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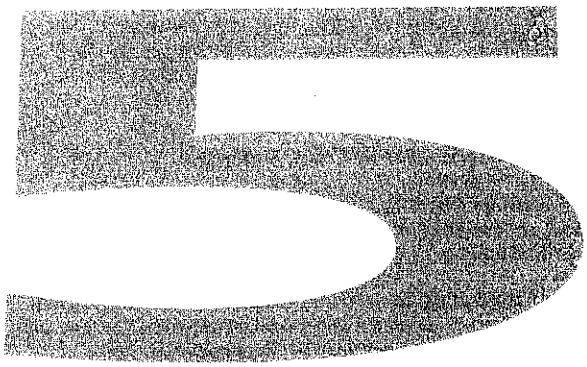
MICRO-ORGANISMS IN FOODS



CHARACTERISTICS OF MICROBIAL PATHOGENS

ICMSF

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Information in this reference supports the application of the research found in Ingham et. al (2010) and Borneman et al. (2009) to products that are stored under air.

p. 145 "The growth of *L. monocytogenes* is little affected by gaseous atmosphere."

p.302 "*S. aureus* grows both aerobically and anaerobically." Survival of *S. aureus* may be improved under anaerobic conditions, suggesting that aerobic storage is preferable for safety.

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mercial milk pasteurization treatment (71°C for 15 s). Resistance in cream is similar to that in skimmed milk, but higher D-values are observed in meats, including salami, and fat is reported to increase resistance (Fain *et al.*, 1991). Inocula grown at low temperatures yield cells having reduced heat resistance (Fedio and Jackson, 1989; Farber and Brown, 1990; Knabel *et al.*, 1990). Heat resistance is enhanced by 'heat shock' immediately before heating (Farber and Brown, 1990) or by prior growth at relatively high temperatures (Smith and Marmer, 1991; Smith *et al.*, 1991; Linton *et al.*, 1992). As expected, elevated levels of solutes increase heat resistance (Sumner *et al.*, 1991; Miller, 1992). A pH suboptimal for growth (particularly <7) may be expected to reduce heat resistance. For example, $D_{52°C}$ values in cabbage juice were 8.2–14.1 min at pH 4.6, but 20–34.5 min at pH 5.6 (Beuchat *et al.*, 1986). Farber and Pagotta (1992) reported enhanced heat resistance after acidification of cells in sterile whole milk prior to heating. Microwave heating chicken to 70°C and cooking beef to 'medium' did not prevent survival of *L. monocytogenes* (Coote *et al.*, 1991).

Irradiation

L. monocytogenes exhibits resistance to gamma irradiation of the same order as other Gram-positive vegetative bacteria (ICMSF, 1980), D-values ranging from 0.34–0.5 kGy in broth to 0.51–1.0 kGy in minced beef (El-Shenawy *et al.*, 1989b,c) and 2 kGy in ice cream at −78°C (Hashisaka *et al.*, 1989). D-values in chicken meat and ground beef have been reported at between 0.27 and 1.06 kGy (*Table 4a*). The results of irradiating whole chilled chickens naturally contaminated with *Listeria* spp. indicate that a dose of 2.5 kGy substantially reduces numbers, but does not completely eliminate the bacteria (Mead *et al.*, 1990; Lewis and Corry, 1991). *L. innocua* was eliminated, but not *L. monocytogenes* (Lewis and Corry, 1991), indicating that *L. innocua* would not be a suitable indicator for the presence of *L. monocytogenes* in irradiated food. An absorbed dose of 3 kGy was not sufficient to eliminate *L. monocytogenes* from vacuum-packed pork (Lebepe *et al.*, 1990).

From the very limited data available (*Table 4b*), *L. monocytogenes* is less resistant to ultraviolet light than many other Gram-positive vegetative bacteria (ICMSF, 1980). Dry cells are 2.5–4 times more resistant than moist cells.

Water activity

L. monocytogenes has a lower a_w limit for growth of approximately 0.90 at 30°C when glycerol is used to control a_w (Farber *et al.*, 1992). Limits of 0.92 and 0.93 have been reported using NaCl and sucrose, respectively (Farber *et al.*, 1992; Miller, 1992). In a meat system (without added solutes) a limit for growth of 0.93 at 20°C was determined by Chen and Shelef (1992). These limits are similar to those for other Gram-positive bacteria (ICMSF, 1980).

Subjection to low water activities at low temperature (4°C) enhanced the bacteriostatic effect (Tapia de Daza *et al.*, 1991). Solutes are best tolerated at 15–30°C (Farber *et al.*, 1992).

L. monocytogenes survived 40 days' storage at 25°C in seafood chowder of low moisture content (2.0–2.35%) (Sikes, 1989).

pH

L. monocytogenes has a broad pH range for growth, the upper limit being about pH 9.2 and the lower pH 4.6–5.0.

Disinfectants

In the absence of organic matter, a wide variety of disinfectants is effective *in vitro* against *L. monocytogenes*, including sodium hypochlorite, iodine, peroxide and quaternary ammonium compounds. Hypochlorite is inactivated by organic matter and decontamination of vegetables requires levels of at least 200 ppm chlorine. *L. monocytogenes* is much more resistant to disinfectants on dry surfaces compared with wet (Best *et al.* 1990).

Effect of atmosphere

The growth of *L. monocytogenes* is reported to be little affected by gaseous atmosphere. Similar generation times have been observed under aerobic, microaerophilic and anaerobic conditions

with no evidence of an inhibitory effect exerted by high levels of CO_2 except at low temperatures (Ingham *et al.*, 1990). Considering a wider range of growth conditions, there is a noticeable difference between the rates of growth in shaken and anaerobic cultures (Buchanan *et al.*, 1989; Buchanan and Phillips, 1990). At pressures of between 3000 and 4000 atmospheres (1 atmos. $= 14.7\ lb/in^2 = 1.033\ kg/cm^2$), D-values of between 100 and 10 min are reported (*Table 6b*).

Interactions, including preservatives

L. monocytogenes is a robust microorganism in terms of its ability to survive mild heating and grow under the environmental conditions commonly found in a wide range of foods. If it survives a heat process, or should it reach food as a post-process contaminant, its multiplication is best controlled by factors in combination. The effects of combinations of factors on the growth response of *L. monocytogenes* is well illustrated in a number of publications (Connor *et al.*, 1986; Buchanan *et al.*, 1989; McClure *et al.*, 1989, 1991; Buchanan and Phillips, 1990; Cole *et al.*, 1990; Wijtzes *et al.*, 1993). The effects of selected preservatives are summarized in *Tables 5a–f.*

Control

The prevention of human listeriosis begins at the farm and continues through processing to the selection and handling of foods by the consumer. *L. monocytogenes* is ubiquitous in the agricultural environment and a complete normal diet that is totally free of *L. monocytogenes* is therefore impossible to obtain. However, the application of controls can reduce the risk of foodborne listeriosis. This is a classical case whereby HACCP should be applied from farm to consumer to minimize the risk of foodborne illness.

Farm

Silage production should be controlled to achieve rapid acidification of the silage to pH <4.0, which prevents the development of high numbers of *L. monocytogenes*. This is particularly important with respect to silage that is to be fed to dairy cattle, because the milk produced may later be used unpasteurized in the manufacture of raw-milk cheeses. Milk should be stored at low temperatures (e.g. $<5°C$) on the farm until transportation to the dairy plant.

Processing

Foods have been placed into four categories (WHO, 1988):

1 Raw foods (e.g. raw vegetables and meats).
2 Processed raw foods not treated listericidally by heating (e.g. coleslaw, fermented sausages, raw-milk cheeses).
3 Processed foods treated listericidally by heating but subjected to potential recontamination during subsequent handling (e.g. certain cheeses and commercially processed meats that are sliced or altered after thermal processing).
4 Processed foods treated listericidally by heating while in an intact package (e.g. cooked ham) or which are aseptically packaged immediately after listericidal treatment (e.g. certain dairy products).

Particular emphasis in control should be placed upon foods identified as of concern through outbreak investigation, prospective epidemiological studies and data demonstrating the multiplication of *L. monocytogenes* (NACMCF, 1991). Attention should be given to the conditions of, for example, producing soft cheeses and pâté and to the maintaining of good hygiene during the slicing of meat products.

Processing plants should base their control programmes on the HACCP concept. Three major objectives must be pursued. The first is to minimize the multiplication of *L. monocytogenes* in raw materials, particularly before and during the processing of raw foods (Category 2 above). The second is to use listericidal processes that assure the destruction of *L. monocytogenes* (Categories 3 and 4 above). The third is to minimize the risk of recontamination of ready-to-eat foods that are further processed after receiving listericidal treatment (Category 3 above). Owing to the prevalence of *L. monocytogenes* in raw materials and its ability to multiply in the environment of many food-processing facilities, traditional cleaning and disinfection methods, equipment design and management practices may be inadequate or even impair the control of *L. monocytogenes*.

(*Table 1a*). At higher temperatures, for example −10°C to 0°C, viability decreases markedly during frozen storage. Staphylococcal enterotoxins are very stable in frozen storage.

Effect of temperature (0–50°C) on growth and enterotoxin production

Growth is optimal between 35 and 40°C with growth limits at about 7 and 48°C. At 10°C there is a long lag time (>20 h) and when growth commences it is very slow (*Table 1b*). At lower temperatures, growth is limited by small reductions in water activity or pH and is further reduced by storage under anaerobic conditions (*Tables 2a* and *3a*). Staphylococcal enterotoxins are produced under a more limited range of conditions compared with growth (*Table 1c*) but are similarly affected by factors affecting growth. Enterotoxins A and D are generally produced under a wider range of growth conditions than is enterotoxin B.

Effect of temperature (50–150°C) on destruction of cells and enterotoxins

The organism is usually readily killed at the temperatures used for pasteurization and in the cooking of foods; resistance is increased in dry and high-fat foods (*Table 1d*). All the enterotoxins are extremely resistant to heat and may survive the heat processes used to sterilize low-acid canned foods (*Table 1e*). After heat treatment toxic activity may persist when serological activity is absent. Treatment of heat-damaged enterotoxin with urea may restore serological activity (Bennett, R., personal communication, 1990). The heat resistance of cells is affected by growth conditions and resistance is somewhat increased by growth at high temperatures (>37°C) and decreased by growth at low temperatures (<20°C).

Effect of irradiation on S. aureus and enterotoxins

S. aureus is readily killed by ionizing and non-ionizing irradiation; resistance is higher in food than in buffers (*Table 4*). Staphylococcal enterotoxin is very resistant to gamma irradiation and will not be destroyed by the amounts used for the treatment of foods.

Effect of water activity on growth and enterotoxin production

S. aureus is a salt-tolerant microorganism and grows at a water activity as low as 0.85 (salt content 25% w/w) under otherwise optimum growth conditions. However, growth is often limited at higher a_w with other humectants (*Table 2a*). The interaction of water activity with other parameters is shown in *Tables 1b* and *2a*. Enterotoxin production occurs under a more narrow range of conditions than growth; production of enterotoxin A may occur at lower water activities than that of enterotoxin B (*Table 2b*).

Effect of pH on growth and enterotoxin production

Under otherwise optimal conditions, *S. aureus* can grow at pH <4.3 with an inorganic acid such as HCl as the acidulent (*Table 1b*). However, in the presence of organic acids pH limits are very much higher (*Table 3a*). The effect of pH on enterotoxin production is shown in *Table 3b* and on the growth of *S. aureus* in the presence of preservatives, at different water activities and at different temperatures in *Tables 5a, 2b* and *1b*, respectively.

Effect of preservatives on growth and enterotoxin production

Examples of the effects of a range of preservatives on growth and enterotoxin production by *S. aureus* are shown in *Table 5a*. This table also shows how the effectiveness of a preservative can be increased by combination with other factors, e.g. atmosphere. Little information is available concerning the specific effects of preservatives on the production of enterotoxins, but there is an indication that some, e.g. ethyl-4-hydroxybenzoate, may be more inhibitory to enterotoxin production than to growth (*Table 5b*).

Effect of gases on S. aureus

S. aureus grows both aerobically and anaerobically, but generally grows more slowly under anaerobic conditions (*Tables 1b* and *6*). In contrast, cell survival may be improved under anaerobic compared with aerobic conditions (*Table 2a*).