EFFECT OF SODIUM LACTATE ON THE HEAT RESISTANCE AND RECOVERY OF *L.MONOCYTOGENES, E. COLI* 0157:H7 AND *SALMONELLA* IN BROTH.

SERGELIDIS D., SARIMVEI A. and GENIGEORGIS C.

Department of Food Hygiene and Technology, Veterinary School, Aristotle University, Thessaloniki, Greece

S-IIA.16

SUMMARY

The present paper deals with the heat resistance and recovery of *L.monocytogenes* (pool of 5 strains), *E.coli* 0157:H7 (pool of 3 strains), and *Salmonella* (pool of *S.typhimurium, S.enteritidis, S.infantis, S.marina,* and *S.havana*) in the presence or absence of sodium lactate (NaL). The test organisms were inoculate in BHI broth with 0, 2.4 and 4.8% NaL and heated at 60C for up to 30 min. Surviving cells were counted on BHI agar with 0, 2.4 and 4.8%, incubated aerobically at 37°C for at least 72 hours.

Increasing levels of NaL in the broth gave increasing protection to the test microorganisms (M.O). Recovery of heat injured cells on BHI agar decreased with increasing levels of NaL in the agar irrespectively of the concentration of NaL in the broth. When heating menstrum and recovery medium had the same level of NaL, recovery for *L. monocytogenes* and *E.coli* diminished with increasing concentrations of NaL, while for *Salmonella* NaL seemed to enhance survival.*L.monocytogenes* was the most heat resistant and Salmonella the most heat sensitive M.O.

INTRODUCTION

In recent years there has been an extensive interest in the antimicrobial effects of lactic acid and its sodium (NaL) and potassium (KL) salts considered as natural products. An adult human body produces an estimated 121g of lactic acid per day.

Natural NaL and KL are considered by the U.S. Food and Drug Administration as GRAS food ingredients with no limitation other than good manufacturing practice in effect when used as an emulsifiers, flavor enhancers, humectants or pH control agents. The US Department of Agriculture allows the use of NaL and KL at levels of 2% of the total formulation in products where flavors are allowed and certain meat products (frankfurter, hot dog, wiener, bologna) covered under regulation 9 CFR 319.180. In addition they are permitted at a maximum level of 4.8% of the total formulation in all cooked products, hermetically sealed and not covered by 9 CFR 319.180.

Sodium lactate has been used primarily as an humectant to increase the water holding capacity and yield in cooked meats, for product flavor and color enhancement, shelf-life extention through its antimicrobial properties (Debevere,1989;Van Burik, et al., 1990; Papadopoulos et al., 1991; Brewer et al., 1991) and for its inhibitory effect on important pathogenic bacteria in cooked meat and other foods (Maas, 1992; de Wit and Rombouts, 1990; Bacus and Bontenbal, 1991; Unda, et al., 1991; Meng and Genigeorgis, 1993a,b; Weaver and Shelef, 1993; Pelroy et al., 1994). Inhibitory effects have been demonstrated for a number of lactic acid bacteria and pathogens like *C.botulinum, L. monocytogenes, Salmonella* and others. Debevere (1989) found that 2% NaL decreased water activity and inhibited the growth of lactics responsible for spoilage of pork liver pate. De Wit and Rombouts (1990) reported the antimicrobial effect of 5% of NaL on various lactics on *S.aureus* and *S.typhymurium.* but not on *E.coli.* NaL delayed microbial deterioration, pH decline and development of sour and off flavors of fresh pork sausage (Brewer, et al., 1991). Extention of lag phase and decrease of the rate of growth of *L.monocytogenes* by NaL and KL at levels of 2-5% in cured and uncured meats and broths has been demostrated (Bacus and Bontenbal, 1991; Weaver and Shelef, 1993; Pelroy et al., 1994). The beneficial effect of3-4% NaL in controlling growth of *L.monocytogenes, S.aureus, Salmonella, E.coli and C.perfringens* in cooked beef hes been shown (Miller and Acuff, 1994). Maas (1992) demonstrated the delaying of toxigenesis of proteolytic types A and B *C.botulinum* by NaL in processed turkey. Models to predict the day to toxicity as affected by the level of NaCl and NaL were developed. In factorial design experiments Meng and Genigeorgis (1993b) demonstrated the beneficial effect of NaL in controlling

toxigenesis by non-proteolytic types B and E *C.botulinum* in processed turkey, developed predictive models, related time to toxicity to levels of NaCl, NaL, spore inoculum and storage temperature. The beneficial effect of NaL in delaying toxigenesis by proteolytic and non-proteolytic *C.botulinum* in "sous-vide" products was also shown (Meng and Genigeorgis, 1993a).

NaL may offer an additional advantage by inhibiting enzyme activity, like that of aminopeptidase, thus resulting in increased shelf-life of fresh and processed meat (Harmayanni et al., 1991). The mechanism of antimicrobial effect of lactate is not clear. It was primarily attributed to pH lowering effect and to the undissociated form of the lactic acid (Grau, 1981; Gill and Newton, 1982). Lowering of water activity, (Debevere, 1989) may explain partially the mechanism for shelf-life prolongation of meat products since at pH 6.3 - 6.7 more than 99.5% of lactic acid (pK value 3.86) is dissociated. Papadopoulos et al. (1991) reported that water activity decrease is not the main factor to the bacteriostatic effect of NaL. It was suggested that inhibitory effects may result from lactate transport into the bacterial cell where it may inhibit biochemical pathways normally producing energy (de Witt and Rombouts, 1991; Maas, 1992). NaL may also interfere with germinants of bacterial spore like alanine by competitive inactivation due to molecular similarity, (Meng, 1992).

There is limited information on the effect of NaL on the heat resistance and recovery of pathogenic bacteria in foods. Meng (1992) studied the effect of NaL (0-4.8%) on the heat injury of proteolytic and non-proteolytic *C.botulinum* spores during heating at 61-100C and the probability of recovery of the spores in broth in the presence of 0-4.8% NaL during storage at 12-30C. His studies indicated that the presence of NaL in the heating broth did not enhance the thermal inactivation of the spores. Under a few conditions of potential heat injury of the spores, the 4.8% NaL seemed to exert a protective effect to heat injury. Sodium nitrite at a level of 156ppm was more inhibitory to spore outgrowth than 2.4% NaL, and less inhibitory than 4.8% NaL. The antimicrobial effect of NaL was enhanced by the lowering of storage temperature. Houtsma et al.(1994) reported that when they compared the effect of 0 and 4% NaL in buffer on the D_{95}-value of proteolytic *C.botulinum* (types A and B), NaL showed a tendency to protect the spores against heat inactivation, but the effect was not statistically significant.

The present paper is part of a long term project concerning lactates and processed meats. The objectives of this study were to determine the effect of 0%, 2.4%, and 4.8% NaL (w/v)in heating menstrua and recovery media on the heat resistance and recovery respectively of pools of five strains of *Listeria monocytogenes*, three strains of *Escherichia coli* 0157:H7 and five serotypes of *Salmonella*.

MATERIALS AND METHODS

Strains and preparation of inoculum: As test organisms we used *L.monocytogenes* strains Scott-A, V7, VPH1, VPH4, and RM, three strains of *E.coli* 0157:H7 and *S.typhymurium, S.enteritidis, S.infantis, S.marina,* and *S.havana.* Stock cultures were kept on Brain Heart Infusion [BHI] agar slants (OXOID) at 4°C. Specific bacterial inocula were prepared by two consecutive subcultures in tubes containing BHI broth (OXOID) incubated at 37°C for 24 hours. Pools of strains of the same species were prepared by mixing 1ml from each individual strain culture. After vortexing the tubes were centrifuged at 3,000 rpm for 10 min. and the cells were resuspended in 0.1% (w/v) peptone water. After a second centrifugation the bacterial cells were resuspended in BHI broth containing 0, 2.4, 4.8% (w/v) NaL (60% solution, PURAC Inc. Lincolnshire IL, USA) so that the initial cfu/ml was about 10^8-10^9 depending on the M.O. One ml from each initial bacterial suspension was pippeted into 2 ml glass ampules (Wheaton, Millvile, New Jersey, U.S.A.) which were then sealed with a burner and placed on ice until heat treatment.

Test media: We used BHI broth with 0, 2.4, and 4.5% (w/v) NaL as heating menstrum. Recovery media were BHI agar containing 0, 2.4, and 4.8% (w/v) NaL. All test media were adjusted to pH 7 ± 0.2.

Heat treatment: The ampules containing the culture suspensions were heated at 60°C in a water bath for a maximum of 30 min. Temperatures of the immersed ampules and of the water bath were monitored with thermocouples (Temperature recorder model CTF 9008, ELLAB Copenhagen, Denmark). One ampule from each NaL concentration remained in slushed ice so as to count the initial population of the test M.O. Come up time for all culture suspensions in the ampules was about 2 min. At preselected times individual ampules were taken out of the water bath, cooled immediately in slushed ice and kept there until plating to estimate the surviving cells .The experiment for each pathogen was repeated four times.

Recovery of heated cells: From each ampule we prepared tenfold serial dilutions in 0.1% peptone water (w/v). Recovery of the surviving cells was made by plating in duplicate on three BHI agars, each containing 0, 2.4 and 4.8% NaL respectively. The plates were incubated aerobically at 37°C for at least 72 hours before final colony counting. This procedure allowed detection of the effect of NaL concentration in the

heating menstrum on the heat injury of cells and at the same time its effect on their recovery in its presence in the recovery agars.

RESULTS AND DISCUSSION

The effect of NaL in heating menstrua and in the recovery agar plates on the heat resistance and recovery of the test M.O is shown in fig.1 for *L.monocytogenes*, fig.2 for *E.coli* O157:H7 and fig.3 for *Salmonella spp* respectively.

The data of fig.1 (A,B,C) indicate that increasing the concentration of NaL in the heating broth from 0 to 4.8% increases the thermal resistanse of *L.monocytogenes*. Thus, while it took 5 min. for 7 decimal reductions(DR) after heating in the absence of NaL in the heating broth and recovery on agar with 4.8% NaL (fig.1A), it took 15 min. for 7 DR in the presence of 4.8% NaL in the broth and the same recovery conditions (fig.1C). In the presence of the same levels of NaL in the heating and recovery medium, as expected to occur in cooked meat products,increasing levels of NaL resulted in faster destruction of the pathogen (fig.1D). Furthermore increasing levels of NaL in the recovery medium accelerated the death of sublethaly damaged cells. The data of fig.2 (A,B,C,D) concerning the behavior of *E.coli* indicate that the trends observed for *L.monocytogenes* apply also to this bacterium. Comparing fig.1 and fig.2 it can be concluded that *E.coli* O157:H7 was by far more heat labile than *L.monocytogenes*. Thus, for approximately 6 DR in the absence of NaL in the heating and recovery media, it took 20 min. for *L.monocytogenes* and only 6 min. for *E.coli*. As the data of fig.3 (A,B,C,D) show, the general trends observed for *L.monocytogenes* and *E.coli* are true for *Salmonella* also with the exception of the data of fig.3D. While we expected that when the same level of NaL is present in the heating and recovery medium, increasing levels enhanced overall destruction, fig. 3D shows a definite degree of protection of *Salmonella* by the presence of NaL. Nevertheless *Salmonella* under the present experimental conditions of heating and recovery was significantly more heat labile than *E.coli* O157:H7. Thus in the absence of NaL (fig.3A) *Salmonella* underwent approximately 8 DR in 3 min., while under the same conditions *E.coli* underwent only 6 DR in 6 min. Overall the figures show that NaL in the heating menstrum gave a slight protection of the test microorganisms when the detection for recovery is on BHI agar without NaL. When NaL was present in the agar medium recovery of heat injured cells decreased with increasing concentrations of NaL irrespectively of its concentration in the heating broth. When heating menstrum and recovery medium had the same concentration of NaL, recovery for *L.monocytogenes* and *E.coli* diminished with increasing concentration of NaL (fig.1D,2D) while for *Salmonella spp* presence of NaL seemed to enhance survival (fig.3D).

With the exception of *C.botulinum* (Meng, 1992; Houtsma et al., 1994) we are not aware of studies which evaluated the effect of NaL and other lactates on the thermal injury or protection of pathogenic bacteria. Addition of lactates to foods decreases water activity (Debevere,1989). The protective effect of decreasing water activity on bacterial thermal injury has been reported (Corry, 1976; Sofos, 1983). The nature of the solute influenced the degree of protection (Corry,1976). The increased protection of the organisms during heating by increasing levels of NaL in this study is in agreement with the literature. The presence of solutes in the recovery media is detrimental to heat injured bacteria (Sofos, 1983). This was true for *L.monocytogenes* and *E.coli* O157:H7 but not *Salmonella* in the present study. For *Salmonella,* increasing levels of NaL in the recovery medium generally enhanced recovery of injured cells though, overall, *Salmonella* was more heat labile than the other two organisms. The reason for the different behavior of *Salmonella* is unclear. It might be related to the degree of cell dehydration by NaL before heating which has been associated with an effect on thermal resistance (Hurst and Hughes, 1983). The practical implications of the present findings are of significance for the safety of cooked meats with added NaL. Irrespectively of the protection from thermal injury during processing there are sufficient experimental findings indicating the detrimental effect of NaL on the growth of even healthy bacterial pathogens during food storage, especially at low temperatures.

In this study we used a model broth instead of food to minimize the variables effecting bacterial survival and growth and thus have a better understanding of the effect of NaL. The effect of lactates on the injury and recovery of pathogenic bacteria during meat processing and storage is under study. The enhanced antimicrobial effect of organic acids with decreasing pH has been attributed to the increased amount of undessociated acid (Razavilar and Genigeorgis, 1992; Sofos, 1993). The decreased D-value of bacteria with decreasing of the pH of the heating menstrum has been shown (Hutton et al., 1992). In this study we used a neutral pH in order to determine the maximum potential protection given by NaL to heat stressed cells. Evaluation of other levels of pH in broths and cooked meats is under study.

CONCLUSION

Increasing levels of NaL from 0 to 4.8% in the heating menstrum or the recovery agar plates increased the thermal resistance and decreased the recovery respectively of *L.monocytogenes, E.coli* O157:H7 and *Salmonella* spp. In the presence of the same levels of NaL in the heating menstrum and the recovery agar, increasing levels of NaL resulted in fewer surviving *L.monocytogenes* and *E.coli* cells. On the contrary under the same conditions NaL exhibited a protective effect on *Salmonella.* Overall under all experimental conditions *L.monocytogenes* was the most resistant and *Salmonella* the most sensitive organism.

REFERENCES

Bacus, J. and Bontenbal, E., (1991). Controlling *Listeria*. Meat Poult., 7:64-65.

Brewer, M.S.,McKeith, F., Martin, S., Dallmier, A. and Meyer,J., (1991). Sodium lactate effects on shelf-life, sensory and physical characteristics of fresh pork sausage. J. Food Sci., 56:1176-1178.

Corry, J.E.L., (1976). The effect of sugars and polyols on the heat resistance and morphology of osmophilic yeasts. J. Appl. Bact., 40:269-276.

Debevere, J.M., (1989). Extended shelf-life from lowered a_w and growth inhibition of lactic acid bacteria by sodium lactate. Fleischwirtsch., 69:223-227.

De Wit, J.C. and Rombouts, F.M., (1990). Antimicrobial activity of sodium lactate. Food Microbiol., 7:113-120.

Gill, C.O. and Newton, K.G., (1982). Effect of lactic acid concentration on growth on meat of gram-negative psychrotrophs from a meat works. Appl. Envir. Microbiol., 43:284-288.
Grau, F.H., (1981). Role of pH, lactate and anaerobiosis in controlling the growth of some fermentative gram negative bacteria on beef. Appl. Envir. Microbiol., 42:1043-1050.

Harmayani, E.J.,Sofos,J.N.,and Schmidt,G.R.,(1991). Effect of sodium lactate, calcium lactate and sodium alginate on bacterial growth and aminopeptidase activity. J. Food Safety., 11:269-283.

Houtsma, P.C., Heuvelink, A., Dufrenne, J. and Notermans, S., (1994). Effect of sodium lactate on toxin production, spore germination and resistance of proteolytic *Clostridium botulinum* strains. J. Food Prot., 57:327-330.

Hurst, A. and Hughes, A., (1983). The protective effect of some food ingredients on *Staphylococcus* MF31. J. Appl. Bact., 55:81-88.

Huttton, M.T., Koskinen, M.A. and Hanlin, J.H., (1991). Interacting effects of pH and NaCl on heat resistance of bacterial spores. J. Food Sci., 56:821-822.

Maas, M.R., (1992). Combined effect of sodium lactate and sodium chloride on toxin production by *Clostridium botulinum* in cured turkey breast. Proc. 2nd ASEPT Inter. Conf. on Predictive Microbiology and HACCP. ASEPT, LAVAL, pp.57-74.

Meng, J., (1992). Effect of sodium lactate on probability of *Clostridium botulinum* growth in BHI broth after heat shock and on toxigenesis in cooked poultry meat products. PhD thesis, University of California, Davis, CA, USA.

Meng, J., and Genigeorgis, C., (1993a). Effect of sodium lactate on toxigenesis of *Clostridium botulinum* in "Sous-vide" products. Dairy Food Environ. Sanit., 13:607.

Meng, J., and Genigeorgis, C., (1993b). Modeling lag phase of non-proteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. Inter. J. Food Microbiol., 19:109-122.

Miller, R.K. and Acuff, G.R., (1994). Sodium lactate affects pathogens in cooked beef. J. Food Prot., 59:15-19.

Papadopoulos, L.S., Miller, G.R., Acuff,C., Vanderzant, C. and Gross, H.R., (1991). Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. J. Food Sci., 56:341-347.

Pelroy, G.A., Paterson, M.E., Holland, P.J and Eklund, M.W., (1994). Inhibition of *Listeria monocytogenes* in cold-precess (smoked) salmon by sodium lactate. J. Food Prot., 57:108-113.
Razavilar, V. and Genigeorgis, C., (1992). Probability of growth of *Listeria* spp as affected by species, pH, acids, temperature and storage time in a model broth. Proc. 3rd World Cong. Foodborne Infections and Intoxications., I:334,355-357.

Sofos, J.N., (1983). Antimicrobial effects of sodium and other ions in foods: A review. J. Food Safety., 6:45-78.

Sofos, J.N., (1993). Current microbiological considerations in food preservation. Int. J. Food Microbiol., 19:87-108.

Unda, J.R., Molins, R.A. and Walker, H.W., (1991). *Clostridium sporogenes* and *Listeria monocytogenes:* Survival and inhibition in microwave-ready beef roasts containing selected antimicrobials. J. Food Sci., 56:198-205.

Van Burik, A.M.C. and De Koos, J.I., (1990). Natriumlactat in Fleischproducten. Fleischwirtsch., 70(11):1266-1268.

Weaver, R.A. and Shelef, L.A., (1993). Antilisteria activity of sodium, potassium or calcium lactate in pork liver sausage. J. Food Safety., 13:133-146.

FIGURES

Fig.1: Effect of O% (A), 2.4% (B) and 4.8% of NaL in the heating broth (BHI) and 0% (circles), 2.4% (squares) and 4.8% (triangles) NaL in the recovery agar on the number of surviving *L.monocytogenes* cells heated at 60C for up to 30 min. Fig.1D: Refers to heating and recovery of the bacterium in the presence of the same level of NaL.

Fig.2 (A,B,C,D): Same conditions as in fig.1 but for *E.coli.*

Fig.3 (A,B,C,D): Same conditions as in fig.1 but for Salmonella.