspending the probe in the duct from the dehumidifier to the dehydrator. Interior relative humidity was measured by comparing readings from a dry bulb thermometer to a home-made wet bulb thermometer.

Treatments were as follows: 1) steam injection for thirty minutes during processing concurrently with heating and air drying: 2) no steam injection, processing the entire time with a pan of water in the bottom of the unit: 3) no moisture added, processing without steam or a pan of water. Treatments were made at 62.5 and 71°C in triplicate. A thermocouple (programmable recorder RD 100A, ΩE Omega, Stamford, CT) was inserted into a meat strip to measure temperature during processing.

Beef bottom rounds purchased from a local grocery store were sliced into 0.5 to 0.6 cm x 1.0 cm x 10 cm strips. After inoculation, the strips were marinated with ingredients specified in a jerky recipe from a commercial cookbook (*5*): 178 ml sauce, 15 g brown sugar, 5 ml liquid smoke, 5 g salt, 2.5 g minced garlic, 2.5 g grated ginger and

2.5 g black pepper. Marinade pH was 5.2. Samples were removed from the dehydrator at thirty minutes, and then hourly for six hours (71°C) or eight hours (62.5°C). A twenty-five gram roast sample was cultured for either *Salmonella* or *E. coli* O157.

Preparation of bacterial inoculum. Five strains each of *Escherichia coli* O157:H7 and *Salmonella* serovars were used. *E. coli* O157:H7 strains were 932 (clinic), E009 (beef), 204 (pork), E0019 (cattle feces) and 380-94 (salami). The *Salmonella* serovars consist of *Salmonella enterica* strains or serovars Typhimurium 654, DT 104-3402, California H3380, and *Salmonella* Enteridis. Each strain was taken from storage on Microbank beads (Pro-lab Diagnostics, Austin, TX) at -20°C and incubated in 9 ml of tryptic soy broth (TSB) (Difco Laboratories, Subsidiary of Becton, Dickinson and Co., Sparks MD) at 37°C for 18 to 24 h. Tryptic soy agar slants (*E. coli* O157:H7) (Difco) and plate count agar slants (*Salmonella* serovars) (Difco) were inoculated with one loopful of each culture and maintained at room temperature. Prior to use each culture was inoculated into 9 ml of TSB and incubated at 37°C for 18 to 24 h. *E. coli* O157:H7 cultures were centrifuged at 9,500 *g* for 10 min, resuspended in 0.1% peptone water (Difco) and inoculated the same day. The *Salmonella* serovars cultures were placed into 120 ml of TSB for a further 18 to 24 h, centrifuged at 3,600 *g* for 30 m, resuspended in 0.1% peptone water and stored at 4°C in separate centrifuge tubes. Two ml from each strain of the respective species were combined before inoculation. *Salmonella* serovars and *E. coli* O157:H7 were tested separately.

Preparation for treatment. Each meat strip was inoculated with 200 µl per side with combined cultures. The inoculated meat strips were incubated for thirty min at 4°C prior to the addition of either 0.4 ml marinade or sterile 0.1% peptone water per side. The

inoculated meat strips were placed in covered stainless steel commercial serving pans overnight at 4ºC.

Sample preparation. Two strips of jerky were removed at timed intervals from the marinated and the peptone water groups. One uninoculated strip was tested at the same intervals for water activity using an Aqua Lab series 3 v. 1.7 water activity meter (Decagon Devices, Pullman, WA). *E. coli* O157:H7 treated samples were tested as follows. Each inoculated strip was placed in a separate sample bag (Filtra-Bag, Labplas, Inc., Quebec, Canada), weighed and ten times the dry weight was added as nutrient broth (NB) (Difco). The bags were stomached using a Stomacher 400 Circulation machine (Seward Co., Norfolk, U.K.) for one minute at average speed. One ml was removed and serially diluted into 9 ml 0.1% peptone water. A Spiral Plater, Autoplate 4000, (Spiral Biotech, Topac, Inc., MA) delivered the dilutant onto sorbital MacConkey (SMAC) (Oxoid, Basingstroke, Hamshire, UK) and tryptic soy agars. Plates were incubated at 35°C for 48 hours. Counts from marinated and control samples were averaged separately. If the organism failed to grow on the final sample time, one ml from the original sample, kept refrigerated at 4°C, was placed into 9 ml EC broth (Difco) incubated for 24 hours at 37°C and replated onto TSA and SMAC. Randomly selected colonies from the nonselective media were tested for identification using latex agglutination by Dry Spot (Oxoid) for *E. coli* O157 antigen.

Salmonella serovars were sampled by adding 225 ml NB to the meat strip After stomaching, serial dilutions were made. The diluent was spread onto either plate count or XLT-4 (Difco) agar and incubated as described above. If growth on selective media

failed at the final sampling period, two enrichments were performed using tetrathionate and Rappaport-Vassiliadis broth.

After incubating the enrichment broths overnight at 37°C, a loopful of each was streaked onto each of XLD (Difco), Brilliant Green (Difco) and XLT-4 agars and incubated for 48 h at 35°C. A colony from one plate was subcultured onto PCA. After incubation at 37°C, a representative isolated colony was selected for identification by culturing it in lysine iron and triple sugar iron agars. A colony pick from the PCA was also tested by latex agglutination (Oxoid) if the biochemical screening gave *Salmonella* serovar typical results. Detection limit is $\leq 1.6 \log_{10}$.

Statistical analysis. Three replications of the treatments were done. Treatments and marinated or not marinated and selective or nonselective media were compared using regression analysis, specifically the general linear model (GLM) procedure (SAS v. 9.1, Cary, N.C.). Marinated and control means were compared using the student's t-test (SAS v. 9.1). Results were considered statistically significant at $P < 0.05$. Results are expressed as mean values and standard deviations.

CHAPTER 4

Results

Addition of steam during processing with the fan on could not raise the unit's interior relative humidity (RH) above an average of 35% (Figures 1 and 2). When the steam was turned off, the relative humidity inside the dehydrator chamber fell to levels equal to the other two treatments within thirty minutes. Without steam, the interior relative humidity ranged from 6-19% with the usual interior relative humidity less than 10%. The interior chamber relative humidity was not different between the two temperatures used for dryng (62.5 and 71^{o}C). Heating the meat and adding steam without the fan blowing will cause the relative humidity to reach saturation; however, it would have caused dripping condensation on the meat. Fan speed ranges from 1 to greater than 2 m/s depending on position (center versus side) in the unit. Meat exposed to 71°C reached that temperature within four to six hours and within four to five hours at 62.5°C. Incoming relative humidity ranged from 20% to 15%. The average water activity at the sampling periods is shown on Tables 1 to 4.

There was no significant difference in the three treatments for population reductions of either of the two organisms ($P< 0.05$). Processing at 71°C caused an average reduction of all treatments in *E. coli* O157:H7 by 4.1 $\log_{10}$/meat strip and in *Salmonella* serovars by 4.4 $\log_{10}$/meat strip for populations recovered on nonselective media from marinated samples. At 62.5°C the average reduction in *E. coli* O157:H7 is 3.3 $\log_{10}$/meat strip and in *Salmonella* serovars is 4.7 $\log_{10}$/meat strip. The nonmarinated

control populations recovered on nonselective media were not significantly different from the marinated samples ($P< 0.05$) (data not shown).

Populations of both organisms recovered on selective media were significantly lower ($P< 0.05$) compared to those recovered on the nonselective media for both temperatures and every treatment. For example, on selective media, at 71°C ending populations of *E. coli* O157:H7 inoculated strips averaged 1.8 $\log_{10}$ lower than the populations from the nonselective agar. *Salmonella* serovar inoculated meat strip populations at the final sample period averaged $\log_{10}$1.5 per sample lower. The relative decrease in selective media populations was evident from the beginning sampling times.

The dehumidifier replicated dry ambient conditions well. The ambient air was approximately 60% relative humidity. The dehumidifier output began between 30% to 20% and within an hour dropped to the low 20's to higher teens and decreased over the course of processing to middle teens. The internal relative humidity replicated that which was reported under low natural conditions. Pathogen attrition in this study is comparable to the one in Colorado (*1*). A five $\log_{10}$ reduction was not achieved. Neither the addition of steam nor the pan of water could overcome the low relative humidity and increase the lethality.

CHAPTER 5

Discussion

Processing under naturally low humidity conditions (19-24% relative humidity) in Colorado, investigators found that neither marinated nor unmarinated whole muscle beef jerky achieved a five $\log_{10}$ reduction of *E. coli* O157:H7 (*1*). The jerky was produced in a home-style dehydrator (from two different manufacturers) at 62.5˚C or 68.5˚C for ten hours reaching a water activity of 0.65 to 0.64. Reductions in the four strain mixture of *E. coli* O157:H7 ranged from 2.2 to 4.6 $\log_{10}$ on marinated meat. During processing at each temperature, the rate of population attrition reached a plateau at four hours (*1*). The population numbers held steady at 2.8 to 4.4 $\log_{10}$ *(1)*. An infrared heat measuring device read the surface temperature during processing. It took between four and five hours to reach the set temperature*(1)*.

The population reductions achieved in this study as recovered on TSA averaged 4.1 $\log_{10}$ at 71˚C and 3.3 $\log_{10}$ per marinated strip at 62.5˚C. The interior relative humidity conditions were similar: 4-10% for the cited study and 6-19%, averaging 10%, for this study. In the present study, populations recovered on TSA reached at stable level at 4.0 $\log_{10}$ at 71°C and 4.6 $\log_{10}$ at 62.5°C at four and five hours, respectively.

Other investigators in the southeast U. S. studied *E. coli* O157:H7 populations inoculated on marinated whole muscle beef strips treated by heating it to 71˚C prior to drying at 60˚C for ten hours to an unspecified water activity in a home-style food dehydrator (*21*). After enrichment, neither the treated meat nor the control which was not

preheated grew the organism. The same investigators repeated the earlier study with a five strain inoculum of *E. coli* O157:H7 (*24*). They reported over a five $\log_{10}$ reduction on the marinated samples, and approximately a 4.25 $\log_{10}$ reduction on the unmarinated samples. No record was published of the final water activity. The present study, also performed in the southeast U.S., failed to reduce marinated populations by 5 $\log_{10}$.

A constant relative humidity of 33% throughout dehydration of beef jerky in a smokehouse reduced numbers of *Salmonella* and *E. coli* O157:H7 significantly more than at the same temperature in a commercial-style dehydrator without added humidity (*29*). The study was performed in the southeastern U.S. The ending water activity was similar after both types of drying. The reduction differences may have been caused by a difference in the relative humidity.

Under USDA Food Inspection and Safety Service guidelines, jerky processing must be made at humidity above 90% 'throughout cooking or thermal heating process (*4*).' Dehydrators, especially home-style ones, are not capable of creating or maintaining a chamber humidity approaching the suggested levels. A pan of water recommended as one method to raise the processing humidity was found in this study sufficient to raise it only to a maximum RH of 17% with the average being RH 10%. The method fails to raise RH to the 90% specified under low relative humidity conditions.

Decreasing the water activity increases heat resistance of gram negative pathogens. The survival of *Salmonella* on the surface of dry (oven-roasted) roast beef was investigated in response to outbreaks caused by commercially processed rare roast beef (*20*). The study's purpose was to establish a method that eliminated *Salmonella*, but preserved the roast's rare state (internal temperature of less than 57.2˚C) for commercial

distribution to restaurants. Dry oven temperatures of 93.2 and 107.1˚C could not eliminate a 7 $\log_{10}$ surface inoculum. At an oven temperature of 121.0˚C, it could be eliminated if the internal temperature reached 54.4˚C. In a smokehouse at 79.4˚C, dry roasting was combined with a thirty minute steam treatment. Roast internal temperatures reached 54.4˚C. The method, regardless of when the steam was injected, even at the end of cooking, eliminated the *Salmonella*. In another type of oven which maintains high humidity, *Salmonella* was eliminated from the surface reaching an internal temperature of only 48.8˚C.

The effect of dehydration and increased heat resistance was investigated by another investigator (*19*) using seven *Salmonella* and one *E. coli* strains. The water activity was increased using a sucrose solution (pH 6.9) while the organisms were exposed to a water bath of 57.2 ˚C. Depending on the *Salmonella* strain, the D value average increase by 26 to 95 times when the water activity decreased from 0.99 to 0.87. The single *E. coli's* resistance increased over 40 times over the same treatments.

Bacteria previously desiccated on paper discs down to a water activity of 0.56 survived subsequent exposure to 70˚C for five hours in a forced-air oven with a one $\log_{10}$ decrease (*25*). The strains studied were five each of *Salmonella*, *E. coli* O157:H7 and 026. Each was associated with an outbreak.

Dehydrators will not reduce potential pathogen populations without another intervention in dry conditions. Modification of design to produce a unit capable of humidifying the product for a period during processing without dripping condensation would be one such intervention. A water trough enclosed in a heat unit at the bottom and an opening/closing vent at the top would be one way produce steam without

condensation during heating. Increase the humidity with a heated water trough during a preheat stage while venting the top for the lethality treatment. After humidified preheating, begin dehydrating by turning on the fan. Future studies should investigate adding 90% relative humidity to including the time needed at that level before drying begins.

Table 1. *Escherichia coli* O157:H7 populations on beef jerky heated to 62.5°C enumerated on nonselective (tryptic soy) and selective (sorbitol MacConkey) agars.

Treatment[a]	Sample time (h)	Aerobic mesophiles	*E. coli* O157:H7	a_w	Internal meat temp (°C)
No treatment	0	7.8	7.0	0.970	4
Steam	0.5	7.4 (0.06)[b]	7.2 (0.21)	0.871 (0.120)	49
Pan of Water	0.5	7.0 (0.21)	7.3 (0.15)	0.967 (0.009)	
No Moisture	0.5	7.4 (0.12)	6.9 (0.17)	0.947 (0.005)	
Steam	1	6.7 (0.21)	6.4 (0.25)	0.808 (0.174)	54
Pan of Water	1	6.7 (0.10)	6.5 (0.55)	0.950 (0.015)	
No Moisture	1	6.7 (0.14)	6.3 (0.28)	0.921 (0.023)	
Steam	2	6.2 (0.00)	5.4 (0.36)	0.778 (0.096)	58
Pan of Water	2	6.2 (0.28)	6.2 (0.07)	0.921 (0.027)	
No Moisture	2	6.3 (0.20)	5.7 (0.29)	0.886 (0.021)	
Steam	3	5.5 (0.10)	5.1 (0.50)	0.775 (0.081)	61
Pan of Water	3	5.2 (0.12)	5.2 (0.17)	0.768 (0.142)	
No Moisture	3	5.4 (0.21)	4.9 (0.15)	0.835 (0.016)	
Steam	4	5.1 (0.30)	4.0 (0.60)	0.634 (0.111)	61
Pan of Water	4	4.8 (0.45)	4.9 (0.27)	0.737 (0.102)	
No Moisture	4	5.2 (0.06)	4.4 (0.15)	0.808 (0.018)	
Steam	5	4.6 (0.42)	3.4 (0.53)	0.621 (0.089)	62
Pan of Water	5	4.8 (0.87)	4.6 (0.55)	0.689 (0.129)	
No Moisture	5	5.0 (0.10)	4.2 (0.15)	0.714 (0.062)	
Steam	6	4.5 (0.40)	3.9 (1.22)	0.634 (0.038)	62
Pan of Water	6	4.5 (0.38)	4.7 (0.42)	0.583 (0.132)	
No Moisture	6	4.8 (0.21)	3.8 (0.60)	0.664 (0.026)	
Steam	7	4.1 (0.23)	2.7 (0.32)	0.570 (0.146)	62
Pan of Water	7	4.5 (0.59)	4.5 (0.77)	0.582 (0.120)	
No Moisture	7	4.5 (0.06)	3.4 (0.60)	0.640 (0.048)	
Steam	8	4.2 (0.10)	2.7 (0.10)	0.450 (0.115)	62
Pan of Water	8	4.7 (0.67)	3.9 (0.87)	0.511 (0.094)	
No Moisture	8	4.7 (0.35)	2.3 (2.00)	0.554 (0.061)	

[a] Counts within time intervals are not significantly different from each other at $P<0.05$.

[b] Values are mean bacterial populations or water activity (standard deviation).

Table 2. *Escherichia coli* O157:H7 populations on beef jerky heated to 71°C enumerated on nonselective (tryptic soy) and selective (sorbitol MacConkey) agars

Treatment[a]	Sample time (h)	Aerobic mesophiles	*E. coli* O157:H7	a_w	Internal meat temp (°C)
No treatment	0	7.7	7.6	0.970	4
Steam	0.5	7.2 (0.35)[b]	6.2 (1.00)	0.957 (0.020)	56
Pan of Water	0.5	6.7 (0.31)	5.8 (0.60)	0.974 (0.004)	
No Moisture	0.5	7.1 (0.07)	6.5 (0.00)	0.843 (0.000)	
Steam	1	6.6 (0.15)	5.6 (0.36)	0.936 (0.038)	62
Pan of Water	1	5.6 (0.55)	4.5 (0.82)	0.950 (0.020)	
No Moisture	1	6.4 (0.61)	5.3 (0.72)	0.845 (0.063)	
Steam	2	5.0 (0.32)	2.4 (2.11)	0.850 (0.059)	66
Pan of Water	2	4.6 (0.15)	1.2 (2.40)	0.855 (0.102)	
No Moisture	2	5.8 (0.66)	4.4 (0.72)	0.795 (0.076)	
Steam	3	4.4 (0.12)	2.9 (0.67)	0.812 (0.042)	67
Pan of Water	3	4.0 (0.67)	2.4 (1.29)	0.846 (0.079)	
No Moisture	3	4.5 (0.55)	3.9 (1.57)	0.734 (0.098)	
Steam	4	4.1 (0.55)	1.7 (1.60)	0.829 (0.105)	69
Pan of Water	4	3.9 (0.85)	2.0 (1.41)	0.567 (0.392)	
No Moisture	4	4.0 (0.49)	3.0 (0.55)	0.738 (0.047)	
Steam	5	3.4 (0.80)	1.4 (1.40)	0.648 (0.055)	69
Pan of Water	5	3.2 (0.65)	1.8 (1.76)	0.680 (0.160)	
No Moisture	5	3.9 (0.61)	2.3 (0.70)	0.627 (0.027)	
Steam	6	3.4 (0.15)	0.9 (0.85)	0.642 (0.137)	70
Pan of Water	6	3.3 (1.48)	1.1 (1.90)	0.593 (0.205)	
No Moisture	6	4.0 (0.44)	2.2 (0.90)	0.594 (0.042)	

[a] Counts within time intervals are not significantly different from each other at $P<0.05$.

[b] Values are mean bacterial populations or water activity (standard deviation).

Table 3. *Salmonella* serovars populations on beef jerky heated to 62.5°C enumerated on nonselective (plate count) and selective (xylose-lysine-tergitol-4) agars

Treatment[a]	Sample time (h)	Aerobic mesophiles	*Salmonella* serovars	a_w	Internal meat temp (°C)
No Treatment	0	7.8	6.6	0.970	4
Steam	0.5	6.7 (0.71)[b]	5.3 (1.10)	0.960 (0.002)	49
Pan of Water	0.5	6.9 (0.31)	6.0 (0.27)	0.948 (0.010)	
No Moisture	0.5	7.0 (0.29)	6.1 (0.21)	0.930 (0.016)	
Steam	1	5.6 (0.65)	4.2 (0.55)	0.931 (0.014)	54
Pan of Water	1	6.1 (0.21)	5.0 (0.25)	0.924 (0.009)	
No Moisture	1	6.3 (0.00)	6.0 (0.00)	0.905 (0.000)	
Steam	2	5.1 (0.21)	3.1 (0.64)	0.900 (0.064)	58
Pan of Water	2	5.8 (0.30)	4.8 (0.65)	0.839 (0.060)	
No Moisture	2	5.5 (0.21)	4.7 (0.32)	0.829 (0.012)	
Steam	3	4.6 (0.47)	2.1 (0.35)	0.861 (0.044)	61
Pan of Water	3	5.2 (0.61)	4.6 (0.82)	0.797 (0.041)	
No Moisture	3	5.2 (0.23)	4.6 (0.27)	0.761 (0.038)	
Steam	4	3.8 (0.63)	2.5 (0.27)	0.826 (0.041)	61
Pan of Wate	4	4.7 (0.46)	3.5 (0.76)	0.692 (0.074)	
No Moisture	4	4.8 (0.42)	3.5 (0.51)	0.779 (0.018)	
Steam	5	3.9 (0.20)	2.0 (0.75)	0.769 (0.030)	62
Pan of Water	5	4.3 (0.42)	2.0 (1.70)	0.716 (0.036)	
No Moisture	5	4.8 (0.40)	3.6 (0.76)	0.693 (0.081)	
Steam	6	3.3 (0.99)	1.0 (0.85)	0.742 (0.014)	62
Pan of Water	6	4.0 (0.15)	2.3 (0.25)	0.597 (0.025)	
No Moisture	6	4.1 (0.65)	3.1 (0.60)	0.516 (0.101)	
Steam	7	2.6 (0.85)	0.6 (1.00)	0.667 (0.026)	62
Pan of Water	7	4.1 (0.12)	2.6 (0.31)	0.555 (0.189)	
No Moisture	7	3.8 (0.06)	2.1 (0.30)	0.512 (0.142)	
Steam	8	≤ 1.6	≤ 1.6[c]	0.663 (0.051)	62
Pan of Water	8	3.8 (0.25)	1.6 (1.40)	0.559 (0.032)	
No Moisture	8	3.8 (0.21)	1.9 (0.57)	0.512 (0.132)	

[a] Counts within time intervals are not significantly different from each other at $P<0.05$.

[b] Values are mean bacterial populations or water activity (standard deviation).

[c] Detection limit is ≤ 1.6 $\log_{10}$; + positive on enrichment

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Table 4. *Salmonella* serovars populations on beef jerky heated to 71°C enumerated on nonselective (plate count) and selective (xylose-lysine-tergitol-4) agars.

Treatment[a]	Sample time(h)	Aerobic mesophiles	*Salmonella* serovars	a_w	Internal meat temp.°C
No Treatment	0	7.6	6.9		4
Steam	0.5	6.0 (0.07)[b]	3.8 (0.07)	0.971 (0.013)	56
Pan of water	0.5	6.8 (0.25)	5.8 (0.20)	0.963 (0.004)	
No Moisture	0.5	6.8 (0.17)	5.7 (0.35)	0.949 (0.018)	
Steam	1	5.5 (0.07)	3.3 (0.07)	0.961 (0.009)	62
Pan of Water	1	5.8 (0.59)	4.8 (0.63)	0.935 (0.016)	
No Moisture	1	5.8 (0.14)	5.0 (0.07)	0.923 (0.021)	
Steam	2	4.7 (0.14)	2.6 (0.42)	0.898 (0.029)	66
Pan of Water	2	4.6 (0.29)	2.4 (2.10)	0.848 (0.042)	
No Moisture	2	5.2 (0.21)	4.1 (0.49)	0.844 (0.035)	
Steam	3	4.9 (0.71)	2.3 (0.28)	0.840 (0.005)	67
Pan of Water	3	4.6 (0.87)	3.0 (0.25)	0.808 (0.073)	
No Moisture	3	4.3 (0.23)	3.3 (0.17)	0.797 (0.058)	
Steam	4	3.4 (0.85)	2.9 (0.85)	0.830 (0.040)	69
Pan of Water	4	3.8 (0.53)	0.6 (0.98)	0.727 (0.127)	
No Moisture	4	4.2 (0.50)	2.6 (0.70)	0.741 (0.005)	
Steam	5	3.7 (0.14)	1.9 (0.85)	0.744 (0.047)	69
Pan of Water	5	3.5 (0.76)	2.0 (0.70)	0.637 (0.101)	
No Moisture	5	3.6 (0.00)	2.2 (0.49)	0.676 (0.069)	
Steam	6	3.0 (0.07)	≤ 1.6[c]	0.688 (0.026)	70
Pan of Water	6	3.3 (0.31)	1.8 (0.40)	0.667 (0.027)	
No Moisture	6	3.2 (0.00)	1.1 (1.00)	0.694 (0.044)	

[a] Counts within time intervals are not significantly different from each other at $P<0.05$.

[b] Values are mean bacterial populations or water activity (standard deviation).

[c] Detection limit is ≤ 1.6 $\log_{10}$; + positive on enrichment.

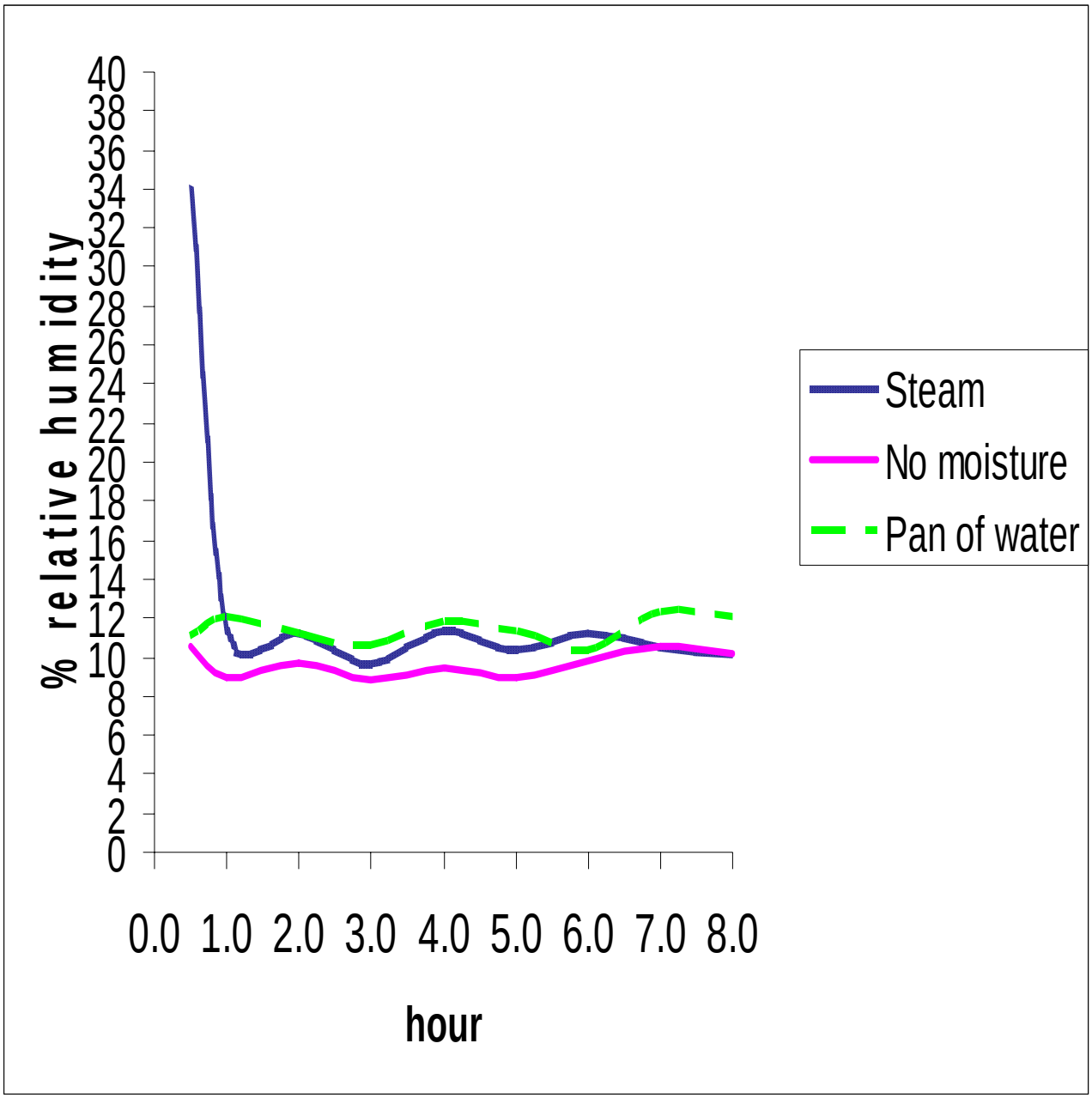

Figure 1. Percent relative humidity inside a commercial-style dehydrator at 65°C under three humidity conditions

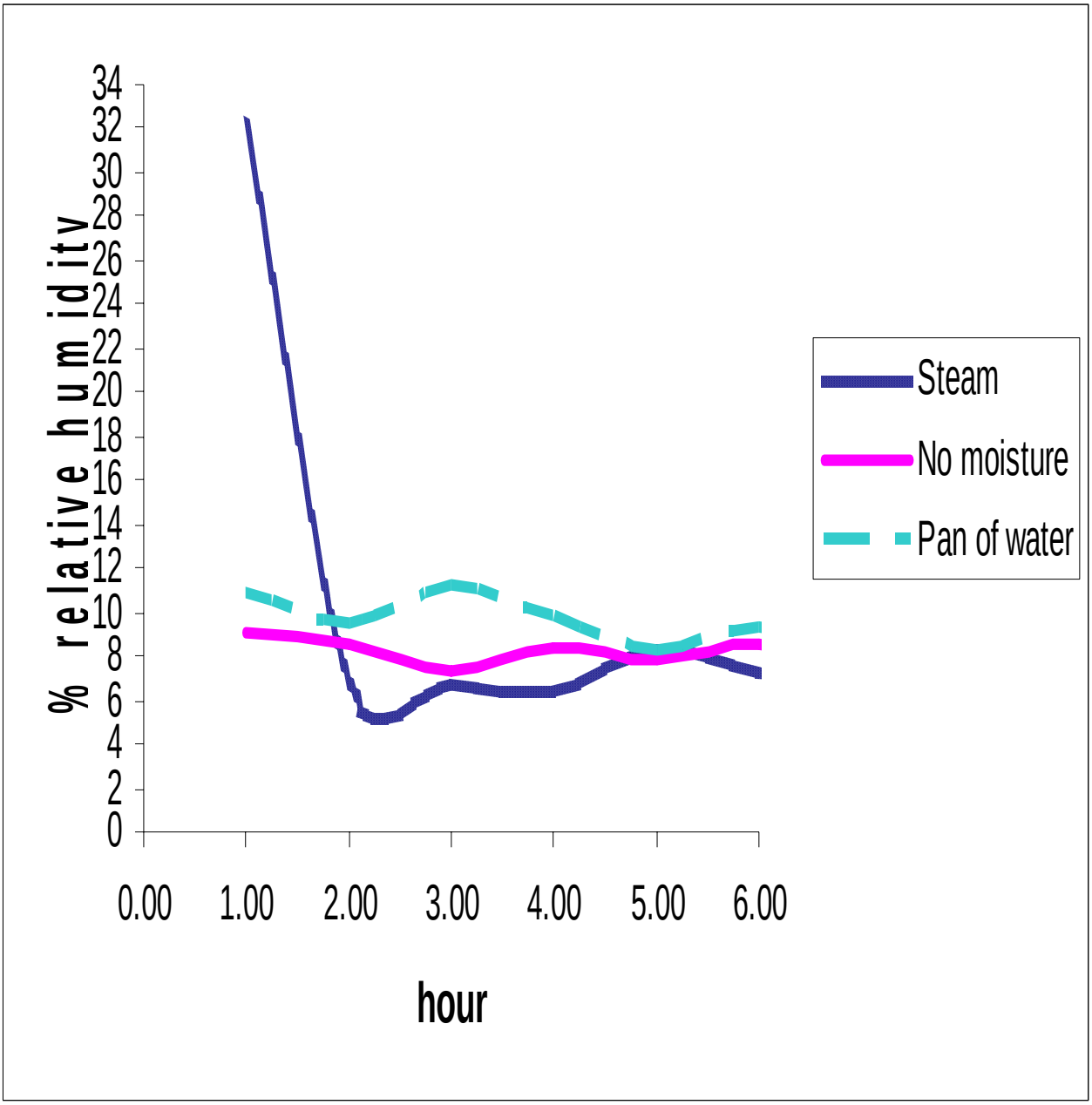

Figure 2. Percent relative humidity inside a commercial-style dehydrator at 71°C under three humidity conditions.

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