OVERVIEW: FOOD SAFETY AND FOOD QUALITY CONTROL - CONTROLING *LISTERIA MONOCYTOGENES* HAZARD

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Abstract

Many hazards cause foodborne illness. When contaminated food is eaten, hazards such as bacteria, viruses, chemicals, or foreign objects can make people ill. In the past two decades, serious outbreaks of foodborne illness have been caused by a bacterial hazard known as Listeria monocytogenes.
By using different preservation methods, foods can become complex eco-systems and experience has shown that interactions among known and unknown hurdles can provide stability against growth of L. monocytogenes.
Factors of significance in this respect can be modified atmosphere, smoke ingredients, bacteriocins, bacterial competition, available nutrients etc.
Key words: *Listeria monocytogenes,* hazard, antimicrobial alternatives

Introduction
L. monocytogenes as microbiological hazard

L. monocytogenes is a facultative intracellular bacterial pathogen of both human and animals and represents one of the most concern microbiological hazards associated with food products (*Table 1, 2* and *3*).

The capability of *Listeria monocytogenes* to grow at temperatures between 4ºC and 10ºC and to endure salt concentrations of up to 12% and *p*H approaching 4.5 makes it a ubiquitous germ in nature. This bacterium occurs widely in both the agricultural (soil, vegetation, silage, faecal material, sewage, water) and food processing environment.

The bacterium is resistant to various environmental conditions such as high salt or acidity, grows at low oxygen conditions and refrigeration temperatures, and survives for long periods in the environment, on foods, in the processing plant, and in the household refrigerator. Although frequently present in raw foods of both plant and animal origin, it also can be present in cooked foods due to post-processing contamination.

Table 1: Microbial hazards typically associated with foods

Listeria monocytogenes	*Salmonella spp.*
Brucella abortus	*Shigellae*
Campylobacter jejuni	*Mycobacterium bovis*
Clostridium botulinum	*Staphylococcus aureus*
Clostridium perfringens	*Vibrio parahaemolyticus*
Coxiella burnettii	*Vibrio vulnificus*
Escherichia coli	*Yersinia enterocolitica*
Entheropathogenic E. coli	Toxin-producing mould
Bacillus cereus	

Table 2: L. monocytogenes concerns (Sofos, 2005)

Survival in adverse environments	Better than other nonspore-formers
Psychrotroph	Grows at refrigeration temperatures
Stress (acid, cold and starvation) adaptation	Hardening; cross-protection; resistance
Plant Environment	Colonize, multiply, persist; attachment and biofilms; drains, conveyors, floor mats, foot baths, freezers, coolers, equipment, chilling rooms, cutting rooms, hands, packaging
Major Concern	Post-processing contamination

Table 3: Survival of *L. monocytogenes* in the environment (Sofos, 2005)

Substrate	Temperature (°C)	Days
Soil	4-6	12 to >720
Water	2 to 37	< 7 to 928
Animal feed	4 to 22	23 to 2190
Fecal material	5 to 56	35 to 2190

Therefore, because of its ability to proliferate under of different environmental conditions contributes significantly to its hazard status (*Table 4*).

L. monocytogenes has been isolated in such foods as raw and pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types) and raw and smoked fish.

Unfortunately, *L.monocytogenes* is the causative agent of listeriosis (implicated in meningitis, rhombencephalitis, and septicemia), which leads to death in 25 to 30% of reported cases.

Even when *L. monocytogenes* is initially present at a low level in a contaminated food, the organism can multiply during storage, including storage at refrigeration temperatures when the food supports growth.

L. monocytogenes causes illness by penetrating the lining of the gastrointestinal tract and then infecting normally sterile sites within the body. The likelihood that *L. monocytogenes* will invade the intestinal tissue depends upon a number of factors, including the number of organisms consumed, host susceptibility, and virulence of the specific isolate ingested.

There is evidence to suggest that it is a transitory resident of the intestinal tract in humans, with 2 to 10% of the general population being carriers of the organism without any apparent health consequences.

All strains of *L. monocytogenes* appear to be pathogenic but their virulence, as defined in animal studies, varies substantially.

Table 4: The severity of different types of microbiological hazards (Brown and Stringer, 2002)

Severe hazards	*Clostridium botulinum* types A, B, E and F; *Shigella dysenteriae; Salmonella typhi and paratyphi A, B; E.coli* O157:H7; *Brucella abortis; Brucella suis; Vibrio cholerae; Vibrio vulnificus*
Moderate hazards – extensive spread from infection	; *Salmonella* spp.; *Shigella* spp.; enterovirulent *E. coli* (EEC); *Streptococcus pyogenes*
Moderate hazards – limited spread	*Bacillus cereus;* potentially toxigenic bacilli; *Campylobacter jejuni, Clostridium perfringens; Staphylococcus aureus, Vibrio parahaemolyticus; Yersinia enterocolitica*
Other microbiological hazards	These include naturally occurring toxicants such as mycotoxins (e.g. aflatoxin), scombotoxin (e.g. histamine)

Listeriosis is an opportunistic infection that most often affects those with severe underlying disease, pregnant women, unborn or newly delivered infants and the elderly. The bacterium most often affects the pregnant uterus, the central nervous system or the bloodstream, and manifestations of listeriosis include but are not limited to bacteremia, meningitis, encephalitis, endocarditis, meningoencephalitis, miscarriage, neonatal disease, premature birth, prodromal illness in pregnant women, septicemia and stillbirth.

Incubation periods prior to individuals becoming symptomatic can be from a few days up to three months and the minimum dose considered to cause disease is less 10^3 cells (table 5).

Table 5: Correlation between minimum dose considered to cause disease and criteria set for end-products (Brown and Stringer, 2002)

Pathogenic organism	Minimum dose considered to cause disease	Probability of infection from exposure to 1 organism	General end-product criteria used*
Infectious organism			
Shigella	1	1.0×10^{-3}	Absence/25 g
Salmonella	1	2.3×10^{-3}	Absence/25 g
Campylobacter	1-10	7.0×10^{-3}	Absence/25 g
Listeria monocytogenes	$> 10^3$		$< 100/g$
Vibrio parahaemolyticus	$>10^4$		$< 10^3/g$
Toxico-infectious organisms			
Clostridium perfringens	$>10^6$		$< 10^5 - 10^6/g$
Bacillus cereus	$> 10^6$		$< 10^5 - 10^6/g$
Organisms causing intoxication			
Staphylococcus aureus	$>10^6$		$< 10^5 - 10^6/g$

* Criteria for pathogenic organisms are not yet well established and they may differ from country to country. The validity of the criteria starts mostly after production and ends at the time of the consumption.

L. monocytogenes can also cause mild febrile gastroenteritis in otherwise healthy individuals. The public health significance of this type of listeriosis is much lower than that of invasive listeriosis. A general description of listeriosis is presented in table 6.

This broad based prevalence in the food system, together with a high mortality rate of listeriosis, suggests that *L. monocytogenes* represents an important hazard to human health that needs to be controlled.

During recent years, the incidence of listeriosis in most countries has not increased, and in a number of countries the incidence appears to have decreased. Apparent reductions in the baseline levels of listeriosis have been observed during the past several years. This likely reflects the efforts of industry and governments to:

(a) implement Good Hygiene Practice (GHP) and apply HACCP to reduce the frequency and extent of *Listeria* spp. in industrially processed foods,

(b) improve the integrity of the cold chain to reduce the incidence of temperature abuse conditions that foster the growth of *L. monocytogenes*,

(c) enhance risk communication, particularly for consumers at increased risk of listeriosis. However, further actions shall be taken to lower the risk of human listeriosis from food consumption world wide.

Table 6: Characteristics of *Listeria monocytogenes* as high microbilogical risk (Sofos, 2005)

Organism	non-spore-forming, gram-positive, rods, facultative, psychrotroph
Illness: *Listeriosis*	infection with many severe syndromes in sensitive individuals
Symptoms	*Non-invasive:* mild, flu-like gastroenteritis *Invasive:* meningitis, bacteremia, endocarditis, septicemia, peritonitis etc. *Pregnant women:* flu-like illness, crosses placenta, attacks fetus, abortion, stillbirth or acutely ill baby (most common in third trimester)
Incubation period	variable: >12 hours or from 3 days to 3 months
Duration of illness	short to long
Infective dose	unknown (possible >100 cells/g food)
Fatality rate	20-30%
Sources	soil, vegetation, humans, animals, water, silage, sewage, etc
Foods involved	raw milk, cheeses, coleslaw, ice cream, vegetables, luncheon meats, seafood
Control	sanitation, hygiene, proper cooking, prevent cross- or re-contamination, post-processing interventions

Antimicrobial alternatives for controlling growth of *Listeria monocytogenes*

There are significant variations in the national allowable tolerances for *L. monocytogenes*. Based upon the known characteristics of the microorganism and the disease some countries maintain a policy of „zero tolerance“for *L. monocytogenes* in ready-to-eat foods. Several countries have concluded that while a complete absence of *L. monocytogenes* (zero tolerance) may be a commendable goal, for certain foods it is an unrealistic and unattainable requirement that limits trade.

Some countries have set the tolerance for *L. monocytogenes* based on the type of food and "use by date" on the labels of the food. The levels of *L.*

monocytogenes associated with "unavoidable" contamination of these products are typically low, particularly if multiplication does not, or cannot, occur during storage, distribution and preparation.

These different approaches towards the management of *L. monocytogenes* may lead to trade barriers that can and should be avoided, if the foods do not endanger a country's appropriate level of protection.

For example, to prevent *Listeria* epidemics, the U.S. Food and Drug Administration advocate "zero tolerance" in ready-to-eat food products, whereas European rules recommend different levels, depending on the product.

To reach this objective, several inhibitory treatments were tested, such as gamma irradiation, organic acids, sodium lactate, sodium nitrite and monoglycerides, but they affected the flavor and the texture of the product. Furthermore, *L. monocytogenes* can survive in many cases, even when these agents were used in combination.

A promising way of preserving foods is through the use of bacteriocins or bacteriocin-producing lactic acid bacteria. Eficiency of bacteriocins produced *in situ* depends on initial levels of *L. monocytogenes*, storage temperature, bacteriocin concentrations, matrix product, and packaging.

Table 7: Antimicrobial alternatives for inhibiting *Listeria monocytogenes* (Sofos, 2005)

Processing Lethality Treatments:
• Heat Treatments
• Chemical Formulation Treatments:
Lactate and Diacetate
Post-Processing/Lethality Treatments:
• Physical Treatments
• Chemical Treatments
• Biological Agents:
Lactics and Bacteriocins
• Combinations
Physical Treatments:
• Heating:
- Pre-packaging steam
- Post-packaging:
Steam
Submersion in hot water
Radiant oven heat
- Consumer re-heating
• High Pressure Processing
• Irradiation

In table 7 are summarized some antimicrobial alternatives for inhibiting the *L. monocytogenes*.

Table 7: - continued Antimicrobial alternatives for inhibiting *Listeria monocytogenes* (Sofos, 2005)

Chemical Treatments:
• Sodium/Potassium Lactate/Lactic Acid; Sodium Diacetate; Sodium Acetate/Acetic Acid; Proprionate; Benzoate; Sorbate; Citrate • Nisin; Pediocin; Lysozyme • Acidic Calcium Sulfate • Essential Oils **applied as**: →Ingredients →Solutions: *Dipping* *Spraying* →Packaging films: nisin →Edible films and coatings →Encapsulated: nisin, lysozyme →Combinations

Salts of organic acids (primarily lactates) are approved for use as food ingredients and have been utilized traditionally to enhance the quality of cooked or cured meat products. Sodium acetate, sodium bicarbonate, sodium lactate, and potassium sorbate, all have generally recognized as safe (GRAS) status.

Examples of products in which sodium lactate has been incorporated at concentrations ranging from 1 to 5% include cooked beef, liver paté, frankfurters, fresh pork sausage, beef patties and cooked poultry.

Some of the major general findings on the nature and degree of antimicrobial activity of lactates have been included in *Table 8*.

Table 8: Lactates as decontaminants in meat and meat products (Smulders and Greer, 1998)

Nature of the antimicrobial effect:	• Largely unknown • Obviously related to the lactate ion, as: *-Sodium lactate exerts a bigger effect than sodium chloride* *-Sodium-, potassium- and calcium lactate produce similar effects* *-Calcium lactate has antimicrobial effects, whereas calcium carbonate, -chloride, -citrate and –phosphate have not* • At almost neutral pH the portion undissociated

Table 8. Continued	acid is too small to account for the antimicrobial effect • The small reduction in aw fails in explain the antimicrobial effect
Degree of antimicrobial effect:	• More pronounced for calcium lactate than for sodium- or potassium salts • Decreases with reduced fat content in sausages, possibly due to insolubility of fat (less fat→ more water→ dilution of lactate concentration) • Increases with decreasing temperatures • Increases with decreasing *p*H • Against *L. monocytogenes* increases with heat treatment and is highest at sterilization temperatures

With respect to the evaluation of the stability of foods against growth of *L. monocytogenes* is important for food manufacturers and food controlling authorities to follow some guidelines (table 9).

Table 9: Guidelines for evaluation of the stability of a product against growth of L. monocytogenes (FAO/WHO, 2003)

Stability achieved without limitation in shelf life	Freezing $pH < 4.5$ $pH < 5.0$ + chilled storage $a_w < 0.90$ $a_w < 0.92$ + chilled storage $a_w < 0.95 + pH < 5.5$
Stability achieved with limitation in shelf life	Lactate 2% + chilled storage (max. 4 weeks shelf life) Lactate 2% + nitrite 150 ppm + chilled storage (max. 5 weeks shelf life) Lactate 2% + glucone-delta-lactone + chilled storage (max. 5 weeks storage)

Conclusions

Raw meat and poultry, raw milk, raw seafood, and raw produce may contain *L. monocytogenes*, although the frequency of occurrence and the levels vary widely. Ingredients likely to contain *L. monocytogenes* should be handled as if they are contaminated.

The presence of a certain number of *L. monocytogenes* in foods is closely related to the stability of foods against growth of this pathogen.
Such stability can be achieved by the use of a combination of several hurdles (i.e.: organic acids and their salts, bacteriocins, storage temperature, pH, a_w,

modified atmosphere packaging etc.) which can inhibit the growth of *L. monocytogenes*. The application of this concept is named hurdle technology, barrier technology or food preservation by combined processes.

Therefore, in order to answer this question knowledge concerning intrinsic and extrinsic factors controlling the growth of *L. monocytogenes* in the product is necessary.

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