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DEATH OF *SALMONELLA* SPP., *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* DURING THE MANUFACTURE OF BASTURMA, A DRIED DRY-CURED PRODUCT

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Introduction

Basturma refers to various meat products originating in Armenia and neighboring countries. However, in this study basturma refers to a commercial product made from intact beef eye of round subprimals (hereafter referred to as rounds) that are dry-cured, rinsed, pressed, and dried with a spice paste coating added. The finished product is sold under refrigeration as a raw product. Basturma made by the process typically has a final water activity of 0.80 to 0.85, below the water activity commonly regarded as the lower limit for pathogenic bacterial growth (*3*). Provided this product also has a Moisture:Protein Ratio (MPR) of $\leq$ 1.9, it could legally be considered shelf-stable (*9*) and, if the processor desired, the product could be sold as a ready-to-eat (RTE) item. Currently, this commercial basturma would be in the "raw product, not ground" category in the Hazard Analysis Critical Control Point (HACCP) food safety system mandated by the United States Department of Agriculture (USDA; *7*). If the product were to be marketed as shelf-stable and ready-to-eat, it would fall under the "not heat-treated, shelf-stable" HACCP plan category. Regardless of the HACCP category, critical limits must be met at designated Critical Control Points to ensure control of significant hazards, namely *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Although USDA critical limit guidance exists for destroying *Salmonella* spp. during cooking (*8*), no USDA guidance exists for critical limits associated with prevention of *Salmonella* spp. and *E. coli* O157:H7 growth or destruction of these pathogens using non-thermal processes.

USDA guidance states that decreasing a product's water activity to < 0.92 will prevent *L. monocytogenes* growth (*10*). However, this guidance is generally applied to previously cooked products. The basturma evaluated in this study received no thermal process and little is known about *L. monocytogenes* survival during non-thermal processing steps leading to reduced water activity.

Objectives

The objective of this study was to determine the fate of *Salmonella* spp., *E. coli* O157:H7, and *Listeria monocytogenes* throughout a basturma-making process used by a collaborating industry partner.

Methodology

Inoculum Preparation. A cocktail was prepared using five strains each of *E. coli 0517:H7, Salmonella* spp., and *Listeria monocytogenes*.

Meat Product and Inoculation. In each of three trials, eighteen commercial beef eye of the round subprimals were used. Rounds ranged from 1.81 kg to 2.72 kg with typical dimensions of 25.4 cm x 12.7 cm x 10.2 cm. Rounds were not trimmed of external fat in trials 1 and 2, but were trimmed prior to inoculation procedures in trial 3. To inoculate each round, a 0.25 ml volume of the undiluted cocktail was pipetted onto one side of the product surface and distributed as evenly as possible over the entire side using a sterile plastic spreader. The inoculated rounds were allowed to dry for 30 minutes to aid microbial attachment.

Basturma-Making Process. Following inoculation, 45.4 kg of rounds were manually rubbed by hand with approximately 6 kg of curing mixture containing proprietary amounts of salt, sucrose, glucose and sodium nitrite, and packed into plastic 52.1 cm x 29.2 cm x 20.3 cm lugs. Lugs were stored at 6.7°C, 50% relative humidity (RH) for 7 days. After one week, accumulated fluid was drained from the lugs; and the rounds were manually dry-rubbed again with a second batch of the curing mixture and allowed to cure for 14 more days.

At the end of the dry-curing phase, rounds were rinsed for 1 h with tap water (approximately 18°C): lugs were filled with water to a level that covered the rounds, allowed to soak for 15 min, and then the water was drained from the lugs. This process was repeated four times. After rinsing, rounds were hung to dry for 2 days at 24°C, 21°C, or 27°C for trials 1, 2, and 3, respectively. The % RH levels for trials 1 and 2 cycled every 12 h between 65 and 80% RH. The % RH for trial 3 was held constant at 70. After drying for 2 d, rounds were pressed for 12 h at 2.2°C to remove additional moisture. Pressures of 517 kg per m^2 were achieved in Trials 1 and 2, while a pressure of 167 kg per m^2 was obtained in Trial 3. When pressing was complete, rounds were re-hung and allowed to continue drying for 4 d. Following the 4 d of drying, rounds were coated with a spice paste consisting of proprietary amounts of fenugreek, cayenne or red pepper, garlic powder, paprika, and water; and were hung to dry for 4 d in trial 1, 5 d in trial 2, and 6 d in trial 3. Finished product samples were sent to a commercial testing laboratory for water activity, MPR, pH, and % water-phase salt determinations (Table 1).

Enumeration of Surviving Cells. Samples were obtained at different stages in the basturma-making process. At each sampling time, three rounds were sampled with three individual samples (3.8 x 3.8 x 0.5 cm thick, by excision) being tested per round. Each sample was placed in a whirl pak filter bag, along with 99 ml BPD, and stomached for 2 minutes at medium speed (Stomacher 400 Circulator lab blender; Fisher). This initial dilution was arbitrarily denoted as 10^{-1}. Initial and subsequent dilutions were spread-plated on MacConkey Sorbitol agar (SMAC; Difco), XLD agar (Difco), and Listeria Selective Agar base (LSA; Oxoid, Ogdensburg, NY) with added Listeria Selective Supplements (Oxford formulation; Oxoid). Plates were incubated at 35°C and then examined for typical colonies of the relevant pathogen.

Data analysis. No difference in numbers of surviving pathogens was noted between sampling sites on individual rounds, so the mean log CFU was calculated for each round. A mean and standard deviation were then calculated for the three rounds at each sampling time. A value of 0.95 log CFU/g (1 CFU less than the 10 CFU detection limit) was assigned when no colonies were present for the least dilute plating.

Results & Discussion

Techniques used in the three trials were very consistent through the dry-curing and rinsing steps. However, the range of pressing and drying conditions purposely used over the three trials resulted in different finished product MPR, % water-phase salt, and water activity values (Table 1). In trial 2, case-hardening may have limited moisture loss.

As seen in Table 2, there was a general trend of pathogen death throughout the process. Through the first 21 days of processing, the reductions in log CFU per sample of *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* were 2.1 – 3.6, 1.8 – 3.4, and 1.8 – 3.2, respectively. The pathogens may have died during the dry-curing or, may have been physically removed from the beef rounds during rinsing. The temperature employed during dry-curing, 6.7°C, is above the minimum growth temperature for *L. monocytogenes* (*2*) and slightly below the accepted minimum growth temperatures for *Salmonella* spp. and *E. coli* O157:H7 (*2*) but the presence of salt and sodium nitrite may have resulted in growth inhibition.

Further reduction in levels of *E. coli* O157:H7 and *Salmonella* spp. occurred in the two days of drying and pressing that following rinsing (Table 2). *Salmonella* spp. levels decreased by an additional 0.7 and 2.3 log CFU in trials 1 and 3. However, in trial 2, levels of *Salmonella* spp. were 0.9 log CFU higher after pressing compared to post-rinsing values. Reductions in *E. coli* O157:H7 levels ranged from 0.1 log CFU in trial 2 to 2.4 log CFU in trial 3. Levels of *L. monocytogenes* fell by 0.8 and 0.9 log CFU in trials 1 and 3, respectively.

The initial six days of drying after pressing, with the surface-application of the spice paste after four days, resulted in further pathogen reductions, with levels of *E. coli* O157:H7 and *Salmonella* spp. in trial 3 falling below the detection limit. Levels of *L. monocytogenes* also continued to fall during the first six days of drying. The continued lethal effect of drying was evident during the final 4 – 6 days of drying. No detectable *E. coli* O157:H7 or *Salmonella* spp. were detected on finished products in trials 1 and 3 and very low levels were found in trial 2. Levels of *L. monocytogenes* also continued to fall during the last 4 – 6 days of processing, with overall reductions of 4.0 – 4.9 log CFU by the end of the basturma-making process.

Of the three pathogens studied, *L. monocytogenes* is clearly the most likely to survive the basturma-making process. However, when the finished product water activity is considered along with the lethality achieved during the basturma-making process, it seems that basturma made as in this study presents little risk as a vehicle for food-borne listeriosis.

Previous microbiological surveys have found that *Salmonella* spp., when detected, is only present at very low levels in ground beef (*6*). Quantitative data on *E. coli* O157:H7 in ground beef is not available. Given this finding and the low prevalence of *E. coli* O157:H7 and *Salmonella* spp. on post-intervention beef carcass samples (*4*), it is highly unlikely that *Salmonella* spp. or *E. coli* O157:H7 would be present at high levels on beef rounds. Thus, our results strongly suggest that basturma prepared by dry-curing, rinsing, pressing, and drying, presents little risk of foodborne infection due to these pathogens, regardless of whether the product is cooked prior to eating.

Other researchers have noted the lethality of dry-curing and drying on Enterobacteriaceae (*1, 5, 11*), but pathogen studies have not been done. Clearly, dry-curing and drying of whole muscle products as applied in the present study causes substantial destruction of pathogenic bacteria and is worthy of closer study.

Conclusions

Basturma prepared by dry-curing, rinsing, pressing, and drying steps as done in this study, presents little risk of foodborne infection due to *Salmonella* spp., *Escherichia coli* O157:H7, or *Listeria monocytogenes*, regardless of whether the product is cooked prior to eating.

References

Aksu, M.I., and M. Kaya. 2001. The effect of starter culture use in pasturma production on the properties of end product. Truk. J. Vet. Anim. Sci. 25: 847–854.

ICMSF (International Commission on Microbiological Specifications for Foods of the International Union of Biological Societies). 1996. Microorganisms in Foods 5. Characteristics of Microbial Pathogens. Blackie Academic & Professional, London.

Jay, J.M. 1992. Modern Food Microbiology, 4th edition. Chapman & Hall, New York.

Rivera-Betancourt, M., S.D. Shackelford, T. M. Arthur, K.E. Westmoreland, G. Bellinger, M. Rossman, J.O. Reagan, and M. Koohmaraie. 2004. Prevalence of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella in two geographically distant commercial beef processing plants in the United States. J. Food Prot. 67: 295–302.

Samelis, J., J. Metaxopoulos, M. Vlassi, and A. Pappa. 1998. Stability and safety of traditional Greek salami – a microbiological ecology study. Int. J. Food Microbiol. 44: 69–82.

United States Department of Agriculture, Food Safety and Inspection Service, Science & Technology, Microbiology Division. 1996a. Nationwide Federal Plant Raw Ground Beef Microbiological Survey August 1993 – March 1994. http://www.fsis.usda.gov/OPHS/baseline/rwgrbeef.pdf. Accessed 29 March 2005.

United States Department of Agriculture, Food Safety and Inspection Service. 1996b. Pathogen reduction; hazard analysis and critical control point (HACCP) systems. Final rule. Fed. Regis. 61: 38805–38855.

United States Department of Agriculture, Food Safety and Inspection Service. 1999. Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products. http://www.fsis.usda.gov/OA/fr/95033F-a.htm. Accessed 13 April 2005.

United States Department of Agriculture, Food Safety and Inspection Service, Office of Policy Program Development. 2003a. Food Standards and Labeling Policy Book. http://www.fsis.usda.gov/OPPDE/larc/policies.htm. Accessed 15 December 2004.

United States Department of Agriculture, Food Safety and Inspection Service. 2004. Compliance guidelines to control Listeria monocytogenes in post-lethality exposed ready-to-eat meat and poultry products. http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97-013F/CompGuidelines.pdf. Accessed 19 April 2004.

Vilar, I., M.C. Garcia-Fontan, B. Prieto, M.E. Tornadijo, and J. Carballo. 2000. A survey on the microbiological changes during the manufacture of dry-cured lacón, a Spanish traditional meat product. J. Appl. Microbiol. 89: 1018–1026.

Tables and Figures

Table 1. Water activity (a_w), Moisture:Protein ratio (MPR), pH, and % water-phase salt (%WPS) of basturma.

Trial	a_w	MPR	pH	% WPS
#1	0.87	1.92	5.6	13.1
#2	0.95	2.00	6.0	8.3
#3	0.84	1.52	5.6	18.0

Table 2. Lethality of the basturma-making process against *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Each value is the mean log CFU/piece for three tested beef rounds. Each beef round was sampled in three locations, with the mean log CFU/piece determined. The standard deviation is shown in parentheses. Reduction in log CFU per piece was calculated by subtracting the mean log CFU per piece at a given step from that determined at day zero.

		E. coli O157:H7		*Salmonella* spp.		*L. monocytogenes*	
Sampling Time		Mean Value	Reduction	Mean Value	Reduction	Mean Value	Reduction
Day 0							
	Trial 1	5.8 (0.3)	---	5.6 (0.4)	---	6.5 (0.3)	---
	Trial 2	6.5 (0.1)	---	6.4 (0.2)	---	6.4 (0.1)	---
	Trial 3	6.3 (0.2)	---	6.0 (0.2)	---	6.4 (0.2)	---
Day 21 (after rinse)							
	Trial 1	3.6 (0.7)	2.2	3.5 (0.6)	2.1	4.3 (0.9)	2.2
	Trial 2	3.1 (0.8)	3.4	2.8 (0.5)	3.6	3.2 (0.7)	3.2
	Trial 3	4.5 (0.1)	1.8	3.7 (0.2)	2.3	4.6 (0.2)	1.8
Day 23 (after pressing)							
	Trial 1	2.5 (0.8)	3.3	2.8 (0.8)	2.8	3.5 (0.1)	3.0
	Trial 2	3.0 (0.6)	3.5	3.7 (0.2)	2.7	Not tested	
	Trial 3	2.1 (0.4)	4.2	1.4 (0.3)	4.6	3.7 (0.3)	2.7
Day 29 (2 d after spice paste applied)							
	Trial 1	Not tested		Not tested		Not tested	
	Trial 2	1.9 (0.2)	4.6	1.7 (0.4)	4.7	2.7 (0.9)	3.7
	Trial 3	0.95* (0)	5.4	0.95* (0)	5.1	2.5 (0.1)	3.9
Finished product							
	Trial 1	0.95* (0)	4.9	0.95* (0)	4.7	2.5 (0.6)	4.0
	Trial 2	1.5 (0.4)	5.0	1.9 (0)	4.5	1.8 (0.2)	4.6
	Trial 3	0.95* (0)	5.4	0.95* (0)	5.1	1.5 (0.1)	4.9

* Value for log CFU per piece assigned when no colonies were observed on least dilute plate.