Biotechnology in Animal Husbandry 23 (5-6), p 103 - 112 , 2007 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.06

SPOILAGE MICROFLORA OF VACUUM PACKAGED FRANKFURTERS AND INFLUENCE ON THE GROWTH OF *Listeria monocytogenes***

D. Radin [1]*, S.E. Niebuhr [2], J.S. Dickson [2]

[1] University of Belgrade - Faculty of Agriculture, Belgrade-Zemun, 11080, Serbia
[2] Iowa State University, Department of Animal Science, Ames, Iowa 50011, USA
*Corresponding author: e-mail: dradin@agrifaculty.bg.ac.yu
** Original Scientific paper

Abstract: Spoilage microflora present on vacuum packaged frankfurters is in most cases, result of post processing contamination, at the same time this is the primary cause of contamination with *Listeria monocytogenes*. Since spoilage organisms are present in the same environment as a pathogen, the aim was to determine their microbial interference. Approximately 100 CFU/cm^2 of a five-strain mixture of *L. monocytogenes* was co inoculated onto frankfurters with different concentrations (10^3 and 10^6 CFU/cm^2) of spoilage microflora (bacteria from genera *Lactobacillus, Bacillus, Micrococcus*, and *Hafnia*). The frankfurters were vacuum packaged and stored at 10°C for up to 48 days. The spoilage microflora that developed during storage consisted predominantly of lactic acid bacteria. The growth of mesophilic aerobic bacteria and LAB was very similar, with populations reaching 8.0 log CFU/cm^2 within 24 days and final population of >9 log CFU/cm^2 within 48 days. The presence of spoilage microflora extended the lag phase of *L. monocytogenes* until 24 days and significantly decreased pathogen level to 4 and 3 log CFU/cm^2, in samples inoculated with initial concentration 10^3 CFU/cm^2 and 10^6 CFU/cm^2 of spoilage microflora, respectively. *L. monocytogenes* populations were significantly higher ($P<0.05$) in the reference sample (no spoilage microflora) and reached a maximum population of 5.9 log CFU/cm^2 after 34 days. These results imply that competing microorganisms present on the processed meat may inhibit the growth of *L. monocytogenes* in the package.

Key words: spoilage microflora, *Listeria monocytogenes*, frankfurter

Introduction

It has been estimated that 25% of all foods produced globally is lost post harvest or post slaughter due to microbial spoilage (*Anonymous,* 1985).

Spoilage of minimal heat-treated, cured, vacuum-packaged meat products, such as frankfurters, bologna sausages, ham, is not uncommon. The heating step applied to these products destroys the normal raw meat microflora with the exception of spore and, possibly, thermoduric bacteria (*Topkin et al.,* 2001). During chilling and preparation for packaging, some contamination will occur on exposed surfaces. Upon prolonged refrigeration, lactic acid bacteria, micrococci, enterococci, *Enterobacteriaceae*, molds, and yeasts may grow and cause spoilage (*Ray*, 2004).

Another very important issue concerning post processing contamination of meat products, including ready-to-eat (RTE) products such as frankfurters, is contamination with *Listeria monocytogenes*. This is a common cause of listeriosis because many of these RTE products are consumed without heating. Numerous sporadic outbreak cases of foodborne illness have been linked to consumption of RTE products with *L. monocytogenes* (CDC, 2002).

L. monocytogenes is ubiquitous, can be resistant to many food preservation methods, and has the ability to colonize meat plants and to survive under unfavorable conditions (*Samelis and Metaxopoulos*, 1999). A variety of environmental factors, such as pH and water activity, influence the rate and extent of *L. monocytogenes* growth, and these factors have been studied extensively. An additional factor is the impact of competing microorganisms. FAO/WHO (2000) quantitative risk assessment activities to predict the growth potential of *L. monocytogenes* in RTE foods included the use of mathematical models which take into account the effect of storage temperature, water activity, pH, lactate, nitrate and microbial interactions. Significant investigations in this field comprise study how *Carnobacterium piscicola*, a common lactic acid bacterium that appears to share the same niche with *Listeria* in refrigerated foods of animal origin, was able to suppress the maximum population density achieved by *L. monocytogenes* (*Buchanan and Bagi,* 1997); growth of *L. monocytogenes* in cold-smoked salmon ceased when the total microflora reach their maximum population density (*Gimenez and Dalgaard,* 2004); similar observation was reported by *Gram et al.* (2002) for *L. monocytogenes* growing in lightly preserved foods with a dominant lactic acid bacterial flora. The latter phenomenon may be caused by bacteriocin production by the LAB, but since several non-

bacteriocin producing LAB were as inhibitory, the inhibition may as well be explained by the LAB out competing the *Listeria* on a few essential nutrients.

As interactive behavior is important in any foods in which a mixed microflora develops during storage, the objective of this investigation was to isolate and determine dominant spoilage microflora of vacuum packaged frankfurters and to examine influence on growth of *L. monocytogenes,* at 10°C storage temperature that represent potential mild abuse during distribution and retail, as well as at the consumer level.

Materials and methods

L. monocytogenes. A five-strain mixture of *L. monocytogenes* was prepared to include strains H7762 and H7769 (serotype 4b, food product isolate), serotype 1/2a FSIS and H7764 (food product isolate), Scott A (serotype 4b, clinical isolate). Individual cultures were grown in TSB containing 0.6% yeast extract at 37°C for 18 h.

Spoilage microflora. Packages of frankfurters were obtained from local retail outlets. Twenty-five milliliters of TSB was added to the packages and gently agitated for approximately 1 min. The samples were combined in a sterile flask and incubated at ambient temperature for 3 days. Bacteria were isolated from TSA, VRBG and MRS agar plates and characterized using BBL Crystal Enteric/Non-fermenter and Gram Positive ID system (BBL, Becton Dickinson, Sparks, Md.) and the API 50CH system (bioMerieux, Hazelwood. Mo.). The isolated cultures were propagated independently and then mixed to achieve the final mixed population.

Frankfurter inoculation. The microflora naturally present on the frankfurters was reduced by immersing the frankfurters in boiling water for 30 s prior to inoculation. Individual frankfurters were placed in vacuum bags commonly used for RTE processed meats (Curlon 861 Film, Curwood, Oshkosh, Wis.; oxygen transmission rate of 3 to 4 ml/100 in^2/24 h at 22.78°C), and inoculated with *L. monocytogenes* at approximately 2 log CFU/cm^2 and spoilage microflora at approximately 3 or 6 log CFU/cm^2. The vacuum-packaged frankfurters were stored at 10°C for 48 days.

Microbiological methods. At the appropriate time interval, according to USDA ARS package rinse method, individual packages were opened and a single frankfurter removed and rinsed in 25 ml of buffered peptone water. The rinsate was used for further analysis. Bacterial populations were enumerated after serial dilution in buffered peptone water as necessary and

surface plating of 0.1 ml on appropriate media; mesophilic aerobic bacteria on TSA plates incubated at 32°C for 48 h, *Enterobacteriaceae* on VRBG agar plates incubated at 37°C for 48 h, *L. monocytogenes* on plates of Oxford Selective Agar – MOX (including selective supplement; Oxoid, Basingstoke, UK) incubated at 37°C for 24 to 48 h, and lactic acid bacteria on MRS agar (Difco, Becton Dickinson, Sparks, Md.) plates incubated anaerobically at 30°C for 48 h.

Analysis of data. The population counts obtained from each analysis were converted to log CFU per square centimeter (the surface area of the frankfurters was determined to be 85 cm^2). The detection limit of the assay was 0.17 log CFU/cm^2, based on plating 100µl of the sample on each of two duplicate plates. The experiments were independently replicated three times. The data were analyzed with the general linear models procedure of SAS (Cary, N.C.).

Results and discussion

The primary bacteria genera isolated from the spoilage microflora of vacuum-packed frankfurters were *Hafnia, Micrococcus, Bacillus* and *Lactobacillus*, what is in accordance with data reported by *Alm et al.* (1961) that during storage of cooked, sliced meat products dominate a mixed autochthonous flora composed of *Bacillus* spp., *Micrococcus* spp., and *Lactobacillus* spp. Major spoilage organisms are lactic acid bacteria whose growth is preferentially selected by factors such as the low storage temperature, presence of nitrite and curing salts, and microaerophilic conditions (*Davies et al.,* 1999). Isolated lactobacilli predominantly were heterofermentative species, which are dominant in vacuum-packaged products (*Ray*, 2004). *Palumbo et al.* (1974) found that out of very low numbers of bacteria that survive the heating step of frankfurters almost exclusively were micrococci. Prolonged storage in a vacuum-pack favored the growth of slowly growing LAB and *Enterobacteriaceae* and *Borch et al.,* (1996) determined that *Hafnia alvei* dominated among *Enterobacteriaceae* at 4°C.

For the recovery of *Listeria monocytogenes* from inoculated vacuum packaged frankfurters the USDA Agricultural Research Service (ARS) package rinse method, which involves sampling of the outer surface of food product was used. *Luchansky et al*. (2002) demonstrated that USDA-ARS package rinse method was significantly more efficient at recovering *L. monocytogenes* from package inoculated with an average of 22 or 20

CFU than was the USDA-ARS product composite rinse method.

In the absence of spoilage microflora, the initial population 2 log CFU/cm^2 of *L. monocytogenes* on frankfurters increased approximately 1log CFU/cm^2 within 14 days, reached a maximum population of 5.9 log CFU/cm^2 (Fig.1 and 2, reference curve) after 34 days. Lag phase duration time was 7 days. In a similar study, *L. monocytogenes* grew from 2.88 log CFU/g to 5.26 log CFU/g in 14 days at 10°C with lag phase about 6.5 days (*Lu et al.,* 2005). *McKellar et al.* (1994) who studied the factors that influenced the survival and growth of this pathogen on Canadian retail frankfurters obtained similar results. Out of four reported samples of beef frankfurters under vacuum at 5°C for 28 days, the population of *L. monocytogenes* increased from 2 to 4.5 log CFU/g and all four samples had a lag period of about 7 days. *Diez-Gonzalez et al.* (2007) in modeling the growth of *L. monocytogenes* on frankfurters obtained that bacteria population increased about 2 log cycles after 28 days at 4°C and had a lag period of 6 days. This data indicate ability of pathogen to proliferate in frankfurters, when introduced on their surface after heating. Lag phase duration on actual food product is important because any contamination of *L. monocytogenes* most likely occurs at very low levels, and the duration of the lag phase will determine how long it takes for the pathogen to grow to hazardous numbers on that product.

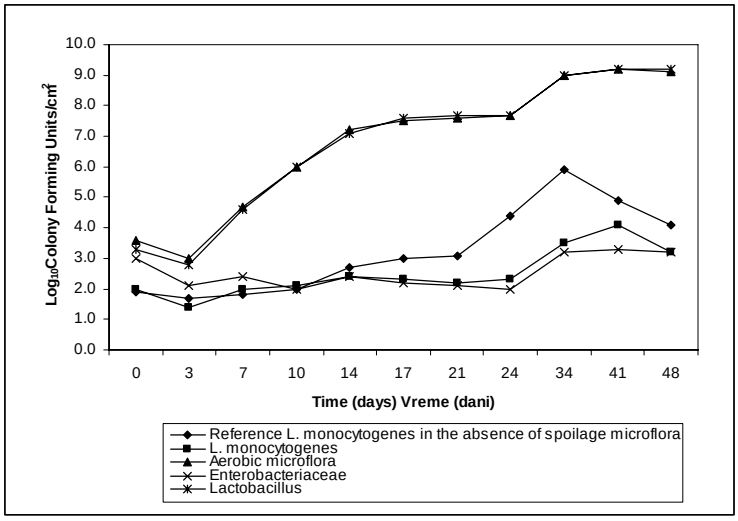

Figure 1. Growth of *Listeria monocytogenes* and spoilage microflora (initial concentration approx. 10^3 CFU/cm^2) on vacuum-packaged frankfurters stored at 10°C

The presence of spoilage microflora extended the lag phase of *L. monocytogenes* until 24 days and significantly decreased pathogen level to 4 and 3 log CFU/cm^2, in samples inoculated with 10^3 CFU/cm^2 (Fig. 1) and 10^6 CFU/cm^2 (Fig. 2) of spoilage microflora, respectively. The suppression of *L. monocytogenes* growth was related to the initial concentration of spoilage microflora; a higher initial concentration of spoilage microflora resulted in a lower overall *L. monocytogenes* population. This suppression (of maximum population density) of a particular organism by an overgrowing microflora is called the Jameson effect (*Gram et al*, 2002).

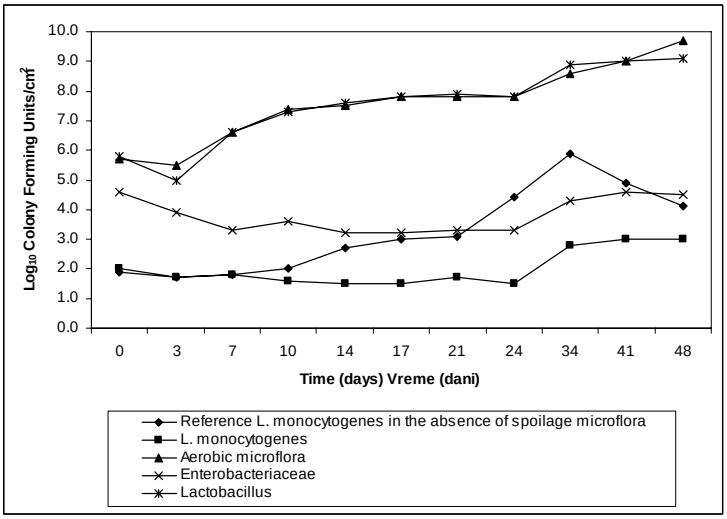

Figure 2. Growth of *Listeria monocytogenes* and spoilage microflora (initial concentration approx. 10^6 CFU/cm^2) on vacuum-packaged frankfurters stored at 10°C

The spoilage microflora that developed in the vacuum packages consisted predominantly of lactic acid bacteria, as indicated by the similarity in the populations of mesophilic aerobic bacteria and LAB. Their growth was very similar, with populations reaching 8.0 log CFU/cm^2 within 24 days and final population of >9 log CFU/cm^2 within 48 days. Generally, depending on the storage temperature and levels of contamination, LAB spoilage microflora can reach $>10^8$ organisms g^{-1} after relatively short times (*Davies et al.* 1999).

The population of *Enterobacteriaceae* bacteria declined until 24 days but ultimately returned to populations approximating their initial concentrations.

Similar data were reported for the spoilage microflora of sliced vacuum

packed cold smoke salmon stored at 10°C, LAB dominated while count of *Enterobactericeae* remains at initial inoculation level (*Gimenez and Dalgaard,* 2004).

Conclusion

From commercially produced vacuum packaged frankfurters dominant microflora that had been isolated were bacteria belonging to genera *Lactobacillus, Bacillus, Micrococcus* and *Hafnia*. This spoilage microflora affected the growth of *Listeria monocytogenes* when coinoculated on vacuum packaged frankfurters. At 10°C storage temperature, *L. monocytogenes* in the absence of spoilage bacteria had shorter lag phase of 7 days and reached a maximum population of 5.9 log CFU/cm^2. However, the presence of spoilage microflora extended the lag phase to 24 days and reduced significantly population levels of pathogen bacteria. Although an even small population of spoilage microflora was sufficient to impact the growth *L. monocytogenes*, a higher concentration resulted in decreased levels of pathogen for more than 3 log CFU/cm^2. The spoilage microflora that developed in the vacuum packages consisted predominantly of lactic acid bacteria whose growth pattern was very similar to growth of mesophilic aerobic microflora.

MIKROORGANIZMI KOJI IZAZIVAJU KVAR VAKUUM PAKOVANIH VIRŠLI I UTICAJ NA RAST *Listeria monocytogenes*

D. Radin, S.E. Niebuhr, J.S. Dickson

Rezime

Proizvodi od mesa, uključujući ready-to-eat (RTE) proizvode kao što su viršle, povezani su sa pojavom oboljenja listerioze. Do kontaminacije ovih proizvoda patogenom bakterijom *Listeria monocytogenes* najčešće dolazi nakon proizvodnje i primenjenog termičkog tretmana a pre pakovanja. S ozirom da se mnogi od ovih proizvoda mogu konzumirati bez ponovnog zagrevanja mogu biti uzrok listerioze. Imajuću u vidu činjenicu da su u vakuum pakovanim viršlama prisutni i mikroorganizmi koji mogu izazivati

kvar, cilj je bio da se ispita njihov međusobni uticaj. Viršle su inokulisane mešavinom pet sojeva *L. monocytogenes* u koncentraciji 100 CFU/cm^2 i različitim koncentracijama bakterija koje pripadaju rodovima *Lactobacillus, Bacillus, Micrococcus* i *Hafnia.* Viršle su, zatim vakuum pakovane i skladištene na 10°C 48 dana.

U odsustvu kompetitivnih bakterija, patogena bakterija *L. monocytogenes* je dostigla maksimalnu koncentraciju od 5.9 log CFU/cm^2 i imala lag fazu koja je iznosila 7 dana. Ovi podaci doprinose činjenici da vakuum pakovane viršle čine veoma dobru sredinu za rast i preživljavanje *L. monocytogenes.*

Mikroflora koja se razvijala tokom skladištenja se uglavnom sastojala od bakterija mlečne kiseline čiji je razvoj bio vrlo sličan razvoju mezofilne aerobne mikroflora, i dostizao maksimalnu populaciju >9 log CFU/cm^2. Prisustvo ove mikroflore je produžilo lag fazu *L. monocytogenes* do 24 dana i značajno smanjilo broj patogena do 4 odn. 3 log CFU/cm^2, u uzorcima koji su inokulisani početnom koncentracijom 10^3 CFU/cm^2 odnosno 10^6 CFU/cm^2 bakterija kvara. Ovi rezultati ukazuju da kompetitivna mikroflora može inhibirati rast *L. monocytogenes* na proizvodima od mesa u vakuum pakovanju.

References

ALM, F., ERICHSEN, I., AND MOLIN, N. (1961): The effect of vacuum packaging on some sliced processed meat products as judged by organoleptic and bacteriological analysis. Food Technol. 4, 199-203.

ANONYMOUS (1985): Subcommittee on Microbiological Criteria, Committee on Food Protection; Food and Nutrition Board National Research Council, an Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academy Press, Washington, D.C.

BORCH, E., KANT-MUERMANS, M., AND BLIXT, Y. (1996): Bacterial spoilage of meat and cured meat products. Food Microbiology, 33, 103-120.

BUCHANAN, R.L., AND BAGI, L.K. (1997): Microbial competition: effect of culture conditions on the suppression of *Listeria monocytogenes* Scott A by *Carnobacterium piscicola*. Journal of Food Protection, 60, 254-261.

CENTERS FOR DISEASE CONTROL, (2002): Outbreak of listeriosis – northeastern United States, Morbidity and Mortality Weekly Report, 51, 950-951.

DAVIES, E.A., MILNE, C.F., BEVIS, H.E., POTTER, R.W., HARRIS, J.M., WILLIAMS, G.C., THOMAS, L.V., AND DELVES-BROUGHTON,

J. (1999): Effective use of nisin to control lactic acid bacteria spoilage in vacuum-packed bologna-type sausage. Journal of Food Protection, 62, 1004-1010.
DIEZ-GONZALEZ, F., BELINA, D., LABUZA, T.P., AND PAL, A. (2007): Modeling the growth of *Listeria monocytogenes* based on a time to detect model in culture media and frankfurters. International Journal of Food Microbiology, 113, 277-283.
FAO/WHO (2000): Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods. FAO Food Nutrition Paper No.71.
GIMENEZ, B., AND DALGAARD, P. (2004): Modeling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon. Journal of Applied Microbiology, 96, 96-109.
GRAM, L., RAVN, L., RASCH, M., BRUHN, J.B., CHRISTENSEN, A.B., AND GIVSKOV, M. (2002): Food spoilage – interactions between food spoilage bacteria. International Journal of Food Microbiology 78, 79-97.
LU, Z., SEBRANEK, J.G., DICKSON, J.S., MENDONCA, A.F., AND BAILEY, T.B. (2005): Inhibitory effects of organic acid salts for control of *Listeria monocytogenes* on frankfurters. Journal of Food Protection, 68, 499-506.
LUCHANSKY, J.B., PORTO, A.C.S., WALLACE, F.M., AND CALL, J.E. (2002): Recovery of *Listeria monocytogenes* from vacuum-sealed packages of frankfurters: comparison of the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service product composite enrichment method, the USDA Agricultural Research Service (ARS) product composite rinse method, and the USDA-ARS package rinse method. Journal of Food Protection, 65, 567-570.
MCKELLAR, R.C., MOIR, R., AND KALAB, M. (1994): Factors influencing the survival and growth of *Listeria monocytogenes* on the surface of Canadian retail wieners. Journal of Food Protection, 57, 387-392.
SAMELIS, J., AND METAXOPOULOS, J. (1999): Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. Food Microbiology 16, 465-477.
PALUMBO, S.A., HUHTANEN, C.N., AND SMITH, J.L. (1974): Microbiology of the frankfurter process: *Salmonella* and natural aerobic flora. Applied Microbiology, 27, 724-732.
RAY, B. (2004): Fundamental food microbiology. 3rd ed. CRC Press LLC

TOMPKIN, R.B., MCNAMARA, A.M., AND ACUFF, G.R. (2001): Meat and poultry products. In: Compendium of methods for the MICROBIOLOGICAL EXAMINATION OF FOODS, fourth edition, Ed. By F.P. Downes and K. Ito.