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FATE OF *L.MONOCYTOGENES, E.COLI* O157:H7 AND *SALMONELLA* SPP DURING COMMERCIAL THERMAL PROCESSING OF CURED COOKED MEAT PRODUCTS IN GREECE WITH OR WITHOUT SODIUM LACTATE

SERGELIDIS, D., A. ABRAHIM AND C. GENIGEORGIS
Department of Food Hygiene and Technology, Veterinary School,
Aristotle University, 54006 Thessaloniki, Greece

Introduction

Effective thermal processing and avoidance of post process contamination are keys of meat products self-life extention and safety. Published data concerning the thermal requirements for the destruction of *L.monocytogenes* in perisable processed meats vary extensively. $D_{62,8}$ in ground beef roast was 2,56 min(Schoeni et al.,1991),while for ground beef plus cure D_{64} was 1,28 min (6). reported Processing of frankfurters to 71,1° C for 25 min killed 10^3 /g *L.monocytogenes* (22). $D_{62,8}$ for *L.monocytogenes* in lean (2% fat) and fatty (30% fat) ground beef was 0,6 and 1,2 min respectively (5). $D_{62,8}$ in lean ground beef for *Salmonella* was 0,6-0,7 min (9). *L.monocytogenes* seems to be more heat resistant than *Salmonella* spp, but less than *S.senftenberg* 775 W (10).
Sodium Lactate (NaL) in cooked meats increases the water holding capacity and yield, enhances flavor and color,extends self-life and inhibits bacterial pathogens like *L.monocytogenes,S.aureus, Salmonella, E.coli* and *C.Perfringens* (11,12,13,19). The protective effect of decreased a_w with increasing concentrations of NaL or other solutes on bacterial thermal injury has been reported(4,18,20). On the contrary the presence of solutes in the recovery media is detrimental to heat injured bacteria (20). The purpose of the present study was to evaluate the efficacy of thermal processes used by Greek manufacturers on the destruction of *L.monocytogenes, Salmonella* and *E.coli* O157:H7 in processed meats in the presence or absence of NaL.

Materials and Methods

Strains and preparation of inoculum. As test organisms we used the same separate pools of *L.monocytogenes, E.coli* O157:H7 and *Salmonella* reported previously (18).
Preparation and inoculation of sausages. Raw sausage mixtures for frankfurters, parisa and beer sausage obtained from two plants were transfered to the laboratory into isothermic box at 0° C within an hour after preparation. The formulations of each sausage had no significant differences between the two plants. Frankfurter mixture contained porc meat 30%, fat 16-17,5%, colagen 10-12%, starch 5%, milk serum 2-3%, dextrose 0,5-1%, salt 1,8-2%, nitrite 0,02%, nitrate 0,015-0,03%, phosphates 0,25-0,28% and seasoning 1,5 %. Parisa contained porc meat 33-35%, total fat 16-17,5%, colagen 10-12%, starch 5%, milk serum 2%, lactose 0,5%, dextrose o,25%, salt 1,2-1,5%, nitrite 0,012%, nitrate 0,008%, phosphates 0,25-0,28% and seasoning 1,2%. Beer sausage contained beef meat 14-15%, sheep meat 16,5-18%, porc meat 29-31%, total fat 20-22,5%, milk serum 3-4%,salt 1,8-2%, nitrite 0,015% and seasoning 1,2 %. NaL(60% solution,PURAC Inc.Lincolnshire IL USA) was added dropwise to formulation batches of about 1OO g at levels 0, 2,4 and 4,8% which were then massaged in a stomacher for 2 min. From each formulation 1g portions were stuffed into finger cots which were next injected with 0,01 ml of each bacterial inoculum to get 2-4 X 10^7 cells/finger cot. Each finger cot received all three pathogens. The entrapped air was evacuated with a syringe, a knot was tied and the finger cot was stored at 0°C.The inoculated finger cots were transfered to the plant at 0°C and were placed into the geometric center of commercial size sausages(22mm diameter for frankfurters, 60mm for parisa and 75mm for beer sausage). The experimental sausages were thermally processed as the commercial lots. One finger cot from each NaL concentration and every type of sausage was stored at 0° C as a non-processed control.
Thermal process calculation. Heat penetration and pasteurization were evaluated by means of FP-value determined by a Temperature Recorder(model CTF 9008,ELLAB,Copenhagen,Denmark). Thermocouples were placed in the geometric center of the sausages which contained the inoculated finger cots. For FP-value calculations we used 70°C as a reference temperature and z=10,0 (3). Based on preliminary studies of FP-values with 8 different thermocouples,the inoculated sausages were located in those areas of the ovens that were considered giving the lowest FP-values.
Detection of survivors. After heat treatment and cooling the finger cots were taken out of the sausages aseptically and stored in sterile screw cap tubes at 0°C until analysis. Two finger cots from each type and NaL concentration were each cut into small pieces and placed into sterile tubes where they were pulverized further with a flat end glass rod. One of the samples was used for a 3-tube MPN enumeration of the surviving M.O. and one for presence or absence after appropriate enrichment. Finger cots were examined for survivors the first day of production and after storage at 4°C for 10 and 20 days, to ensure detection of recovered injured cells.
Detection of *L.monocytogenes*. Nine ml of 0,1% peptone water with 0,1% Tween 80 were added to one of the two tubes containing pulverized sample. After vortexing for 2 min ten-fold serial diliutions were prepared in the same diluent. One ml aliquots from each dilution were pipetted into 3 tubes of 9ml of FDA Listeria enrichment broth(LEB) without antimicrobials. The tube containing the dublicate finger cot received 9ml LEB without antimicrobials. All tubes were incubated at 30°C for 24 hr (first enrichment step). Next day 0,1ml from all tubes were transfered into tubes containing 9ml LEB with antimicrobials(nalidixic acid 40 mg/lt,cycloheximide 15 mg/lt and acriflavine 50 mg/lt) and tubes containing 9ml of Frazer broth (BBL) which were incubated at 30°C for 24hr (second enrichment). After incubation a loopful was streaked onto LPM agar (BBL) which was incubated at 37°C for 24-48hr. Three suspected colonies(under Henry's transillumination) were transfered onto BHI agar(OXOID) to ensure purity and confirmed as *L.monocytogenes*.
Detection of *Salmonella* spp. The same manipulations of finger cots and dilution of samples as for *L.monocytogenes* were followed for the application of MPN for *Salmonella* spp detection. Buffered Peptone Water was used for primary enrichement. After incubation at 37 C for 24hr, 0,1 ml was transfered from each tube to Selenite-Cystine broth(OXOID) and Selenite F-broth(BBL). They were incubated at 37 ° C for 24 hr (second enrichement). A loopful from each tube was then streaked onto SS agar (DIFCO) and BGA (OXOID). The

plates were incubated at 37°C for 24-48 hr. Presumptive *Salmonella* isolates were then biochemically tested on TSI- agar (OXOID) and LI--agar(OXOID) together with urease and indole tests.

Detection of *E.coli* O157:H7. Nine ml of TSYEB(BBL) was added in each tube containing the pulverized samples. The tubes were left for 1hr at room temperature.Then ten-fold serial dilutions were prepared in peptone water 0,1% plus Tween 80. For the 3- tube MPN method 1ml from each dilution was pipetted into tubes containing 9 ml MacConkey broth(BBL). All tubes were incubated at 37°C for 24-48 hr. From the positive tubes 0,1 ml was transfered to tubes containing E.C. broth(OXOID) and Tryptone Water which were incubated at 37°C for 24-72 hr. Tubes showing growth and gas formation in EC and indole positive reaction in Tryptone Water were considered as positive for the pathogen.

Results and Discussion.

In this study we evaluated the thermal processing of frankfurters, parisa and beer sausage in two meat plants on the survival of *L.monocytogenes, Salmonella* spp and *E.coli* O157:H7 under commercial conditions.,The plants were selected because of the national and international marketing of their products. The sausages were selected because they represent about 50% of the total plant daily production. For each product and plant, thermal destruction experiments were repeated at least three times. No surviving cells of any of the pathogens were detected either immediately after processing or after 20 days at 4°C. Based on an inoculum of 2-4X10^7 cells/finger cot it can be assumed that the commercial processes resulted in over 7-decimal reductions(DR) of the inoculated bacteria. The impact of NaL on the survival of the pathogens could not be evaluated since none survived the thermal processing. There is a need for an agreement on the number of DR of the initial population of specific pathogens to be accomplished by thermal process For the USA there is a requarement for 7-DR for *Salmonella* in processed roast beef and cooked corned beefs (8). A 4-DR was recommended for *Listeria* in cooked uncured meat and poultry products(14),a 5-DR for *E.coli* O157:H7(15), while 6-DR for *Listeria* and non-proteolytic *C.botulinum* in sous-vide products has been recommended in Europe. The current UK Department of Health (2) quidelines on cooked-chill and cooked-freeze catering systems state that in order to ensure the destruction of *L.monocytogenes* the temperature throughout the food should be held above 70° C for not less than 2 min when cooking and maintained at not less than 70° C for 2'min when reheating. In recent years *L. monocytogenes* , *Salmonalla* and *E.coli* O157:H7 have become of special significance to the meat industry and regulators. Extensive outbreaks of hemorrhagic colitis due to the above *E.coli* which survived in cooked hamburgers have been reported recently from the USA (16). Rules for cooking such products have been issued (1). *L.monocytogenes* has been shown to be more heat resistant than *Salmonella* and *E.coli* O157:H7 (21). The recorded internal temperatures and the estimated FP_{70}-values for each thermally processed product are presented in Table I.The minimum FP_{70}-value calculated was 23, 33,8 and 31,6 for frankfurter, parisa and beer sausage with corresponding internal temperatures of 71,4, 72,5 and 69,1°C respectively. These FP-values at least for *L.monocytogenes* with reported D_{70} of 0,14-0,27 min(Gaze et al.,1989) in ground beef can justify the absence of any pathogen as a result of current thermal processes. On the other hand recorded maximum FP_{70}-values of 59 - 373,5 min may be indicative of over processing with probable impact on product quality. Borgh-Sorensen (1993) suggested that FP_{70}-values for pasteurized meat products should be >40 min. Sausage heat penetration during their thermal treatment was uneven. This seemed to be influenced by the initial core temperature, sausage volume, location in the oven and cooling rates. As a consequence there were different internal temperatures and FP-values even in the same lot. Also placing and keeping the thermocouples in the geometric center of each sausage was not an easy task.

TABLE (I) Recorded internal temperatures and estimated FP_{70}-values during thermal treatments of inoculated sausages.

	FP_{70}-values (Internal Temperature)			
	Plant (A)		Plant (B)	
Sausage type	Min	Max	Min	Max
Frankfurter	23 (71.4°C)	78 (78.1° C)	155 (82.4° C)	373.5 (85.6° C)
Parisa	34 (72.5° C)	59 (74.6° C)	45 (74.5° C)	78.5 (76.6° C)
Beer Sausage			31.5 (69.1° C)	51 (72.6° C)

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