USE OF ORGANIC ACIDS TO CONTROL *LISTERIA* IN MEAT

A low pH (acidic) environment has an adverse effect on the growth of *Listeria monocytogenes* but it is not only the specific pH of the medium which is important but also the type of acid, temperature, and other antimicrobial compounds which are present (*7*). Several researchers have noted that, in culture media, acetic acid has more potent antilisterial effects than lactic acid, which, in turn, is more inhibitory than hydrochloric acid (*1,19,20,36*). Although similar concentrations of citric and lactic acids reduce the pH of tryptic soy broth more than acetic acid does, addition of acetic acid results in greater cell destruction (*19*). Malic acid, the predominant organic acid in apples, is not as effective as lactic acid in suppressing growth of *L. monocytogenes* (*4*). Sodium diacetate (a mixture of acetic acid and sodium acetate) also significantly inhibits the growth of *L. monocytogenes* in broth cultures (*32*). Several experiments in culture media demonstrated that inhibitory effects of an acid are greater at lower temperatures (*5,6,13,16,17,31*).

Other factors, such as the presence of salt and other compounds used as preservatives, may modify the effects of organic acids on *L. monocytogenes* (*6,16,21,31*) and several models have been developed to describe these interactions (*5,17,26*). These models may provide useful estimates of the relative importance of different factors and the magnitude of inhibition to be expected but they may overestimate or underestimate the effects on *L. monocytogenes* in meat, such as bologna (*17*) and sausage (*26*).

Organic acids can interact with other preservatives to enhance their effects. Acetic and lactic acids enhance the antilisterial effects of monolaurin (*25,27,28*). Lactic acid increased the susceptibility of *L. monocytogenes* to heat shock in culture media (*20*). But no effect on thermal tolerance was observed in ground pork (*39*).

However, it should be noted that the effects of organic acids are not always positive in terms of food safety. Listeriae which are exposed to these acids and survive may repair themselves during storage at low temperatures and begin to multiply if other barriers are not present (*9,14,29*). Exposure to acid also induces stress responses in listeriae which make the bacteria more tolerant of more acidity, ethanol, and hydrogen peroxide (*22*).

Antilisterial effects of organic acids have been examined in several types of meats raw, cooked, and cured. Since carcass meat may be contaminated with *L. monocytogenes* during slaughter and packaging into retail cuts of meat, solutions of organic acids have been tested as washes or dips for removing listeriae from meat and/or inhibiting its growth during refrigerated storage. When lactic or acetic acids (1.5-4%) were sprayed on contaminated beef carcass or beef trim, large numbers of inoculated *L. monocytogenes* persisted and grew on the meat stored under refrigeration (*10,11*). On the other hand, if the beef was sprayed with 2% lactic or acetic acid before it was contaminated with *L. monocytogenes*, the residual activity of the acids suppressed the growth of the bacteria (*12*).

Organic acids (1-3%) used as dips are usually more efficacious than carcass washes because some residual activity remains on the meat. These acid concentrations, generally cause no adverse effect on the sensory properties of the meat. *L. monocytogenes* and *E. coli*, however, are more resistant to acid treatments than *Yersinia* and *Salmonella* (*14,34*). Both lactic acid (1.7%) and acetic acid (2%) reduced *L. monocytogenes* populations on lean beef tissue by 2–3 logs for up to 7 days (*33*). In other experiments with raw beef, 2% fumaric acid was found to be a more effective antilisterial agent than 1% acetic or lactic acid (*30*). When lean pork tissue and pork fat were artificially inoculated with *L. monocytogenes* and then dipped in 3% lactic acid or water for 15 sec, numbers of listeriae were reduced by 1-2 logs for the lean meat and up to 7 logs for the fat during 15 days of refrigerated storage (*14*). The more potent effects observed for pork fat were probably due to the fact that acid-treated fat was approximately 2.5 pH units lower than acid-treated lean tissue. A similar effect was observed in pork liver sausage with 22-67% fat treated with propionate or lactate: At higher fat levels, the kill was approximately 2-3 times greater (*18*).

The best treatment for artificially contaminated raw chicken legs was reported to be a wash with a 10% lactic acid/sodium lactate buffer, pH 3.0 followed by packaging in 90% carbon dioxide, 10% oxygen. This procedure extended the shelf life of the chicken from 6 days to 17 days. Chicken treated with the lactate buffer without modified atmosphere packaging had a shelf life of 10 days (*40*).

Artificial contamination of frankfurters with *L. monocytogenes* followed by a 2 min dip in 1% lactic, acetic, tartaric, or citric acids resulted in a 1-2 log kill of the bacteria. However, surviving bacteria started growing during refrigerated storage. A dip in 5% acetic or lactic acids not only killed *L. monocytogenes* but prevented its regrowth during 90 days storage (*29*).

Addition of 1.8% or 2% lactic acid to raw or cooked ground beef did not appreciably affect the survival and growth of *L. monocytogenes* (*15,37*). Data from another experiment indicated that lactic acid slightly reduced the thermal tolerance of *L. monocytogenes* in ground beef (23). Sodium diacetate (0.3%) delayed growth of *L. monocytogenes* in turkey slurry (*31*).

Sodium lactate (4%) was reported to suppress the growth of *L. monocytogenes* in cooked strained beef (*8*) and beef roasts (*24*). In both cases, however, there were viable listeriae left in the meat during refrigeration. *L. monocytogenes* inoculated onto cooked chicken which was treated with lactate were observed to have a longer lag phase but were still able to grow during storage (*2*). Brines containing monolaurin and lactate pumped into beef roasts (microwave-ready beef roasts) enabled a greater kill of *L. monocytogenes* during cooking in bags in water baths than brines with only lactate (*35*).

Cured meats, such as sausage, ham, and frankfurters, which contain salt and other preservatives are more susceptible to the listericidal effects of organic acids (*3,17,18,26, 29,38*)

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USE OF OTHER PRESERVATIVES TO CONTROL *LISTERIA* IN MEAT

Since *Listeria monocytogenes* can grow on a variety of processed meat products at refrigeration temperatures (9), a variety of chemicals which destroy or limit the growth of harmful microbes have been tested for the preservation of meat. Many of these compounds are well known and their effects on various bacteria and on meat quality have been thoroughly investigated; others have been introduced recently and are not as well studied. Some compounds are not very potent by themselves but in combination with other preservatives or storage conditions can suppress the growth of foodborne pathogens. Several researchers have developed models which describe the effects of different combinations of preservatives on the growth of *L. monocytogenes* in laboratory media (*2,8,26,31*). Although these models are useful, growth of *L. monocytogenes* in meat nearly always differs from that in culture media.

Sodium chloride (NaCl). NaCl in growth media or foods can be a source of osmotic stress by decreasing water activity (a_w). However, *L monocytogenes* is remarkably salt-tolerant and able to withstand higher salt concentrations than *Salmonella* spp. and *Yersinia* spp. (*13*). In an experiment to determine the antilisterial effects of brine solutions which could be used as dips, *L. monocytogenes* easily survived 6 hours at 10°C in solutions containing 6, 16, or 26% sodium chloride (*15*). *L. monocytogenes* even grew in the 6% brine solution (*15*) and in meat peptone media containing 8% NaCl (*40*). The presence of sodium chloride in growth media also partially protects *L. monocytogenes* from other stresses such as heat in ground pork (*45*), lactocin 705 in minced beef slurry (*41*), and hydrogen peroxide in culture media (*21*).

Although *L. monocytogenes* is halotolerant, salt is a stress and does depress growth rates (*4,40*). In combination with other compounds used in curing meats, NaCl is one factor contributing to the destruction or inhibition of *L. monocytogenes* (*3,8,17,26,31*).

Nitrite. Nitrite alone is also not a very effective antilisterial agent. In turkey slurries (pH6.2), 30 ppm sodium nitrite was unable to inhibit the growth of *L. monocytogenes* at 4 or 25°C (*35*). In beef slurries, 800 ppm was required to inhibit growth of *L. monocytogenes* (*41*). However, as with salt, in the presence of other curing agents (*8,26,31,44*) or lactocin 705 (*41*), nitrite can contribute to the suppression of *L. monocytogenes* at refrigeration temperatures.

Trisodium phosphate (TSP). Trisodium phosphate has been used for decontamination of poultry carcasses (*34*) and can reduce bacterial contaminants by 1-2 logs. Spraying of TSP on beef carcass tissue contaminated with *L. monocytogenes* removed 1.3 log of cells but by the 7th day of cold storage, the remaining bacteria started to grow (*6*). Use of 10% TSP as a 15 sec dip removed only about 39% and 81% of *L. monocytogenes* at 10°C and 4°C, respectively (*5*). In other experiments, in which *L. monocytogenes* was suspended on solutions of TSP, exposure to 8% TSP for at least 10 min was required to reduce

bacterial numbers by at least 1 log (*36*). *E. coli* O157:H7, *Campylobacter jejuni* and *Salmonella typhimurium* were all more sensitive than *L. monocytogenes* to TSP.

Smoke/Liquid Smoke. Smoking of meat and fish is a well known preservation technique and has been shown to inhibit the growth of *L. monocytogenes* (*27,32*). Several experiments have also documented the antilisterial effects of liquid smoke additives. Of 5 Red Arrow smoke products evaluated, CharSol-10 was the most effective against *L. monocytogenes* and reduced viable cells on the surface of beef franks by >99.9% after 72 hours storage at 4°C (*23*). Another product, CharSol Supreme also had potent antilisterial effects in wiener exudate (*7*). Analysis of this product revealed that its active ingredient was isoeugenol and that this compound was more effective in the presence of acetic acid at pH 5.8. Experiments with 7 commercial smoke preparations used in Spain indicated that some were better antilisterial agents than others and that the most potent had higher concentrations of phenols (*37*).

Plant Extracts. A variety of herbs and spices have been tested for their efficacy in suppressing the growth of *L. monocytogenes* in culture media. Plant extracts exhibiting antilisterial activity include: hop extracts (*20*), eugenol (*1,10,11*), pimento leaf (*10,11*), horseradish distillates (*43*), rosemary (*21,30*), cloves (*21,30*), cinnamic acid (*19,33*), furanocoumarins (*38*), and carvacol (*18*). Numerous other plant extracts have been tested but results were not always consistent. (*10,18,21*). Different commercial samples of plant essential oils and different varieties of the same herbs may exhibit differences in antilisterial potency because of varying amounts of critical compounds. Some plant extracts were also found to be effective against *Listeria* spp. in meat including rosemary in ready-to-eat pork liver sausage (*30*), horseradish distillates on roast beef (*43*), and eugenol and pimento leaf of refrigerated cooked beef (*11*). It should be noted that *L. monocytogenes* was usually less sensitive to these extracts in meat (compared to culture media) and sensitivity also varied with fat content of the meat. For hop extracts tested in dairy products, antimicrobial activity was higher in lower fat meats (*20*).

Monolaurin and other monoglycerides. Several monoglycerides (glycerol with one esterified fatty acid) are effective inhibitors of *L. monocytogenes* in culture media (*25,28,29,42*) and in foods. In beef frank slurries (pH 5.0 and 5.5), mono-caprin, monolaurin and coconut monoglycerides, individually all inhibited the growth of *L. monocytogenes* (*42*). These individual compounds were not as effective in turkey frank slurries but combinations of monoglycerides were effective. Brines containing monolaurin and lactate pumped into beef roasts (microwave-ready beef roasts) enabled a greater kill of *L. monocytogenes* during cooking in bags in water baths than brines without monolaurin (*39*). Monolaurin appeared to be a more potent antimicrobial at lower temperatures and pH values (*25,29,42*). Also, planktonic cells of *L. monocytogenes* were more susceptible to monolaurin than cells attached to stainless steel surfaces (*28*).

Chelators (Citrate and EDTA). Chelators, which bind metal ions, are not by themselves lethal to *L. monocytogenes* in the concentrations used in foods (*46*). However, these compounds interact with other preservatives and sometimes aid in suppressing the growth of *L. monocytogenes* in meats (*1,25,31*). In other cases, for example EDTA combined with nisin, the opposite occurs and EDTA reduces the antimicrobial effects of nisin (*46*).

Lysozyme. Hen egg white lysozyme suppressed the growth of *L. monocytogenes* in fresh pork sausage (bratwurst) for 2-3 weeks (*16*).

Sorbate (Sorbic acid). Experiments using culture media revealed that *L. monocytogenes* was more susceptible to sorbate at lower pH (pH 5 vs pH 6) and at lower temperatures (5°C vs 30°C). (24) In beaker sausage sorbate was also a more effective inhibitor of *L. monocytogenes* at lower temperatures (*14*). Fat content of the sausage did not affect the potency of sorbate at 4°C but at 10°C, sorbate was a more effective in sausages containing 67% fat as compared to 22% fat.

Other additives. Minimal inhibitory concentrations of **methyl paraben (p-hydroxybenzoate)** for growth of *L. monocytogenes* in culture media were lower at pH 5 than at pH 6 and at 5°C than at 30°C. Under similar conditions, methyl paraben was a more potent inhibitor of *L. monocytogenes* than sorbate (*24*). **Sodium erythorbate** did not appear to be an effective antilisterial agent in raw or cooked ground beef. (*12*).

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USE OF BACTERIOCINS TO CONTROL *LISTERIA* IN MEAT

Bacteriocins are proteinaceous, antimicrobial compounds produced by many kinds of bacteria. Attempts to harness these compounds to control *Listeria monocytogenes* in meats have taken two approaches: (*i*) Add the bacteriocin directly to the food in a purified or partially purified form. (*ii*) Add the bacteriocin-producing bacteria to the meat so they will grow and produce bacteriocins in situ. Some recent reviews summarize results of experiments using bacteriocins to control *L. monocytogenes* in foods and discussed modes of action of these compounds, factors affecting their effectiveness, and development of resistance in *L. monocytogenes* (*2,15,26*). In particular, Muriana (*26*) discusses the use of bacteriocins for controlling *L. monocytogenes* and includes some earlier references which are not cited in this report.

Bacterial Cultures. Since lactobacilli are known to produce many different bacteriocins and some are also used in starter cultures for sausage production, addition of these bacteriocin producers has been effective in reducing *L. monocytogenes* populations in many fermented meats (*8,12,14,30,37*). Some bacteriocinogenic strains do not grow well at refrigeration temperatures and thus may be more useful in controlling listeriae at temperature abuse conditions rather than in refrigerated storage (*4*). Other bacteria produce higher levels of bacteriocins at low temperatures (*5*). Bacteriocinogenic strains have also been used to control spoilage organisms (*20*).

Lactobacilli also produce lactic acid which acidifies the meat and, in some cases, antilisterial effects of lactobacilli have been traced to lactic acid rather than to bacteriocins (*18*).

Lactocin 705. Lactocin 905, produced by *Lactobacillus casei* CRL 705, exerted a moderate inhibitory effect on the growth of *L. monocytogenes* in minced beef slurry (*36*). Further experiments with sodium chloride, nitrite, and lactate added to minced beef demonstrated that these curing salts reduced the effectiveness of lactocin 905 (*35*).

Nisin. Nisin is currently being used for the preservation of some foods because of its GRAS status and well-known antilisterial effects. Several factors affecting the inhibitory activity of nisin were investigated in broth cultures (*28*) and a model was developed to predict possible effects in food systems.

Nisin is more effective in more acidic foods but *L. monocytogenes*, which has adapted to acidic conditions, becomes more tolerant of nisin (*34*). This tolerance, along with the development of nisin-resistant strains (*23*) and mutants (*27,29*) of *L. monocytogenes* may limit the effectiveness of nisin in some applications. One solution is the use of nisin in combination with another bacteriocin, e.g. leucocin F 10 (*28*) or with starter cultures of bacteria producing other antilisterial bacteriocins (*29*).

Recently, powders containing nisin and pediocin were produced from milk-based media and applied to food packaging materials (*25*). The bacteriocins did not diffuse through casings and packaging films and effectively inhibited listerial growth on meat surfaces.

In experiments with nisin used as a dip for meats, growth of *L. monocytogenes* on raw pork tenderloin (*11*), fresh ground pork (*27*), and cooked pork tenderloin (*10*) was inhibited. However, after a short time under aerobic conditions at 5°C, nisin-resistant listeriae started to

grow on the pork. Modified atmosphere packaging provided an additional hurdle and margin of safety.

Nisin also inhibited the growth of *L. monocytogenes* on beef steaks (*1*) and cubes (*6,40*). Although vacuum packaging alone did not prevent listerial growth on steaks, nisin added to the meat before vacuum packaging effectively suppressed the growth of *L. monocytogenes* for 4 weeks at 4°C (*1*). Inhibition of listerial growth on beef cubes was greater at refrigeration temperatures but even at room temperature growth was delayed for one day (*6*) This may afford some protection during short periods of temperature abuse. EDTA does not enhance the antilisterial activity of nisin on beef (*40*).

Other experiments indicated that a rinse with nisin reduced populations of *L. monocytogenes* attached to turkey skin and growth was further inhibited during refrigerated storage (*22*).

Pediocin AcH. Pediocin has strong antilisterial effects in culture with a lower minimal inhibitory concentration (MIC) than nisin A or Z (*24*). However, in meat such as ground pork, this bacteriocin reduces *L. monocytogenes* populations by as much as 2 logs within 24 hours (*19*) but it loses its effectiveness over time apparently due to its rapid degradation by meat proteases (*27*). Encapsulation of pediocin in liposomes or the addition of an emulsifier (Tween 80) increased its antilisterial effects in beef slurries (*7*). Pediocin can also be used in combination with other preservatives, such as diacetate, lactate and nitrite, to ensure greater inhibition of *L. monocytogenes* in turkey slurries (*31*). Pediocin and pediocin-producing cultures added to wiener exudates killed *L. monocytogenes* at both refrigeration and room temperatures (*38*). In addition, pediocin-producing bacteria, added as part of starter cultures for the production of chicken summer sausage, killed listeriae during fermentation (*3*).

One advantage of using pediocin in meats is its resistance to thermal degradation. It can be added to raw chicken and will retain its activity after the chicken is cooked (*13*). Pediocin containing powders have been produced and applied to food packaging films which inhibit the growth of *L. monocytogenes* on the surface of meat (*25*).

Reuterin. Reuterin (produced by *Lactobacillus reuteri*) is a broad spectrum antimicrobial agent which is water-soluble, effective over a wide pH range, and resistant to proteolytic and lipolytic enzymes. When added to the surface of cooked pork or mixed with ground pork, reduced populations of *L. monocytogenes* by 0.3 and 3.0 logs, respectively. Lactic acid enhanced the effectiveness of this bacteriocin (*9*).

Sakacin. Sakacin P, produced by *Lactobacillus sake* LTH 673, inhibits the growth of *Listeria ivanovii* and this inhibition is increased by high NaCl concentrations and a low pH (*12*). Sakacin K, produced by L. sake CTC 494, inhibited the growth of *Listeria innocua* in raw minced pork, poultry breast meat, and cooked pork. The greatest reduction in listerial populations occurred in meats packaged in vacuum or modified atmospheres (*17*). *L. sake* CTC 494 appears to be a very useful organism for sausage starter cultures because the temperature and pH conditions present during fermentation of dry sausages are ideal for sakacin K production (*21*).

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USE OF THERMAL PROCESSES TO CONTROL *LISTERIA* IN MEAT

Heat resistance of *Listeria monocytogenes* depends upon many factors including characteristics of different strains and serovars (*2,32,44*). Conditions known to affect the susceptibility of *L. monocytogenes* to thermal treatments include stage in the growth cycle, temperature during growth, and exposure to other stresses. Cells in stationary phase (*31*), those grown at higher temperatures (19 or 37°C) (*2,27*), and those previously exposed to stresses such as acid, ethanol, and hydrogen peroxide (*31*) are generally more resistant to thermal treatments. Thermotolerance is increased significantly after heat shock (30 min exposure to 48°C) in cells grown at 4°C (*26*) and tends to increase in cells grown at higher temperatures (*4,5,13,14*). Ranges of D values measured for *L. monocytogenes* in various types of meat are presented in a table at the end of this report.

Beef. In <u>raw ground beef</u>, higher concentrations of fat (30.5%) appear to protect *L. monocytogenes* from heat while higher concentrations of lactate enhance bacterial destruction by heat (*12*). In the production of <u>beef jerky</u>, *L. monocytogenes* populations are reduced during heating and marination and become undetectable after a 10 hour drying period (*23*). For production of <u>microwave-ready roast beef</u>, cooking in a bag was twice as effective as without the bag since *L. monocytogenes* could survive on beef surfaces which had been cooked for up to 45 min to a temperature of 62.8°C (*47*). Heating of <u>beef loin chunks</u> for 16 min at 85°C reduced *L. monocytogenes* populations by as much as 4 logs. However, some cells survived and might be able to grow under appropriate conditions (*9*). In a process simulating <u>sous vide</u> preparation of <u>cooked beef</u>, with slow heating, *L. monocytogenes* was killed as efficiently by the slow heating process as by faster heating. the reason for this difference from tests in pork (*30,40*) appears to be the low pH of 5.64 of the beef (19).

Pork. *L. monocytogenes* is more heat resistant when mixed with raw ground pork than when suspended in broth medium (*40*). Addition of soy hulls to ground pork further protects listeriae from heat (*38*). When pork inoculated with *L. monocytogenes* is heated slowly, the thermal tolerance of these bacteria is much greater as compared to bacteria in pork heated rapidly (*30,40*).

Cured meats. Investigations with beaker sausage demonstrated that heating the sausage to an internal temperature of 62.8°C was required to completely inactivate *L. monocytogenes* (*17*). Heating pepperoni at 51.7°C for 4 hours after drying destroyed listeriae but heating before drying was insufficient to eliminate the bacteria (*17*).

Curing agents (usually a mixture of sodium chloride, sodium nitrite/nitrate, dextrose, etc.) protect *L. monocytogenes* in various types of sausage, ham, bologna and other cured meats from thermal destruction (*13,29,32,43,48,49*). When curing ingredients were considered separately, all except sodium nitrite and sodium erythorbate enhanced listerial thermotolerance in ground pork (15% fat) (*48*). Addition of κ-carrageenan to cured ground pork lessened the protective effects of curing salts (*49*).

While many thermal processing treatments are very effective in killing foodborne pathogens, high temperatures or prolonged heating may alter some sensory characteristics of foods. Therefore, research is underway to determine appropriate combinations of heat and high

pressure treatments (*1,28,36,45*), irradiation, bacteriocins, or other antimicrobials (*46*) to produce safe and more organoleptically acceptable foods.

D value ranges (min) for thermal inactivation of *L. monocytogenes* in different meats

Meat	D values at 60°C	Reference(s) number
ground beef - raw	0.24 – 12.53*	3,11,25,32
ground beef - cooked	6.27 – 8.32	15
ground chicken - raw	5.6 – 8.7	32
ground chicken - cooked	5.02 – 5.29	15
ground pork - raw	4.3 – 9.2 (62°C)	30
ham	1.82	5
sausage	7.3 – 9.13	2,40
sous vide beef	6.4 – 7.1	19
roast beef	1.625	18
beaker sausage		43

*Variability related to differences in strains, pH, log vs stationary phase cells, heating rate.

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USE OF IRRADIATION TO CONTROL *LISTERIA* IN MEAT

Irradiation can damage and destroy most foodborne bacteria including *Listeria monocytogenes* (Lm). (See reference 6 for a recent review.) Irradiation dosage, expressed in kiloGrays (kGy), is a function of the energy of the radiation source and the time of exposure. Effectiveness of a given radiation dose varies depending on the density, antioxidant levels, moisture, and other components or characteristics of the foods. External factors, such as temperature, the presence or absence of oxygen, and subsequent storage conditions also influence the effective-ness of radiation. A split dose application of irradiation increased the radiosensitivity of Lm to irradiation under some conditions(*1*).

Different isolates of Lm exhibit some variation in resistance to irradiation. Under similar experimental conditions, the range in D_{10} values in: (a) culture media was 0.28-0.34 kGy (*11*); (b) mechanically deboned chicken meat was 0.41-0.53 kGy (*11*); (c) minced raw chicken was 0.48-0.54 kGy (*15*); (d) ground beef was 0.5-1.0 kGy (*3*); (e) ground pork was 0.42-0.64 kGy (*19*). *Listeria innocua*, a nonpathogenic species, is similar to Lm in its sensitivity to irradiation and so may be used for the safe evaluation of irradiation processes for different meats (*13*).

In nearly all experiments, *Salmonella* and *Listeria* proved to be more resistant to irradiation than *E. coli, Arcobacter, Campylobacter, Yersinia,* and *Staphylococcus* (*5,6,7,8,14, 22*). *Listeria* and *Salmonella* appear to have a similar susceptibility to irradiation; in some experiments, Lm has a larger D_{10} value while in other cases, Salmonella appears to be more resistant (*4,6,7,8,9,22*).

Irradiation of Lm in laboratory media offers some useful preliminary information but Lm is significantly more resistant to irradiation in meats than in culture media (*2,3,10,11,12,13,15*). However, neither the fat content of the meat (*14*) nor the source (beef, chicken, lamb, pork, turkey breast, turkey leg) of raw meat (*12,22*) had a significant effect on D values for irradiation.

Factors which do affect the effectiveness of a radiation dose in meat include cooking, concentration of bacteria in the meat, and temperature during irradiation. Lm added to raw turkey nuggets was more susceptible to irradiation than that added to cooked turkey nuggets (*23*). At lower temperatures, the radiation resistance of Lm increased (*2,12,20*). With larger concentrations of Lm in solution or on meat, larger doses of radiation are required to destroy the cells (*2,16*). Therefore, if food is highly contaminated, the usual radiation dose may not kill all the Lm and, as several researchers reminded us, Lm can grow in the cold and surviving and damaged cells may begin to multiply if the irradiated meat is stored under refrigeration (*10,24*).

Heat treatments as in sous vide processing (*9,17,18*) and modified atmosphere packaging (*7,21,24*) have been found to enhance the safety of irradiated foods. In addition, salt, nitrites, and other compounds added to preserved meats may increase the effectiveness of a radiation dose: Lm is more radiation-resistant in uncured pork than in ham (*4*). These additives may act by amplifying the kill by irradiation or by preventing the repair and growth of damaged, surviving cells. However, there has been very little published research on the effects of irradiation on cured and processed meats.

Some recommended doses of irradiation include: (a) 3 kGy for elimination of 10^3 cells Lm/g in air-packed frozen chicken (*12*); (b) 2.5 kGy to kill $10^{4.1}$ Lm/g in ground beef (*14*); (c) 2 kGy to destroy 10^4 Lm in mechanically deboned chicken meat at 2-4°C (*11*).

Food processors should be aware that various food additives and changes in processing parameters may affect the effective-ness of a radiation dose and that any surviving *Listeria* may grow to dangerous levels during storage at refrigeration temperatures if some other hurdle(s) to growth are not present. In addition, only a few types of plastic wraps and packaging are approved for use in irradiating packaged foods.

Irradiation has been approved by the FDA (*25,26*) for the purpose of microbial disinfestation of:

fresh or frozen uncooked poultry	to a limit of	<3.0kGy
pork carcasses and meat (for Trichinella)	"	<1.0kGy
packaged meat for NASA flights	"	<44kGy
fresh or frozen red meat	"	4.5kGy (fresh)
		7 kGy (frozen)

Irradiation of red meat (not including processed ready-to-eat meats) was approved by the FDA in December, 1997 and the recommended procedures for irradiating meat have been published by the USDA in the Federal Register. (26) Since the periods for comments on these procedures has been extended, the final rules have not been published as yet (July 1, 1999).

A number of individual European countries have regulations in place permitting (or in some cases prohibiting) irradiation of foods under specified conditions. The European Community is at this time working to establish a common set of guidelines.

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USE OF MODIFIED ATMOSPHERE PACKAGING TO CONTROL *LISTERIA* IN MEAT

Packaging of meats in modified atmospheres (MAP) containing low oxygen and/or high carbon dioxide levels can suppress the growth of foodborne pathogens as well as extend shelf life and preserve food quality. Several review papers discuss the advantages and disadvantages of various MAP systems with respect to the gases used, types of foods and packaging materials (*8,13,30*), effects on *Listeria monocytogenes* (*7,30*), and effectiveness of the combined use of MAP and irradiation (*23*). In addition, models have been developed to predict the growth of *L. monocytogenes* in culture media containing: (a) different concentrations of carbon dioxide (0–100%) and sodium chloride (0.5–8%) at pH 4.5–7.0 and 4–20°C (*15*); (b) carbon dioxide (10–90%) at pH 5.5–6.5 and 4–10°C (*12*); and (c) anaerobic nitrogen atmosphere with sodium chloride (0.5–4.5%) and sodium nitrite 50–1000 μg/ml at pH 6–7.5 and 5–37°C (*4*). Predicted listerial growth rates from one model were in good agreement with observed growth in chicken nuggets and raw and cooked beef (*15*). However, growth rates of *L. monocytogenes* on raw chicken were greater and on raw pork were much greater than those predicted by the model.

Results of numerous studies on the efficacy of different MAP systems in suppressing the growth of *L. monocytogenes* on different meats have been published in the past decade. However, data are not always consistent. This may result from variations in fat content and acidity of foods, storage temperatures, and the presence of other preservatives. MAP containing high levels of CO_2 effectively inhibit growth of *L. monocytogenes*, particularly at low temperatures. However, *L. monocytogenes* does grow in the absence of oxygen and has been observed to multiply on vacuum packaged meat at pH >6. One general concern about MAP is that some atmospheres may inhibit spoilage bacteria but not significantly suppress *L. monocytogenes* or *Clostridium botulinum.* Therefore, after an extended period of refrigerated storage, the meat may appear to be unspoiled and safe to eat but, in fact, it harbors high levels of these pathogens (*7,30*).

A brief summary of recent experimental results follows. Parameters that appeared to affect results are noted but original papers should be consulted for full experimental details.

Raw poultry. Storage temperature and carbon dioxide and oxygen levels in MAP significantly affect growth of *L. monocytogenes* on raw minced chicken (*33*), minced turkey (*32*), and turkey slices (*24*). An atmosphere containing 75% CO_2 inhibited growth at 4, 10, and 27°C but the addition of just 5% oxygen allowed growth at all of these temperatures (*33*). However, the presence of 60 or 80% oxygen prevented growth of *L. monocytogenes* at 1°C (*24*). Although irradiation (2.5 kGy) of ground turkey drastically reduced numbers of *L. monocytogenes*, surviving cells were able to grow at 7°C under atmospheres containing no oxygen and ≤64% CO_2 (*32*).

A lactate buffer, pH 3.0, combined with an atmosphere of 90% CO_2 inhibited growth of *L. monocytogenes* on chicken legs for nearly two weeks (*34*). Lactate by itself suppressed growth for about a week while the MAP alone suppressed growth for 2–4 days.

Cooked poultry. Temperature was also very important in limiting the growth of *L. monocytogenes* on cooked chicken breast (*4,6*), precooked chicken nuggets (*26,27*), chicken loaves (*18*), poultry cuts (*4*), and turkey roll slices (*14*). Despite vacuum packaging or atmospheres containing as much as 80% CO_2 and no oxygen, *L. monocytogenes* was able to grow on cooked poultry at temperatures between 6.5 and 11°C (*3,4,6,18,26,27*). At lower temperatures (6.5–7°C), MAP and the presence of lactate slowed the growth of *L. monocytogenes* somewhat even though they were not able to completely inhibit it (*3,6,18*). At 4°C, 70% CO_2 levels and vacuum packaging did suppress the growth of *L. monocytogenes* for 28 days in turkey roll slices (*14*) and chicken breast (*6*).

Raw pork. A study of the incidence of contaminated pork loins and Boston butts packaged in MAP revealed that very few butts were contaminated with *L. monocytogenes* while loins packaged under vacuum or in an atmosphere of 66% oxygen, 8% nitrogen, and 26% CO_2 had fewer contaminants than those packaged in air (*29*). Vacuum packaging did not prevent the growth of *L. monocytogenes* on hot or cold packed pork loin (*22*) or pork chops (*25*). Neither did vacuum packaging or a modified atmosphere (25% CO_2 : 75% nitrogen) prevent the growth, in ground pork, of listeriae injured by heat (*20*) or irradiation (*16*).

At 4°C, an atmosphere of 100% CO_2 did inhibit the growth of *L. monocytogenes* on raw pork tenderloin (*11*). Addition of nisin to pork tenderloin significantly suppressed growth of listeriae under both air and MAP (100% CO_2 and 80% CO_2 : 20% air) at both 4 and 20°C (*11*).

Cooked pork. *L. monocytogenes,* inoculated along with *Pseudomonas fragi,* on cooked pork tenderloin and grew as well under modified atmospheres (100% CO_2 and 80% CO_2 : 20% air) as in air at both 4 and 20°C (*10*). Nisin solutions, used as 20 min dips for pork, prevented growth of *L. monocytogenes* under air and MAP at both temperatures.

Raw beef. Saturated carbon dioxide packaging but not vacuum packaging suppressed the growth of *L. monocytogenes* on beef steaks stored at 5 and 10°C for 3–6 weeks (*2*). Further experiments demonstrated that when contaminated steaks, which had been stored under a saturated carbon dioxide atmosphere at 1.5°C, were removed from storage and kept at 12°C (gross temperature abuse), *L. monocytogenes* still failed to grow or grew extremely slowly (*1*).

Although vacuum packaging alone was insufficient to prevent listerial growth in ground beef stored at 4°C for 9 weeks, the addition of *Lactobacillus alimentarius* L-2 to the beef caused about a 2 log decline in final numbers of *L. monocytogenes* (*19*). Since

these lactobacilli do not produce bacteriocins, their inhibition is believed to be due to the production of lactic acid.

Cooked beef. Vacuum packaging of roast beef slices failed to prevent growth of *L. monocytogenes* at –1.5°C (*17*) or 3°C (*17,28*). A saturated carbon dioxide atmosphere caused *L. monocytogenes* populations to decline at –1.5°C and lengthened the lag phase at 3°C so that by the time *L. monocytogenes* grew the meat already appeared spoiled (*17*).

Cured meats. Tests with atmospheres containing 20, 30, 50 or 80% CO_2 demonstrated that only the highest carbon dioxide level was sufficient to inhibit growth of *L. monocytogenes* on frankfurters at both 4.7 and 10°C (*21*). An atmosphere with 50% carbon dioxide inhibited listerial growth only at the lower temperature. Neither vacuum packaging nor an atmosphere with 30% CO_2 : 70% nitrogen inhibited listerial growth on ham or lunch meat at 7°C (*4*).

Other uncured meats. Experiments with raw lamb pieces and mince demonstrated that listerial growth at 5°C was suppressed by an atmosphere of 100% CO_2 but not by atmospheres of 50% CO_2 : 50% nitrogen or 80% oxygen : 20% CO_2. Vacuum packaging was effective in preventing growth of listeriae in lamb mince but not in pieces of lamb (*31*). An atmosphere of 80% oxygen : 20% CO_2 was insufficient to prevent the growth of *L. monocytogenes* in lamb meat juice at 4°C (*9*).

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USE OF HIGH PRESSURE TO CONTROL *LISTERIA* IN MEAT

High hydrostatic pressure causes widespread damage to cells with adverse effects on membranes, enzymes and other structures and molecules (*7*). *Listeria monocytogenes* is sensitive to high pressure treatments of 400-500 MPa but like other Gram positive organisms (such as *Staphylococcus aureus*) is one of the more resistant species of bacteria. Some strain variation in sensitivity to pressure is evident at lower temperatures (25°C) but largely disappears at 50°C (*2*). Bacterial spores are very barotolerant, requiring pressures as high as 1000 MPa to destroy them (*5,13*).

With current interest in minimally processed foods, high pressure treatment has become a more attractive technique because of its minimal effect on the characteristics of the final product. The effects of high pressure are instantaneously and uniformly transmitted throughout foods regardless of their geometry or size. Although high pressure destroys living cells, it does not degrade small molecules like vitamins and flavors and (under the conditions tested) has minimal effects on the sensory quality of meats (*3,8*). One disadvantage is the difficulty in completely sterilizing foods; pressure destruction curves usually demonstrate some tailing and damaged but viable cells may recover and start growing during storage (*10,13,16*). Nevertheless, several pressure-treated foods are currently being marketed including fruit juices and jams and raw squid.

According to experiments with *L. monocytogenes* and *L. innocua* in laboratory media, several factors affect the lethality of a given level of high pressure. Modest increases in temperature (from 25 to 50°C) decreased D values from 50.8 to 22.4 min at 137.9 MPa and from 14.3 to 1.3 min at 344.7 MPa (*1,2*). Therefore, a 7 log kill could be achieved by exposure to 345 MPa pressure for approximately 9 minutes at 50°C. Increased acidity also enhances the effect of pressure. At 45°C and 252 MPa for 30 min, an 8 log kill of *L. monocytogenes* occurred at pH 4.0 and only a 2 log kill at pH 6.0 (*14*). Addition of the bacteriocin, pediocin AcH, also increases the effectiveness of high pressure: an 8 log reduction in *L. monocytogenes* cells was achieved in only 5 min at 345 MPa in the presence of pediocin (*6*). Compounds similar to those in foods (bovine serum albumin, glucose, and olive oil) exert a protective effect on *L. monocytogenes* as indicated by larger D values (*11*).

Experiments with minced beef, chicken, and pork and with pork chops inoculated with *L. monocytogenes* or other bacteria have confirmed the increased lethality caused by a moderate rise in temperature (*4,8,10,12*) and the presence of bacteriocins (*15,17*). Generally, somewhat higher pressures (400-500 MPa) were required to achieve a useful kill rate in a reasonable length of time in meat as compared to laboratory media. Some representative D values are presented in the table below.

D values (min) in meat treated with high pressure

Bacteria*	Pressure	Temp. (°C)	Meat	D value	Ref. #
L. monocytogenes	414 MPa	25	ground pork	4.17	8
L. monocytogenes	414 MPa	50	ground pork	0.63	8
L. monocytogenes	400 MPa	4	pork chop	3.52	9
L. monocytogenes	414 MPa	25	pork chop	2.17	3
S. typhimurium	414 MPa	25	pork chop	1.48	3
L. monocytogenes	375 MPa	18	raw chicken	5.0	12
L. monocytogenes	375 MPa	18	cooked chicken	9.2	12
L. monocytogenes	375 MPa	18	raw minced beef	4.9	12
L. monocytogenes	375 MPa	18	cooked minced beef	9.4	12
L. innocua	330 MPa	20	ground beef	6.5	4

*L. = *Listeria; S. = Salmonella*

It should be emphasized that effectiveness of high pressure treatments depend on temperature, length of exposure, and pressure intensity as well as the strain of Listeria used and various ingredients in different foods.

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USE OF PULSED ELECTRIC FIELDS AND ELECTROLYZED OXIDIZING WATER TO CONTROL *LISTERIA*

Pulsed electric field (PEF) pasteurization is a non-thermal process which destroys contaminating bacteria by a short bursts (< 1 sec) of high voltage. Exposure to PEF destabilizes cell membranes and with sufficient intensity and duration of treatment, membranes are irreversibly damaged, important cellular compounds leak out, and cells die (*2,6*). At lower PEF doses, these effects on cell membranes have been exploited by genetic engineers to induce hybridization of cells and introduction of DNA fragments into cells (*2*).

Bacterial spores, Gram positive cells (including *L. monocytogenes*), and cells in stationary phase of growth are more resistant to the effects of PEF (*1*). For *L. monocytogenes* suspended in milk, a continuous flow PEF system resulted in a 3 log reduction in bacterial numbers at 25°C and a 4 log decrease at 50°C (*5*). A model of microbial survival after exposure to PEF has been developed (*3*).

As yet this new technology has been applied primarily to liquids such as juices, milk, yogurt, beaten eggs, sauces, and soups (*4*). A PEF system has also been used to destroy *E. coli* in a homogeneous semisolid medium (potato dextrose agar) (*8*). Pumpable food pastes such as vegetable or fruit purées and minced meat are also possible candidates for this type of pasteurization (*1*). Bacteria in dry powders (flour, spices), however, appear to be less susceptible to PEF compared to those in liquids (*2*). Further research is needed to determine the potential for use of PEF for the pasteurization of viscous and particulate foods.

Electrolyzed oxidizing water (EO water) is acidic water (≤ pH 2.7) collected from the anode during electrolysis of deionized water containing a low concentration of NaCl. Tests demonstrated that a 2 min exposure of *L. monocytogenes* to EO water at 35°C resulted in ≥ 7 log kill (*7*). This process is still in early developmental stages but may have future applications in food processing.

An excellent review of the methods of PEF and its prospects for use in food processing is presented by Barsotti and Cheftel (*1*).

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USE OF ULTRAVIOLET LIGHT TO CONTROL *LISTERIA* IN MEAT

Although the bactericidal effects of ultraviolet light have long been utilized to control microbial contamination in some medical and food industry areas, it is only recently that techniques using UV to reduce the microbial load on foods, such as the surfaces of meat, have been developed. Since UV light cannot penetrate into foods, only microbes on an exposed surface are susceptible to its effects. Bacteria on a smooth surface such as agar plates in laboratories or flat plate beef absorb more UV light than bacteria on a rough surface such as some cuts of beef, pork, or chicken skin (*3,5,6*). Therefore, the UV exposure required for effective killing of bacteria on meat will most likely exceed that required for killing cells on laboratory media.

Studies have shown that UV exposure does not have a deleterious effect on the color of meat nor does it cause oxidative rancidity (*3,5*). This is because UV light does not induce production of oxidizing free radicals. Rather, the toxicity of UV light is primarily due to the formation of thymine dimers which disrupt the structure and functioning of DNA in bacterial cells.

Experiments with *Listeria monocytogenes* demonstrated that cells in a moist environment were killed more easily than those in a dry film or crust (*7*). In addition, shorter wavelengths (254 nm) were more effective than longer wavelengths (365 nm) of UV light (*7*). In the presence of psoralen compounds (from parsley, limes, celery, etc.), longer wavelengths of UV can kill *L. monocytogenes* and other bacteria (*4*).

A recent innovation which greatly increases the peak power in the UV light source is the pulse power energization technique (PPET). PPET light sources operating a 1 pulse/second kill *L. monocytogenes* on an agar surface much faster than a continuous light source and can reduce cell populations by 6 logs in a 512 μs (*1*). PPET sources can be developed to operate at 100-1000 pulses/sec and these high energy sources may be practical for disinfecting meat surfaces.

A comparison of susceptibility of foodborne pathogens (grown on agar plates) revealed that *L. monocytogenes* was the most resistant to UV light (*2*): *L. monocytogenes> Staphylococcus aureus≥ Salmonella enteritidis> E. coli> Bacillus cereus*.

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USE OF ULTRASOUND TO CONTROL *LISTERIA* IN MEAT

The lethal effects of ultrasound have been known since sonar was developed to detect submarines and nearby fish were killed. Ultrasound kills by disrupting cell membranes apparently as a result of the formation and subsequent implosion of small bubbles (cavitation). Heat and some chemicals may enhance the lethal effects of ultrasound.

Currently, ultrasound is used in food processing for emulsification, accelerating freezing and cleaning (*1*). Some recent investigations have focussed on the possible uses of ultrasound for the destruction of foodborne pathogens. Because viscous liquids and solids impede the propagation of ultrasound waves, this technique is potentially most useful for sterilization of liquids, such as milk and juices. At some future time, ultrasound, in combination with other preservation methods, may be useful in surface sterilization of other foods. Therefore, some recent research papers describing the effects of combinations of ultrasound and high pressure and/or heat on *L. monocytogenes* in liquids have been listed below (*2,3,4*).

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