Water Relations of Foodborne Bacterial Pathogens - An Updated Review

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ABSTRACT

The effects of \( a_w \) limitation on growth and metabolic activities of foodborne bacterial pathogens continue to be actively investigated in laboratories throughout the world. Perhaps the most intensive work over the past 10 years has centered on growth of \( C. \) botulinum in multicomponent systems. This emphasis undoubtedly has been the result of concern about the role played by sodium nitrate in formation of nitrosamines and the possibility of a prohibition of the addition of this preservative to foods. While investigations have continued on \( C. \) botulinum and more "traditional" foodborne pathogens, a "new" group of pathogens, some of them opportunistic, has emerged. Several of these organisms are covered in this review whereas others are not for the simple reason that the water requirements of these organisms have not as yet been investigated. Particularly surprising is the lack of \( a_w \)-related information on \( L. \) monocytogenes, \( A. \) hydrophila and col-ohemorrhagic \( E. \) coli. In fact, the water requirements of gram-negative bacteria in general and the \( E. \) teria in particular seem to have been somewhat neglected. Researchers intending to do \( a_w \)-related research should consider trends in the American diet and in commercial food processors that supply much of it. For example, no one can deny that consumption of fish and seafood products has increased in the diets of many Americans yet potential pathogens indigenous to these products have received little investigative work in terms of their water requirements. Scombroid poisoning, a form of histamine poisoning, may be caused by several species of gram-negative bacteria, yet we know nothing of the effect of \( a_w \) on these organisms, their heat resistance, combinations with modified atmospheres, pH, preservatives, etc. Similarly, limited application of gamma irradiation for sterilization of spices has been approved by the FDA, however, the effect of \( a_w \) in these irradiated systems is largely unknown. Certainly greater thermal resistance at low \( a_w \) levels has been reported; however, it is surprising that investigators have not searched for a similar effect with irradiation. Despite these shortcomings, a sizeable body of literature and knowledge of \( a_w \) and its effects on microorganisms has emerged. Some of the research has only begun to exploit the hard-won knowledge of how microorganisms adapt to, and cope with, environments of low \( a_w \). Based on extrapolations of these efforts and our greater awareness of these physiological facts, one can only predict even greater advancements during the next decade.

Use of water activity-related concepts to predict the behavior of foods and their components has now received widespread acceptance. Food texture, color, viscosity, nutritional quality, oxidation, flavor and a host of other characteristics are influenced, to a greater or lesser degree, by the manipulation of water activity (\( a_w \)). Although various definitions of \( a_w \) exist, the simplest describes this parameter as the ratio of the vapor pressure of pure water to that of a solution at a specific temperature.

Perhaps in no other discipline has this relationship been so widely exploited and investigated as in food microbiology. The spoilage of foods has for centuries been related, usually empirically, to their moisture content, yet only within the last 30 years or so has a serious effort been made to explain this "propensity to rot" on a rational, objective basis. During the early phases of this work, it was recognized that microbial species might react differently to \( a_w \) reduction, depending upon the specific solute used to obtain moisture binding. In a sense, microorganisms compete with solutes for unbound or loosely bound water in a multi-component system such as food, however, we know so little about the forces affecting this binding that differences in microbial response, seemingly related to the composition of solutes in a product, are not unexpected.

The work of Christian and Waltho (28), Gould and Measures (42), Anagnostopoulos and Dhavises (7), Brown (21) and Moran and Witter (77) have given us insights into the mechanisms by which microorganisms cope with reduced \( a_w \). Despite these advances, and they are significant, we still have only fragmentary knowledge of occurrences at the level of the micro-environment. Clegg (30) has warned that we often make the assumption that "intracellular water is chemically and physically equivalent to the water in bulk aqueous solutions" and has raised questions about this assumption. To these concerns, one might add that water "experienced" by the microorganism within its microenvironment might also be singularly unique in its physical and biological properties and that to ignore this possibility might create a void in our understanding of \( a_w \) and how it influences microorganisms.
Similarly, perhaps we have concentrated too heavily on the physiological alterations created intracellularly by exposure to limited moisture environments while ignoring the role that the cell membrane must surely play in this scheme of things. We would have to designate a leading role in resistance to this structure if for no other reason than that the energized accumulation of compatible solutes must be critically dependent on a whole and "healthy" membrane.

Osmotic adaptation, in addition, has been considere by Anagnostopoulos and Roller (8) who noted that cultures of Escherichia coli adapted to environments of low aw by simply accumulating carbon sources rather than using them during biosynthesis.

We might also question whether there is such a thing as osmotic injury (96). If so, an understanding of this phenomenon could be of great significance to the food industry and to those of us who attempt to recover microorganisms from environments of limited aw.

Throughout these introductory discussions we have ignored the role that moisture limitation plays in securing our food supply from foodborne diseases. This is not from lack of significant emphasis in the literature on this aspect. In fact, the very earliest use of the term aw to explain moisture-related effects (98) as they relate to microorganisms dealt with Staphylococcus aureus, one of the most infamous foodborne pathogens. This knowledge expanded in the ensuring years and in 1974, the author made an attempt to summarize most of the existing literature on this subject in a published review (119). Now, once again, a sufficient backlog of information has accumulated to justify its collection and summarization.

This review concentrates on those reports published since 1973. However, on occasion and as background, significant contributions, previously reviewed, may be included to provide a more complete understanding of the data. Also, descriptions of the nature of specific diseases have been included. It is not my intention that this be a comprehensive treatment, but only that it serve as a brief, introductory statement. Much more thorough descriptions of the various foodborne diseases can be found in the volume edited by Riemann and Bryan (88) and in the many excellent texts on food microbiology.

It should also be noted that an entire category of important diseases, those caused by mycotoxins, has not been considered in this review. It is hoped that this subject can be addressed in a subsequent review.

**STAPHYLOCOCCUS AUREUS**

Staphylococcal food intoxication is caused by ingestion of one or more preformed enterotoxins. Six serologically distinct enterotoxins have been identified with the type A toxin most frequently implicated in outbreaks. Symptoms of the disease appear within 2 to 6 h and include vomiting, nausea and diarrhea. Mortality is very low.

The toxin is a protein of moderate molecular weight and very great heat resistance. Because the vegetable cells of staphylococci are not particularly heat resistant, it is not unusual to find toxin in the absence of viable cells in a pasteurized food. Custards, ham, poultry and cream-filled bakery products are most frequently implicated as causative agents of the disease.

**Growth**

Staphylococci characteristically possess the ability to grow at relatively high sodium chloride concentrations. Scott (98) indicated that, while the optimum aw for growth was about 0.995, the minimum was 0.86. Subsequent investigations (66) and a review (112) have indicated that growth may occur at slightly lower aw levels in meat products adjusted with NaCl. Troller (121) indicated that growth of some strains could occur at 0.83 aw, however, the presence of relatively high concentrations of beef extract in the medium was required. Chirife et al. (26) also examined the minimal aw for growth of S. aureus and were unable to detect growth in a laboratory medium and two meat extracts at aw levels of 0.837 to 0.839. As a practical matter, the exact minimum for S. aureus growth may be less important than recognition that this organism is the only foodborne bacterial pathogen that is routinely capable of growth at aw levels below 0.90. In mixed cultures, this organism normally is a very poor competitor (116), however, in environments of low aw, suppression of the competing microflora could permit relatively unimpeded growth, and potentially, enterotoxigenesis by staphylococci.

Whether a specific aw level is attained by water addition (absorption) or subtraction (desorption) can influence growth of a microorganism. This was demonstrated by Acott and Labuza (2) who found that an intermediate moisture food, when adjusted to an aw of 0.92 by desorption, contained 67.5% moisture, however when prepared to the identical aw by desorption, a moisture content of 55.9% was measured. A strain of S. aureus inoculated into this system grew at 67.5% moisture but did not grow in the desorption system at 55.9% despite the fact that the aw of both systems was identical. Relying on hysteresis to preserve a food can be risky since equilibration will occur eventually to a level which may support growth with possible spoilage or safety problems resulting. If, however, equilibration is both predictable and slow, perhaps some practical use can be made of the way that moisture is added or subtracted to preserve intermediate or low moisture foods.

One other general characteristic of staphylococci grown at low aw is the greater tolerance of NaCl than glycerol (71), while sucrose is tolerated about as well as NaCl (81). Whether this solute-related effect extends to enterotoxin production in addition to growth has not been investigated (105). Apparently, the dissimilarity of solute effects applies to other aspects of growth since both sucrose and NaCl, at similar estimated aw levels, are reported by Annear and Grubb (9) to increase the
maximum temperature at which a large number of staphylococcal strains will grow. Glycerol was not tested.

Why these solute-related differences occur is not known with certainty at present, however the intracellular changes that occur in the cells’ response to reduced $a_w$ may be involved.

**Growth and enterotoxin production in foods.**

The advent of less arduous methods of analyzing foods for enterotoxins (16, 105) has stimulated a number of studies on the effect of $a_w$ limitation on enterotoxin production. Meat products have been most frequently investigated; however, cheese and even fish products also have come under scrutiny.

An intermediate moisture meat product ($a_w = 0.86$ to 0.90) was tested (19) for its ability to support growth of an *S. aureus* strain inoculated into it during manufacture. Growth occurred in products adjusted to pH 5.2 and 5.6; however, addition of small amounts of methyl paraben, sodium benzoate, potassium sorbate and capric acid proved to be effective in preventing growth, especially in those products adjusted to pH 5.2. A polyhydric alcohol, 1,3-butylene glycol, also is an effective inhibitor of staphylococcal growth, but at a much higher level than the other inhibitors tested. These latter data confirm the earlier results of Plitman et al. (85) who had noted intrinsic antistaphylococcal activity when 1,3-butylene glycol was added to laboratory-scale chicken and pork-based intermediate moisture foods. A similar result also was reported by Vaamonde (126) who found that both polyethylene glycol 200 and polyethylene glycol 400 exhibited intrinsic antimicrobial activity beyond that which could be attributed to $a_w$ reduction alone.

Another type of cured minced meat product similar to a bologna-type sausage was adjusted to various NaCl levels and inoculated with several pathogens by Nielsen and Zeuthen (78). These experiments showed that the three concentrations of NaCl added to the minced meat, 3.6, 4.7 and 6.4% had no inhibitory effect on two strains of *S. aureus*; however, toxin formation was inhibited depending on NaCl level, temperature of incubation and pH. These authors suggested that the current trend toward lower NaCl concentrations, while not influencing growth of staphylococci, could make these products more susceptible to toxin formation in the absence of competing microorganisms.

Fermented sausage products have been implicated in outbreaks of staphylococcal gastroenteritis (125) and the role that $a_w$ reduction plays in the preservation of these products has been investigated in several laboratories. Niskanen and Nurmi (80) omitted starter cultures from laboratory-prepared sausages, inoculating instead with enterotoxigenic staphylococci. They found that growth and enterotoxin A and C production occurred despite a decreasing $a_w$ gradient from 0.96 to <0.88 $a_w$. Enterotoxin B could not be detected in inoculated samples probably because the reduction in $a_w$ prevented its synthesis (117). Interestingly, thermonuclease reactions were very weak in some samples in which extensive enterotoxin synthesis had occurred, leading the authors to question the reliability of the thermonuclease test. Results similar to those of Niskanen and Nurmi were reported by Lee et al. (65) who inoculated enterotoxin A and B-producing strains into Genoa salami that had not been treated with a starter culture. Differences in oxygen tension and/or oxidation-reduction potential seemed to influence the ability of staphylococci to grow since counts were higher at the surface of the sausage than in core samples. Enterotoxin production also was suppressed in core samples. The effect of oxidation/reduction potential on growth and enterotoxin production by staphylococci will be discussed below.

A dry Greek sausage inoculated with *S. aureus* was investigated by Karaioanoglou et al. (60) who noted that after an initial growth period of 24 h, the organism steadily decreased in numbers. This may have been due to the $a_w$ of the sausage which, when inoculated, was 0.93 and decreased rapidly during the first 24 h through a range of $a_w$ levels that would not permit staphylococcal growth. Other factors that might have influenced the decrease in populations of staphylococci were a decrease in pH and a decrease in the numbers of lactic acid bacteria. These authors concluded that enterotoxin could develop during the initial phases of sausage production if staphylococci were present in large numbers initially.

The studies cited earlier involved inoculation in a system at 0.97 to 0.98 $a_w$, which progressed to a lower $a_w$ very slowly during smoking and curing and involved significant time periods within the $a_w$ range for staphylococcal growth. The effect of $a_w$ on smoked products in addition to sausages has been investigated in several laboratories. Snoek, a type of fish widely distributed in waters off southern Africa, is commonly cured and smoked, yet is rarely implicated in cases of staphylococcal enterotoxocosis. Investigations by Theron and Prior (113) showed that abundant growth of an *S. aureus* strain, previously isolated from samples of this fish, occurred at 12°C and at $a_w$ values of 0.966, 0.956, and 0.944, however, thermonuclease could not be detected. Reduction in $a_w$ resulted in slower growth at 37°C and delayed thermnuclease production. The relationship between $a_w$ and thermonuclease production was further investigated by Troller and Stinson (122), who found that synthesis of this enzyme was strongly suppressed in laboratory media by $a_w$ reduction and it could not be found in a culture of an enterotoxin B-producing strain grown at 0.91 $a_w$. Enterotoxin synthesis was suppressed commensurately with thermonuclease production suggesting a correlation between enterotoxigenesis and production of the enzyme.

Pre-cooked canned bacon was intensively investigated in a series of studies at the U.S. Army Food Sciences Laboratory at Natick, MA. This product is a military ration item in which staphylococci could grow unless $a_w$ was poised at an unfavorable level. Studies to determine the ability of an enterotoxin A-producing strain to grow and produce toxin at altered $a_w$ levels, temperatures and
atmospheric oxygen contents in pre-cooked, canned bacon were reported by Lee et al. (66) who observed rapid aerobic growth of inoculated anaerobically under similar conditions, the minimal aw for growth was raised to 0.90. Unlike the reports of Troller (117, 118), Notermans and Heuvelman (81) and others, these authors found that enterotoxin production and growth were closely related for all strains of S. aureus tested. In a subsequent study, Silverman et al. (101) extended the studies of Lee et al. (65) by limiting the oxygen concentration to 5.5%. Under these conditions, minimal aw levels for growth were 0.87 and 0.91 at 37 and 20°C, respectively. These authors also noted that aw was the most effective predictor of growth potential in canned bacon and recommended that this parameter be measured directly rather than the previously employed (by the U.S. Army) moisture/salt ratio.

Growth of S. aureus in processed cheese was evaluated by Kreisman and Labuza (63) who found that in a series of cheese products adjusted to various aw levels, staphylococcal growth could not occur below 0.94 aw. In a later study of similar products ranging from 0.950 to 0.970 aw and manufactured in Argentina, Magrini et al. (70) concluded that refrigeration normally suppressed staphylococcal growth; however, temperature-abuse could result in growth and, presumably, enterotoxin production. Although aw, temperature, pH and other factors combine to limit staphylococcal growth during cheese manufacture, inadequate development of starter cultures may be one of the principal factors predisposing these products to enterotoxin development.

Combination effects

The combination of aw limitation with other microbiological control factors is a frequently exploited food preservation technique. Generally, as the minimal aw for growth of the organism is approached, it becomes more sensitive to other inhibitors and inhibitory conditions that might be present in its environment. However, there are exceptions to this rule, which will be noted.

pH. Of the combined factors investigated recently, the adjustment of pH under limiting aw conditions to inhibit staphylococci is the most common. As a practical matter, the benefits of combined aw and pH are most acutely realized in the processed meat industry where, as noted previously, mildly processed cured meat products may be susceptible to staphylococcal growth.

Earlier reports such as the one by Genigeorgis et al. (40) had indicated that the pH range for enterotoxin production was reduced as the concentration of NaCl was increased. In 1974, Bean and Roberts (15) investigated the combined effects of pH, sodium chloride and sodium nitrite on heat resistance of an enterotoxin A-producing strain of S. aureus in buffer and a pork macerate. They found a protective effect in buffer attributable to the presence of NaCl at pH 6.0, 6.5 and 7.0; however, this reaction was not present at pH 5.0 or 5.5. The effect of pH alone within the range 5.0 to 7.0 was small. The meat macerate adjusted to pH 6.5 did not appear to protect inoculated S. aureus in the presence of 4% NaCl and/or 500 ppm sodium nitrite. However, a four-fold increase in D NaCl was observed if 8% NaCl was added (4.61 min increase to 18.62 min) and a six-to-eight-fold increase (4.51 min to 28.49 min) was found if both 8% NaCl and nitrite were added. These authors concluded that the heat resistance of S. aureus in cured meat containing the latter combination was close to that required for survival of pasteurization and recommended that post-pasteurization storage of cured meats be at <10°C. Also, in 1974, Smolka et al. (106) investigated the effect of NaCl and pH level on heat-stressed and unstressed cells of S. aureus. A narrowing of the optimum pH range of both cell types was observed as the NaCl concentration was increased from 0 to 7.5%. In an investigation of the combined effect of butylated hydroxyanisole (BHA), NaCl and pH, Stern et al. (108) found that BHA at 50 and 100 ppm became more inhibitory as the NaCl concentration was increased and the pH was lowered. At pH levels of 5.0, 6.0, and 7.0, 100 ppm BHA with 5 or 7% NaCl was most inhibitory. In one of the few studies in which enterotoxin was measured, Pereira et al. (84) examined the effects of temperature, pH and sodium chloride on production of enterotoxins A and B by a single strain of S. aureus. No production of enterotoxin A occurred in 4% NaCl at pH levels of 6.0 and 6.5 after 18 h, however, low levels could be detected in media that had been adjusted to pH 6.5 and 7.0 followed by incubation for 72 h. Production of enterotoxin B appeared to be more sensitive to NaCl concentration than did the production of enterotoxin A. A concentration of 4% NaCl in the media used by Pereira et al. (84) would be expected to be about 0.985 aw, a level at which enterotoxin A production would be little affected whereas some effect on enterotoxin B production could be expected.

Most of the studies described above quantified humectant levels as per cent NaCl rather than in terms of aw. An exception is the work of Notermans and Heuvelman (81) who investigated the combined effects of pH, sub-optimal temperature and aw (using two humectants, NaCl and sucrose) on staphylococcal growth and enterotoxin production. At 30°C, growth of a mixture of S. aureus strains occurred at pH 4.6 but not at pH 4.3 in the control (0.99 aw) medium. Identical results were found when the aw was reduced to 0.96. At 0.93 aw, the presence or absence of growth at pH 4.6 depended on the humectant used, no growth being present in NaCl-containing media whereas growth did appear in sucrose-containing media. Further reductions in aw to 0.90 and 0.87 resulted in increases in the minimal pH for growth to 4.9 and 5.2, respectively. Interestingly, these workers reported that sucrose was the least inhibitory humectant at higher aw levels (0.99 to 0.90) whereas at 0.87 aw, NaCl but not sucrose permitted growth at pH levels as low as 5.2. Enterotoxin A production occurred at pH 5.2 and 0.90 aw (NaCl) and enterotoxin B production occurred at 0.90 only at pH 7.0 with the same humectant.
It is commonly accepted that the undissociated forms of acids are the active antimicrobial moieties. Despite this, no work had been done to compare the interactive effects of various acids and \( a_w \) levels with the exception of a paper by Robach and Stateler (89) and a more recent report by Troller (124). In the former, a synergistic inhibition between \( \text{NaCl} \) and sorbate was reported in which growth of two enterotoxin-producing strains of \( S. \text{aureus} \) was suppressed. Either 5 or 7% \( \text{NaCl} \) produced this effect when combined with 0.2% sorbic acid. The work of Troller (124) also deals only with growth suppression and compares the effectiveness of citric, acetic, phosphoric and hydrochloric acids as they interact with \( a_w \) reduction. At higher \( a_w \) levels, citric and acetic acids were reported to be more effective than either of the two inorganic acids; however, as the \( a_w \) was decreased (by addition of \( \text{NaCl} \)), hydrochloric and phosphoric acids, especially the latter, tended to be more inhibitory. In terms of relative inhibition per milliequivalent of acid, phosphoric acid was most effective at all \( a_w \) levels.

**Antioxidants.** Stern et al. (108) considered tertiary combinations of sodium chloride, butylated hydroxyanisole (BHA) and pH. It was found that inhibition of \( S. \text{aureus} \) was greatest at all pH and \( \text{NaCl} \) levels in the presence of 100 ppm BHA. This concentration of antioxidant was most effective when combined with 5 or 7% \( \text{NaCl} \).

**Atmospheres.** Controlled or modified atmospheres are receiving increasing attention as potential inhibitors of food spoilage organisms and foodborne pathogens. Carbon dioxide is most commonly used and has received commercial application in controlling slime formation by psychrotolerant bacteria on the surfaces of beef (29). It is well known that staphylococci grow optimally and produce maximal levels of toxins at relatively high oxygen tensions (12). An oxygen concentration of 5.5% (comparable to about 20 in. of vacuum) was tested for suppression of inoculated populations of \( S. \text{aureus} \) in canned, pre-cooked bacon by Silverman et al. (101). As noted earlier, they found that the minimal \( a_w \) in the inoculated cans was 0.87 at 37°C and 0.91 at 20°C at the decreased \( O_2 \) level. This was intermediate to results obtained by Lee et al. (66) when the same strain was grown aerobically (about 20% oxygen) in canned bacon and anaerobically (no oxygen present) in canned bacon. With anaerobic incubation, the minimal \( a_w \) for growth was 0.90, whereas an aerobically incubated strain was able to grow at 0.84 \( a_w \).

The effect of \( CO_2 \) atmospheres in combination with \( a_w \) reduction has not been studied, although the effect of \( CO_2 \) atmospheres alone is reported to be inhibitory for many microorganisms in bacteriological media (74) and packaged beef (69).

**Preservatives.** In addition to work on the combinations of potassium sorbate and \( \text{NaCl} \) described by Robach and Stateler (89), another preservative, sodium nitrite, in combination with \( \text{NaCl} \) and pH was investigated by Tompkin et al. (114). These workers found that enterotoxin A production by \( S. \text{aureus} \) 100 was suppressed by 9% \( \text{NaCl} \) in the absence of \( \text{NaN}_2 \) at pH 7.0. Only slight additional suppression of toxin production occurred if either 75 or 300 ppm \( \text{NaN}_2 \) were added. However, pH reductions to 6.1 and 5.7 in media containing only 3 or 4% \( \text{NaCl} \) were very restrictive to enterotoxin production. The addition of \( \text{NaN}_2 \) to these systems may have produced an even greater effect on toxin production, although the results appeared to be somewhat inconclusive in this regard.

As noted earlier, Bean and Roberts (15) investigated the combined effect of nitrite, pH and sodium chloride content on the heat resistance of \( S. \text{aureus} \) in buffer and macerated pork. In the latter, addition of 8.4 to 8.5% \( \text{NaCl} \) (pH 6.5) produced significant increases in heat resistance; however, addition on \( \text{NaN}_2 \) to create a tertiary system did not produce consistent results, and so the effect on heat resistance in the presence of \( \text{NaCl} \), pH reduction and \( \text{NaN}_2 \) remains somewhat uncertain.

**Production of extracellular metabolites.** In addition to enterotoxin, the production of other extracellular metabolites may be influenced by the \( a_w \) of the growth medium. Enzymes seem to be particularly susceptible. For example, Mates and Sudakevitz (72) found that production of an extracellular lipase was inhibited as the molar concentration of \( \text{NaCl} \) in the growth medium was increased. The largest increment of decrease in enzyme activity was between 0.2 and 0.4 M \( \text{NaCl} \) when the percentage of inhibition increased from 8 to 52%. Troller and Stinson (123) included a lipase in their study on the influence of \( a_w \) on production of a number of enzymes by staphylococci. They found that both tributyrinase and trioleinase activity were absent at 0.91 \( a_w \) and that a reduction in total and relative (to cell protein levels) lipolytic activity occurred as the \( a_w \) was lowered through a range of from 0.996 to 0.94. These authors found similar results with production of other enzymes such as heat-stable nuclelease, catalase, coagulase and acid phosphatase. Protease activity responsible for hydrolysis of hemoglobin and casein was the sole exception, reacting more extensively at 0.94 \( a_w \) than at either 0.996 or 0.97.

Heat-stable thermonuclease (HSTN) may be a useful indicator of enterotoxin production in foods, consequently the various factors which influence its production, including \( a_w \), have been investigated. Niskanen and Nurmi (80) for example, found that the \( a_w \) of a “dry” sausage decreased with time until it had dropped from 0.96 \( a_w \) at the time of manufacture to <0.88 after 30 d of ripening. Samples inoculated with enterotoxigenic strains of \( S. \text{aureus} \) were positive for thermonuclease after 30 d of storage, however, the enzymes could have been synthesized earlier during the ripening period when the product was poised at a higher \( a_w \) level. In 1978, Kamman et al. (58) published the results of their investigations on the effect of polyol humectants on production of HSTN. They found that media adjusted with 20% glycerol (0.95 \( a_w \), 10% propylene glycol (0.97 \( a_w \)) or 10% butylene glycol (0.98 \( a_w \)) resulted in reduced relative rates of thermonuc-
lease production. A direct correlation with enterotoxin production was not done; however, comparing this work with the earlier data of Troller (117) would suggest that enterotoxin and HSTN production react to reduced aw in a similar manner. This was later confirmed by Troller (122) in a study on the effect of aw on the production of a number of S. aureus metabolites. Barve and Kulkarni (14) added NaCl to a medium in a range of 1 to 10%. Coagulase activity, another predictor of the presence of enterotoxin was inhibited by NaCl concentrations as low as 5.0% (0.97 aw). Daoud and Debevere (34) recently measured HSTN activity in a cooked meat medium containing various concentrations of NaCl. They found optimal activity of the enzyme between 0.5 and 5.0% NaCl with a significant decrease occurring at higher concentrations of NaCl.

In the presence of relatively low levels of NaCl (up to 3.0%), Galligan et al. (38) found that catalase activity actually increased, possibly in response to increased aerobic respiration by the organism. At NaCl levels >3.0%, however, there was a measurable decrease in catalase activity. A similar effect on catalase production by two strain of S. aureus was reported earlier by Troller and Stinson (122).

Temperature effects

Temperature. Generally, as the temperature for incubation is moved from optimal for growth and enterotoxin formation, about 39°C, staphylococci become more sensitive to aw reduction. Notermans and Heuvelman (81) found that at 0.85 aw, neither growth nor enterotoxin production occurred at 18°C. When incubated at 24°C, some active to aw reduction. Notermans and Heuvelman found that at 0.85 aw, neither growth nor enterotoxin production occurred at 18°C. When incubated at 24°C, some staphylococcal growth was observed at 0.87 aw depending on the pH of the system.

The effect of aw on thermal destruction of enterotoxins has not been thoroughly investigated. Similarly, the effect of aw limitation on staphylococcal thermal resistance requires elucidation. Based on work done on Salmonella species by Corry (31) and others, one would expect an increase in resistance as the aw is reduced, however, this has not been proved for the staphylococci.

Although the vegetative cells of S. aureus are not particularly heat resistant, enterotoxin is very thermostable. Contaminated foods that have been heated may not contain viable staphylococci yet may contain sufficient enterotoxin to cause an out-break of disease; therefore, the effect of temperature on growth, enterotoxin production and the thermostability of enterotoxin have received considerable attention.

Effect of temperature on staphylococcal growth. In a brief report appearing in 1975, Annear and Grubb (9) found that growth was retarded or absent on agar media without NaCl at 45°C, whereas addition of 6% NaCl resulted in recoveries identical to those at a more optimal temperature (37°C).

Sucrose in concentrations of 20-30% produced similar results. Hurst and his co-workers in Canada also reported on this effect. They found (49) that the addition of 1M NaCl to staphylococcal growth media extended the upper limits for growth and enterotoxin production about 2°C. Other humectants such as KCl, MgCl2 (at greater concentration), glucose and sucrose were found by Hurst et al. (49) to produce similar results. Glycerol was one of the solutes tested that did not extend the maximum temperature range for growth. In a subsequent report, Hurst and Hughes (51) confirmed these findings in foods and noted that cultures grown in a medium containing 5.8% NaCl and incubated at 46°C had a D60°C value four times greater than an identical strain incubated at 37°C in the absence of NaCl. Addition of both 5.8% NaCl and 5.0% monosodium glutamate to cultures incubated at 37 and 46°C resulted in a D60°C value about seven times the value in the absence of these solutes. Interestingly, these workers found morphological changes in the presence of NaCl which included thickened cell walls, irregular septation and cell clumps surrounded by capsular material. These results, in part, confirm an earlier, similar finding reported by Troller and Christian (122). Notermans and Heuvelman (81) also investigated the effect of aw and sub-optimal temperatures on staphylococcal growth and enterotoxigenesis. They found that growth at pH 7.0 would occur at 12°C at 0.93, but not at 0.90 aw. Adjustment of the medium aw with sucrose was more inhibitory at minimal aw levels than adjustment with NaCl.

Heat resistance. Like salmonellae, staphylococci become more heat resistant as the aw is lowered until at an aw level between 0.70 and 0.80, resistance begins to decline. Although not working with S. aureus, Verrills and Van Rhee (127) found that the heat inactivation curves of Staphylococcus epidermidis were biphasic under all conditions of aw and temperature tested. They further noted that the second phase, or tail, resulted in very slow inactivation times with a D60°C value of at least 500 s.

Heat injury. Heat-induced injury usually is defined in terms of failure to initiate subsequent growth in a medium containing 7% NaCl. Smith et al. (103) have reported data in which increasing levels of NaCl from 1 to 9% provided increasing protection from thermal injury. Glycerol and sucrose behaved similarly in reducing heat-injury to S. aureus. In a later paper, Smith et al. (104) attempted to determine the mechanism for the thermal protective effect of glycerol and sucrose by determining if these compounds were metabolized. Their results showed that both metabolizable and non-metabolizable components were protective against thermal injury (50°C).

Thermal inactivation of enterotoxin. As noted earlier, enterotoxins are relatively resistant to thermal inactivation yet despite this important fact, the effects of aw on thermal inactivation are poorly understood. In one of the few studies on this subject reported in the literature, Jamlang et al. (55) studied the heat inactivation of enterotoxin B at various ionic strengths of NaCl between 0.02 and 1.0 M (aw 0.996 to 0.96). They noted that at pH 4.5, inactivation was increased by NaCl addition to 0.05 M and greater.
In 1974, Troller and Stinson characterized destruction of enterotoxin B at oil frying temperatures using NaCl as the humectant. Like the findings of Verrips and Van Rhee (127) for inactivation of staphylococcal vegetative cells, they found that inactivation plots for the toxin were biphasic. At the highest temperature tested, 160°C, toxin was reduced to serologically undetectable levels within 2.8 min, however, at 121°C, 62 min were required to inactivate the toxin. At reduced aw levels, a definite protective effect was observed, with continued heating, diminished somewhat in rate of inactivation. The D149°C for inactivation of the toxin at 0.99 aw was 100 min, whereas at 0.90 aw the D149°C was 225 min. Similar differences in activation energies and rate constants also were reported.

**VIBRIO SPECIES**

*Vibrio parahaemolyticus*

This organism is the causative agent of a type of food infection most commonly associated with consumption of raw seafood, although cooked seafood can be cross-contaminated during preparation. It is a mesophilic, gram-negative organism that has been described as "halophilic" by Sakazaki (95); however, it seems to be much more sensitive to sodium chloride than most halophiles and probably would fit Troller and Christian's (122) definition of a "mildly halophilic" organism, i.e. it requires 0.2 to 0.5 M NaCl for growth.

The symptoms of the disease caused by this organism are severe abdominal pain, diarrhea, vomiting, and nausea. These occur approximately 12 h after ingestion of food and persist for a day or two followed by eventual recovery. Mortality is very low.

The moisture requirements for *V. parahaemolyticus* have not been established; however, a number of papers have appeared which discuss the NaCl requirement for growth. Some of these refer to *V. parahaemolyticus* whereas others may relate to other, similar *Vibrio* species. One of the latter (48) describes testing 5, 10 and 15% NaCl solutions for their ability to support growth and attachment to copepods by a strain of *Vibrio cholerae*. These data showed that maximal levels of both parameters were obtained at the highest salinity tested, 15%.

Rodel et al. (94), on the other hand, found that salt tolerance among the nine *Vibrio* strains was highly variable. *V. parahaemolyticus* would not grow (increase in turbidity of media) at 0.9328 aw (11% NaCl) but some, slight increase was observed at 0.9388 aw (10% NaCl). They suggested that aw-tolerance might be a useful means of identifying the various strains of food origin. Beuchat (17) found that the minimal aw at which this organism was capable of growth was highly dependent on the solute employed. Reduced aw was best tolerated at 29°C. At this temperature the minimal aw for growth using glycerol as the humectant was 0.937; using KCl it was 0.945; NaCl, 0.948 and sucrose, 0.957.

Until further work appears on the effect of aw on growth and survival of *V. parahaemolyticus*, one should probably view it as a moderately halophilic, osmotolerant organism.

*Vibrio vulnificus*

This pathogen can be transmitted to humans by several means including consumption of raw shellfish. Distinguished from *V. parahaemolyticus* by the fact that if ferments lactose, septicemia caused by *V. vulnificus* has a mortality rate (>50%) that is the equal of *Clostridium botulinum* intoxications. Persons with liver disease or immunodeficiencies appear to be particularly susceptible (36).

Kelly (62) investigated the effect of NaCl concentration on growth rate of this organism and found its growth to be favored by relatively low salinity. Optimal growth occurred in media containing 1.0 to 2.0% NaCl (estimated aw = 0.9994 to 0.989) with none occurring in media containing 0.1% (0.9991aw) or 5.0% (0.970 aw) NaCl. These results applied to both clinical and environmental isolates.

**CAMPYLOBACTER JEUNI**

According to Buchanan (23), symptoms of gastroenteritis caused by *Campylobacter fetus* subsp. *jejuni* occur 2 to 5 d post-ingestion and persist for about 2 to 3 d. They consist of diarrhea, cramps, nausea and vomiting. Most commonly implicated foods are unpasteurized milk (61% of the reported *Campylobacter* outbreaks have been traced to raw milk consumption), improperly chlorinated water, poultry and clams. Infected food handlers also may be an important source of this organism.

A microaerophilic environment containing about 8% CO2 is required for isolation of *C. fetus* subsp. *jejuni* as well as a fairly specific temperature range of 42 to 45°C. This organism is one of the most salt-sensitive bacterial species, being inhibited by as little as 2.5% NaCl (23). Doyle and Roman (35) found that a slightly lower NaCl concentration of 2.0% prevented growth even though it grew best in the presence of 0.5% NaCl. Abram and Potter (1) also examined the effects of NaCl on growth and survival of this organism with emphasis on low-temperature incubation. They found that the extent of inhibition (decrease in survival) was greater at 10 than at 6°C. These data confirmed earlier findings by Hanninen (44) in which survival in *Brucella* broth, which contains 3.5% NaCl, was enhanced at low temperatures. The aw requirements of this organism have not been investigated; however, minimal levels for growth apparently are very high. It would be interesting to determine the minimal aw for growth when humectants other than NaCl, such as sucrose or glycerol, are used. Highly osmosensitive organisms such as these also could be valuable investigative tools in determining the mechanisms of growth at reduced aw.

**YERSINIA ENTEROCOLITICA**

*Y. enterocolitica* food infections most frequently occur after consumption of contaminated milk, milk products...
and meat products. Symptoms of the disease are abdominal pain, fever, diarrhea and vomiting. Because of the type and anatomical location of the abdominal pain plus fever symptoms, this disease is frequently misdiagnosed as appendicitis.

Of primary importance is the fact that Y. enterocolitica is psychrotrophic, yet possesses a fair degree of heat resistance. As a result, pasteurized milk could and has, become a significant source of disease outbreaks. Because many food safety strategies rely heavily on pasteurization to kill pathogens plus refrigerated storage to prevent subsequent growth of both spoilage and safety-related organisms, the public health implications of this organism are obvious. In one of the few papers relating to solute effects on Y. enterocolitica, Stern et al. (109) found that both 5.0 and 7.0% NaCl in a medium reduced its growth rate. The degree of inhibition was somewhat greater at 3 than at 25°C. Work relating to specific aw effects has not been published.

Nielsen and Zeuthen (78) studied growth of Y. enterocolitica in a cured meat product and found that a brine concentration of 6% prevented growth of this organism at 2 to 12°C. When the brine concentration was reduced to 4.5%, growth only was prevented at an incubation temperature of 2°C. Extrapolating these results to probable toxin production, the authors reasoned that at refrigeration temperatures, only the highest NaCl level tested, 6%, would leave the product free of toxin.

**Bacillus Cereus**

Foodborne illness caused by strains of Bacillus cereus has become increasingly common over the past 10 to 20 years. The source of this increase most probably is improved recognition, diagnosis and reporting rather than a "real" increase in incidence of disease. There are two clinical manifestations, a severe emetic type and a milder diarrheal type. The former has a rapid onset (1-6 h) characterized principally by vomiting and acute gastric pain. This type is sometimes associated with consumption of fried rice in Chinese restaurants. Although originally of concern in the United Kingdom, greater awareness of this type of food intoxication has brought about its discovery in outbreaks throughout the world. The diarrheal type seems to be a much milder form than the emetic type and in many respects the symptoms mimic those of Clostridium perfringens gastroenteritis. The types of foods implicated are quite diverse, including cooked meat, poultry and vegetables, soups and desserts. Although different in symptomology and serological reactions, limited comparisons indicate no differences in response to changes in growth conditions by either type of B. cereus. Based on this, one would not expect differences in the moisture relations of the two types; however, this has not been proven experimentally. Apparently, there are extensive solute-related differences which influence both germination and outgrowth. For example, Jakobsen et al. (52) tested a number of commonly used humectants and found that the organism responded quite differently depending on the type of humectant used. In general, glycerol, erythritol and dimethyl sulfoxide were less inhibitory, that is, lower minima for germination and outgrowth were obtained when these humectants were used to lower the aw levels of media. Highly ionic solutes produced the most inhibition. In a subsequent study, Jakobsen and Murrell (53) noted that the sequence of germination-related events did not change with changes in humectants. This disagrees with Hashimoto (45), who found that the second phase of microgermination in B. cereus was inhibited when 0.3 M CaCl2 was present. NaCl was generally the most inhibitory of the solutes examined, whereas glycerol was the least inhibitory. The sole exception to this was a slightly greater inhibition of B. cereus observed at identical aw levels when CaCl2 was the humectant. The actual minimum for germination and outgrowth of B. cereus was not determined in these studies. The effect of NaCl on growth of B. cereus vegetative cells was studied by Raevuori and Genigeorgis (89), who found that a decreasing rate of growth occurred when the organism was exposed to NaCl concentrations increasing from 0 to 10%. Also, at the highest level tested a much larger inoculum was required to initiate growth. In terms of aw, these authors stated that the minimum permitting growth was 0.955, a value which agrees well with the 0.95 indicated earlier by Scott (99).

The effect of NaCl on germination of B. cereus spores also was studied by Farkas and Roberts (37). They found that germination was more salt-sensitive in simple than in complex germinating media. They further noted that spore germination by B. cereus was less sensitive to NaCl than outgrowth and development of vegetative cells. When these authors evaluated the salt-sensitivity of cells exposed to irradiation, they found that it increased dramatically and in a manner similar to that of cells that had been heated. They suggested that combinations of heat and aw reduction could perhaps be used to reduce the amount of radiation required to obtain a given level of preservation.

Relatively little additional information on this potentially important foodborne pathogen has appeared. Those data that have been published continue to support the original contention of Scott (99) that the minimal inhibitory level for vegetative growth is 0.95. Germination and outgrowth, especially the former, may occur as low as 0.91 aw when glycerol was the humectant and at 0.95 aw when glucose, sorbitol or NaCl were used to poise the aw. These effects appear to be highly humectant-dependent with glycerol and erythritol being tolerated to the greatest degree by the organism under a variety of conditions.

**Clostridium Perfringens**

Clostridium perfringens intoxication occurs most frequently in foods prepared by foodservice organizations or in institutional kitchens. Incorrect storage and holding...
temperatures are often implicated. These temperatures, normally in the range >45°F to <140°F, may provide conditions for the rapid, almost explosive, growth of this organism so that populations in the 10^3 to 10^7/g range may be reached in relatively short periods. Upon ingestion, further growth and toxin production occurs in the alkaline environment present in the small intestine. Toxin formation is invariably associated with sporulation.

Cooked foods, usually meats, are the most frequently implicated vehicles. Because of its ubiquitous nature, C. perfringens is virtually impossible to exclude, totally, from food, hence control procedures rely heavily on holding foods at temperatures that prevent growth. Symptoms are relatively mild and include, primarily, stomach cramping and diarrhea.

It is apparent from previous work (58,110) that the minimal a_w for growth of C. perfringens depends heavily on the controlling humectant. Ionic solutes such as KCl and NaCl appear to be most inhibitory at a specific a_w whereas nonionic materials such as glucose and glycerol seem to permit growth at a_w levels as low as 0.95 to 0.93. Sporulation generally requires a higher a_w level than does either germination or outgrowth. The effect of a_w on production of C. perfringens enterotoxin in vitro has not been investigated.

In terms of tolerance to NaCl and NaN02, Roberts and Derrick (92) showed that C. perfringens strains from a variety of sources possessed a tolerance to NaCl that was both uniform and similar to C. botulinum. These authors noted that only one of the 21 strains evaluated was capable of growth of 7% NaCl (0.95 a_w) and that combinations of NaCl and NaN02 were especially active in preventing growth.

Two additional reports have added to our knowledge of the effect of a_w on C. perfringens. The first of these by Jakobsen and Trolle (54) determined the minimal a_w levels for growth of a number of species of the clostridia including C. perfringens. These data suggested that the minimum for the growth of vegetative cells is about 0.94 a_w. Germination of spores and vegetative growth occurred at identical a_w levels. Somewhat higher minimal a_w values for growth were reported for two strains of C. perfringens by Bartsch and Walker (13), who found that 0.975 was the minimal a_w for growth in media adjusted with KCl and incubated at either 37 or 45°C.

CLOSTRIDIUM BOTULINUM

With the possible exception of staphylococcal enterotoxigenic, intoxications as a result of consuming food containing botulin toxin are probably the most broadly known and feared of all foodborne disease. The chief reason for this notoriety probably is the very high rate of mortality encountered with this disease which in some past outbreaks, has reached as high as 90 to 100%. According to Tompkin and Christiansen (115) mortality rates have been steadily decreasing for about the past 20 or so years as a result of improved treatment regimens and more rapid recognition of the disease which results in the timely use of botulin antitoxin.

The symptoms of botulism occur within 18 to 36 h after consuming a food containing the toxin. Although, they may vary depending on the toxin type, weakness, blurred and double vision, difficulty in swallowing and, eventually, respiratory failure are the most common indicators of botulism. There are seven, serologically identified, toxins with types A, B and E being the most common in humans. Foods frequently implicated are cured meats, low-acid canned vegetables, milk products, fish and seafoods.

As a result of the early work of Ohye and Christian (81) and Baird-Parker and Freame (10), knowledge of the water requirements of C. botulinum is quite complete. These workers established that the minimal a_w levels for growth (NaCl humectant) were 0.95 for type A and 0.94 for type B strains. Minimal levels for growth were somewhat lower when glycerol was the humectant rather than NaCl and the type E strain was more sensitive to a_w reduction than either type A or B strains. Germination of all types occurs at a_w levels below those for growth about 0.95 a_w when NaCl is the humectant. Estimates of minimal a_w levels for sporulation have not been reported.

In the 1970s and early 1980s there was considerable speculation that toxicological concerns surrounding generation of nitrosamines from nitrates in cured meats could force elimination of nitrates from all food products. Because nitrates limit growth of C. botulinum, intensive work was carried out in a number of laboratories to identify alternatives that would be safe and effective. Work by Roberts and Ingrum (90) using a bacteriological medium had confirmed earlier observations that a strong interaction existed between NaCl, pH and NaN02 and suggested that it was incorrect to change one of these components without considering the other two. These workers further demonstrated that nitrite in a cured meat product could not be safely eliminated unless the NaCl concentration was >7% (0.95 a_w), a level which results in obvious flavor problems. Subsequently, a number of additional papers on this subject have appeared in British journals which have emanated from the Leatherhead Food group and the Meat Research Institute. These articles have further defined the nitrite-NaCl interaction in a variety of meat products.

Greatest inhibition of C. botulinum was observed by Roberts et al. (91) in a pork slurry at either 1.8 or 3.5% NaCl at an incubation temperature of 15°C. At higher temperatures (20, 22.5 and 25°C) little inhibition occurred at these salt concentrations even if 300 ppm NaN02 were present. Using a similar minced pork slurry system, Rhodes and Jarvis (87) found that there was little effect of several different thermal processes on inhibition by 75, 175 and 300 ppm NaN02 in the presence of 3.5% NaCl. It should also be noted that some variation in inhibition was found with different batches of meat. Thermal sensitization of C. botulinum spores to 2.5 and 3.5%
NaCl was obtained by heating at 85, 90 or 95°C according to Jarvis et al. (56). If spores were heated at 70° or 80°C, sensitization only to 4.5 and 5.5% NaCl could be obtained. Somewhat later, Roberts et al. (93) investigated the effect of multiple component systems containing sodium nitrite, sodium isoascorbate and polyphosphate on growth of C. botulinum. Toxin production was significantly reduced in a meat slurry if either nitrite, NaCl (2.5, 3.5 and 4.5%) or the above materials were added individually. The relative effect of increasing nitrite content was reduced in the presence of isoascorbate or 4.5% NaCl. Oddly, increasing salt levels were less inhibitory if isoascorbate was present. While these results are helpful in ascertaining the effect of various “curing” components on growth of C. botulinum in meat slurries, some care should be exercised in interpreting data obtained in fat-containing systems. Emulsifying agents, such as phospholipids normally present in meats, can grossly alter the moisture “condition” to which the organism is exposed within the microenvironment. These alterations cannot be determined by moisture analysis nor can they be fully “visualized” by standard a_w measure- ment techniques. Chen and Karmas (25) refer to these problems in their article on the effect of surface active agents on intermediate moisture foods. As these authors found, a_w increased as the concentration of surfactant (emulsifier) increased and they advocated that reduction of surface tension should be avoided to obtain a low a_w level in foods that are dependent on moisture sorption for their safety and/or stability. If these proscriptions are indeed significant, and there are contradictory data (6), one would expect batch-to-batch differences in the reaction of the various meat slurries used in these studies and, in fact, at least one paper (87) reports exactly this problem.

The thermal resistance of C. botulinum spores at various a_w levels also has been investigated. The earlier work of Murrell and Scott (79) had shown a maximal range of thermal resistance at a_s levels between 0.2 and 0.4. Another means (in addition to a_s adjustment) of altering the thermal resistance of spores of aerobic bacilli was reported originally by Alderton and Snell (3), who found that by changing the chemical form from calcium (heat resistant) to hydrogen (heat sensitive) forms, thermal resistance could be altered significantly. This work was extended to C. botulinum spores in a subsequent publication (4), and was compared at different a_s levels by Alderton et al. (5) in 1980. In this later work, it was found that very low heat resistance occurred at a_s 0.00 and 0.70 to 0.90 a_s with highest resistance occurring at 0.1 to 0.5 a_s. Throughout most of the a_s test range, it was found that the calcium form remained more heat resistant than the hydrogen form.

The effect of very low pH and water activity on the thermal resistance of a mixture of type A and type B spores was investigated by Snell et al. (102). At all pH levels, the heating menstrua poised at reduced a_w with 30% sucrose (about 0.98 a_w) indicated greater resistance within a range of 3.5 to 4.4. Extensive strain-to-strain differences were seen in this study, especially between the Type B strains.

Several foods have been evaluated for their ability to support growth and toxin formation by C. botulinum. One of the most interesting is pasteurized process cheese spread which possesses a relatively high pH level (>4.6) and an a_w greater than 0.85. Two incidents implicating cheese spreads are on record; one in 1951 in the U.S. and another in 1974 in Argentina. These incidents stimulated a number of studies, some of them currently in progress, to investigate the growth factors involved.

It appears that the technique used to inoculate test spreads and to achieve uniform distribution of spores is extremely critical in determining if C. botulinum will grow. Tanaka (111) showed that toxin formation was possible in spreads in which the spore inoculum was poorly mixed. On the other hand, this worker was unable to detect toxin in spreads that were thoroughly mixed. Before this publication, Kautter (61) had shown that some products of this type could support growth and toxigenesis when inoculated; however, the procedures used for distribution of the inoculant within the spread could have resulted in localized areas of high a_w and uneven distribution of spores. Additional studies currently underway at the University of Wisconsin should shed some light on the factors responsible for the safety of this product; however, at this time it would appear that pH and a_w are only two of the components involved and that other, even more critical, factors may be operative in the cheese. In any event, nonuniform spore distribution and/or localized areas of conditions favorable for growth of C. botulinum would be unexpected in manufactured spreads because of vigorous agitation during manufacture.

One additional study in Argentina (20) using pH and a_w levels similar to those in processed cheese, but in bacteriological media, found that no growth occurred at < 0.949 a_w after 52 to 59 d of incubation at 30°C. In subsequent studies with actual cheese spreads inoculated by thoroughly mixing spore suspensions with the cheese, toxin production was obtained in as little as 30 d. The average a_w of these spreads was 0.970 and the average pH was 5.7. None of the spreads examined in the FDA study (61) (see above) approached these a_w levels and most were well below the 0.949 level indicated in the Argentine work.

Another product receiving only a mild heat process, caviar, was investigated by Hauschild and Hilshemer (46) to determine the factors that control growth of C. botulinum. Products similar to caviar have, on rare occasions, been implicated in outbreaks of botulism (usually Type E) with home-prepared, fermented fish eggs being most frequently incriminated. A number of caviar products were surveyed to determine their brine salt concentration and pH, the two factors most likely to control growth. An overall range of 6.0 to 38.8% NaCl was found with a median of 9.5% for lumpfish, 20.0% for salmon, and 14.0% for sturgeon caviar. As noted above,
Type E strains have the greatest potential for toxigenesis in marine products such as these and Type E strains also are the most intolerant of aw reduction, normally refusing to grow below 0.97 aw. The pH medians reported were from 5.5 to 5.8; well within the ranges for growth and toxin production; however, as with brine concentrations, the samples examined exhibited a very wide pH range, hence the risk for growth could be high in some packages of caviar. Toxin was not formed under any circumstances if the per cent of NaCl in the water phase was ≥5.56% (about 0.96 aw) or if the pH was ≤5.0.

Other marine products have caused serious botulism outbreaks. The type E outbreaks associated with smoked, cured fish in the 1960s are examples. Consumption of traditional, subsistence foods preserved by native Alaskans also is a significant source of botulism. Zottola et al. (128) observed preservation techniques for a number of native fish products and fermented whale meat. Analyses indicated that they contained high counts of anaerobic bacteria and that they depended on reduced aw (NaCl addition) to create conditions that prevent growth of C. botulinum. Fermented whale meat (0.976 aw), half-smoked whitefish (0.942 aw) and half-smoked silver salmon (0.995 aw) were within the aw range for growth of C. botulinum. These authors concluded that the preservation processes they observed in native villages do not destroy spores and that safety problems could occur if NaCl concentrations are not sufficient to reduce aw levels to below those required for germination and toxigenesis.

Hot process salmon was examined by Pelroy et al. (83) to determine the amounts of sodium nitrite and NaCl required to inhibit toxin formation. Sodium chloride concentrations of 3.8 and 6.1% prevented toxigenesis by type E and type A strains, respectively. In all instances, the amount of NaCl required to prevent toxigenesis was reduced by addition of NaNO2; however, greater amounts of both were required if the inoculum level was increased from 102 to 104 spores/gram. Type A toxin production was prevented by a combination of 150 ppm nitrite and 3.5% NaCl.

Montville (75) examined the relationship between aw and pH reduction and its effect on cell growth and lysis and, like others, reported that growth rates decreased with decreasing pH and increasing salt levels. Unlike growth, cell lysis was affected only by pH with the rate of lysis decreasing with pH reduction. This is especially significant because others (18) had previously associated toxin release with autolysis. Montville (76) in a later paper again noted the interaction between NaCl and pH and found that growth under these restrictive conditions could be attributed only to a very small subpopulation within the total, inoculated population. Plating efficiencies at pH levels of 6.5, 6.0 and 5.5 decreased from 0.20 to 0.5 to 0.005%, respectively. NaCl addition also brought about a reduction in plating efficiency. For example, at pH 6.0 an efficiency of less than 0.01% was observed in a medium containing 3% NaCl.

Water activity interactions with other environmental factors also have been studied. One of these, percent of NaCl interaction with oxidation-reduction potential (ORP), has been investigated in detail by Smoot and Pierson (107). They found that the Eo7 definitely influenced the aw (NaCl or sucrose) required to inhibit a test strain of C. botulinum. Growth and toxin formation were delayed or decreased at higher Eo if 5 or 6% NaCl or 30% sucrose were added. In a very comprehensive paper, Lund and Wyatt (68) studied the interaction of ORP with NaCl concentration and its effect on the probability for growth of a type E strain. They found that, in a medium adjusted with 3.25 or 4.0% NaCl and poised at -400 mV, the probability for growth was decreased by factors of 102 and 104, respectively. At a much higher ORP (between +65 and +122 mV), 3.25% NaCl decreased the growth probability by 106. In the absence of NaCl, an increase in ORP above +60 mV resulted in a significant decrease in growth probability. The authors theorize that the combined effects of increased ORP and NaCl addition are due to death of vegetative cells subsequent to spore germination.

LISTERIA MONOCYTOGENES

The possibility that Listeria monocytogenes could be a significant foodborne pathogen has been considered for some time; however, documented outbreaks are extremely rare. Based on the capability of this organism, the paucity of outbreaks seems almost paradoxical unless many incidents are undetected or misdiagnosed.

L. monocytogenes is a frequent inhabitant of the intestinal tract of domesticated animals and fowl, hence, human exposure to sources of this organism must occur fairly frequently. Because L. monocytogenes is excreted in the feces of animals, plants fertilized with manure can carry the organism, which multiplies rapidly even though the product may be refrigerated. An outbreak of this type occurred in Canada (97) in 1981 in which cole slaw made from cabbage grown in a field fertilized with untreated sheep manure was implicated.

Resistance to moderately high temperature (80°C for 5 min) and growth at low temperatures (4°C minimal) are not the only unusual characteristics possessed by L. monocytogenes. The work of Shahamat et al. (100) indicates that this organism survives salt levels normally used to preserve meat products. In addition, it was found that sodium nitrite, while having little effect alone, was especially inhibitory to this organism in trypticase soy broth containing 3, 5.5 and 8% NaCl (estimated aw levels = 0.983, 0.967 and 0.950).

SALMONELLA

Salmonellosis continues to be one of the most commonly occurring foodborne diseases throughout the world. Meats and meat products, especially those that are undercooked are frequently implicated. Dairy products and poultry also are significant sources.
Because this disease is a food infection, some growth occurs in the alimentary tract; hence, the time of onset may be 24 h or more. Symptoms, which persist for 3 to 5 d, consist of abdominal distress, vomiting, diarrhea and fever. Mortality is very low; however, outbreaks in populations of elderly persons or the very young can be life-threatening.

The moisture requirements for growth of salmonella are, like many other gram-negative genera, in the 0.94 to 0.995 range (27). Growth in foods may occur at a slightly lower minimum a_w, 0.93. Throughout these early studies, few solute-related effects were observed; however, this was not true with work done in the early 1970s by Goeppert et al. (41) and Corry (31) on the effect of a_w on the heat resistance of Salmonella species. As pointed out by Troller and Christian (122), these data are especially critical in the confectionary industry which often heats suspect ingredients under conditions of low a_w. In her studies on the heat resistance of Salmonella typhimurium, Corry (31) noted that the effect of various solutes on resistance at 65°C was in the order sucrose > glucose > sorbitol > fructose > glycerol. Earlier studies (11) had indicated that NaCl was more protective than glycerol. Subsequent work by Corry (32) showed that the extent of protection from the effects of heat is related to cell plasmolysis and shrinkage. It was concluded that heat resistance is associated with dehydration rather than replacement of intracellular moisture by solute. These and other aspects of the growth and survival of Salmonella spp. at low a_w are discussed in a review by Corry (33). Additional work in this area was reported by Kwast and Verrips (64), who expressed, mathematically, the relationship between sucrose concentration and inactivation rate by the equation:

\[ \ln k = \ln k_0 - T [\text{sucrose}] \]

where \( k \) is thermal inactivation rate, \( k_0 \) is the extrapolated rate for zero sucrose concentration and [sucrose] is the concentration of sucrose. \( T \) is a temperature-dependent constant. Using this formula, these authors were able to predict what Goeppert, Corry and Baird-Parker had shown experimentally 8 to 12 years earlier.

Survival of Salmonella species maintained at various a_w levels was considered by Campanini (24). Whereas growth occurred at a minimum a_w of 0.945 to 0.934 in media, survival at 5 and 25°C in uncooked ham at a_w 0.88 to 0.92 occurred to a greater degree than in culture media. Survival of Salmonella montevideo and Salmonella heidelberg in dry food and feed was studied by Juven et al. (57). They found that survival of S. montevideo was greatest at 0.43 a_w as opposed to 0.52 and 0.75. Numbers of S. heidelberg appeared to be little affected by a_w, suggesting that survival may be highly species-specific.

Survival of S. typhimurium in a bacteriological medium adjusted to low a_w with glycerol and containing 0.3 M acetic acid was studied by Meyer et al. (73). Acetate was chosen as a combinant in this system because of its previously demonstrated (39) capacity to lower the heat resis-

tance of Salmonella species in egg products. In a control solution adjusted to 0.86 a_w with glycerol only, approximately 15% of the inoculated cells survived after 6 h. Under similar conditions a solution containing acetate permitted survival of only 3% of the inoculum. In a solution containing only acetate (no glycerol), only 1% survival was noted. Greatest acetate effectiveness appeared at the minimal growth limit.

A composite of Salmonella species was one of the inocula used by Nielsen and Zeuthen (78) in their study on growth of foodborne pathogens in minced, bologna-like meat product. The inoculum did not grow in product that contained 6% NaCl although rapid growth occurred at 5%. Temperature was an important additional factor influencing growth.

**SHIGELLA**

Little has been reported on the moisture requirements of this organism. Although it has, on occasion, been implicated in outbreaks of foodborne disease, it is primarily of concern at a waterborne organism. Based on this, one would expect a high minimum a_w for growth and, in fact, Leistner and Rodel (67) quote a publication which apparently lists the minimum a_w for growth of an unspecified species of Shigella as 0.96 a_w. This level would place it slightly above that of most Salmonella species.

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