Food Microbiology

Volume I
Concepts in Physiology and Metabolism

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PREFACE

Volume I

The genesis of this book lies in the convergence of three forces. First, the era of molecular biology developed and eventually came to dominate the life science scene during the period of my scientific formation. Secondly, the philosophy of the food science departments where I was educated (and currently reside) is one of stressing the basic science and underlying mechanisms of food related phenomena. Finally, the Gordon Research Conferences on The Microbiological Safety of Foods have provided stimulating contact with food microbiologists who are passionately interested in getting to the bottom of things, and elucidating their mechanisms at the molecular level. These have enabled me to recognize the need for a volume that deals with the physiology of foodborne microbes at the molecular level.

The chapters in *Food Microbiology*, Volume I deal with the regulation of important intracellular processes ranging from osmoregulation of bacterial cells to germination of spores. This volume starts with an examination of how bacteria cope with reduced water activity. This is a basic question with very real ramifications in applied areas of food microbiology. Chapter 2 outlines the major physiological and biochemical mechanisms that bacteria use to regulate the movement of sugars and amino acids across their membranes. The importance of this process to foodborne microbes and those who seek to control them should be self-evident. Redox potential is another extrinsic parameter that is an important, but poorly understood aspect of microbial physiology. An introduciton to redox potential, the electron transport pathways, and electron carriers important to anaerobic bacteria is presented in Chapter 3 along with some applied aspects of this area. Chapter 4 details the genetic regulation of toxins synthesis by foodborne pathogens. This volume concludes with a review of how bacteria differentiate to form spores and vice versa.

One contributor to this volume remarked that writing her chapter was akin to having a baby. Editing this volume has been like fathering one; you plant the seed, support, cajole, and wait — and then you realize that the work has just begun. The authors, of course, did the real work and deserve the lion's share of the credit for this book. I also thank the other people who've helped along the way. Dr. Donald D. Bills at the USDA encouraged me during the early stages of planning this work. My department chairperson, Dr. Stephen S. Change, and my colleagues at Rutgers made my transition to academic life a smooth one enabling me to keep this project on track. The constructive reviews provided by Peggy Foegeding, Stephanie Doores, Terry Amoroso, James Smith, Hans Blaschek, and Scott Scioli were of great assistance and deeply appreciated. Finally, I thank my wife, Nancy, for her understanding and patience.

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Chapter 1

OSMOREGULATION BY MICROORGANISMS AT REDUCED WATER ACTIVITY*

Lloyd D. Witter and Carl B. Anderson

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* This chapter was received in final form in May, 1985.

I. INTRODUCTION

Just as in humans, the metabolism of microorganisms proceeds only in an aqueous environment. Active organisms contain more water than anything else. Bacteria range from 70 to 90% water, plant protoplasm from 80 to 90% water, and human body cells average 60% water.[1,2] The water in a living cell assumes many roles. It acts as a solvent of cellular metabolites, in the maintenance of turgor pressure, as a reactant or product (e.g., photosynthesis, hydrolysis, and polymerization), and to regulate the stability and reaction rate of other compounds through hydration or solvation. Water is a participant of any biochemical reaction that takes place in water even if it is not a specific reactant.[3]

An attempt was made to present a comprehensive review, but this was abandoned since it would have produced a reference list to challenge even that of Mossel.[4] Despite the rather massive literature on water relations and microorganisms, precious little appears firmly established. The information available is extensive and quite reliable, but the conclusions are not.

For the microbial cell, the external environment must be in thermodynamic parity with the internal cytoplasm or there will be a net movement of water either in or out of the cell. In a complex environment, such as a food product, much of the water present is associated with various solutes and even with insoluble solids.[5-13] The associated or bound water is unavailable to the microbial cell.[14] Clearly, an expression was needed to express available water and this need was not met by a measurement of the moisture content.

II. AVAILABLE WATER

In 1957, Scott[15] summarized his work, which was started in 1953,[16] in a review and convincingly established the value of water activity (a_w) for measuring the available water for supporting the growth of microorganisms. Water activity and the fate of microorganisms has since been extensively studied with subsequent reviews and books effectively reporting the progress.[17-30] The a_w was subsequently correlated to osmotic pressure,[31] pore size,[32,33] lipid oxidation,[34-38] vitamin degradation,[39,40] nonenzymatic browning,[41-47] enzymatic reactions,[48-50] and the heat resistance of microorganisms.[51-66]

A. Water Activity

Scott[15] defined a_w as the ratio of the vapor pressure of the solution to that of pure water, i.e.,

$$a_w = \frac{p}{p_o} \tag{1}$$

When no solutes are present, the activity of the water is 1.0. When solutes are added, there is an interaction between the solute and water and the a_w is reduced. For ideal solutions, the a_w is equal to the mole fraction of water, N_w, and follows Raoult's Law,

$$a_w = N_w = \frac{n_w}{n_s + n_w} \tag{2}$$

where n_w is the moles of water and n_s is the moles of solute. For nonideal solutions, (i.e., most solutes in other than very dilute solutions), the a_w is proportional rather than equal to the mole fraction of water and the proportionality constant is the activity coefficient (γ);

$$a_w = \gamma N_w \tag{3}$$

The activity coefficients of most relevant solutes at various concentrations and temperatures are available or can be calculated.[67-69] Regardless of whether the solution is ideal or not, the a_w is related to the equilibrium relative humidity, ERH, by the equation

$$a_w = \frac{ERH}{100} \tag{4}$$

The ERH is the relative humidity of an atmosphere in which, at equilibrium, the solution would have neither a net gain nor a loss of water.

B. Variations of Chemical Potential

There are alternative expressions for adequately representing the available water in a solution which some authors have suggested are more thermodynamically rigorous.[18,31,70-72]

1. Chemical Potential of Water

One of these alternate expressions is the chemical potential of water, μ_w, which is easily derived from the second law of thermodynamics.[73-75] The μ_w and a_w are related by the equation

$$\mu_w = \mu_o + RT \ln a_w \tag{5}$$

where μ_o is the standard state chemical potential of water, R is the gas constant, and T is the absolute temperature. Other variables which are parameters of the chemical potential such as gravitational potential, electrical potential, and the PV_w work, where P is the pressure and V_w is the partial molal volume of water, are not included in Equation 5. While the gravitational and electrical potentials are insignificant in microbial environments, a change in pressure can cause a substantial change in μ_w.[31]

2. Water Potential

The pressure and the difficulty of determining μ_o are taken into account by plant physiologists[3,18,73,76,77] by using the water potential, ψ, which is defined by the equation

$$\psi = \frac{\mu_w - \mu_o}{V_w} \tag{6}$$

The ψ has units of pressure and is always negative in real systems. The water moves from high water potential to low water potential. (One author in discussing water potential used the expression "psi" to identify the greek letter and received the manuscript back with an editor's correction of "lb/in^2" replacing it.) The water potential and a_w are related by the equation

$$\psi = P + \left(\frac{RT}{V_w}\right) \ln a_w \tag{7}$$

Temple[78] discusses the interconvertibility of ψ and a_w, suggesting that no useful purpose is served by continuing to use both in the literature. But, he does not suggest which to use and does not include the turgor pressure in his relating equation. In the equations for a_w, Equation 1 to 4, the temperature is only inferred while in Equation 7 for ψ the temperature is explicitly stated as a variable to be accounted for.

3. Osmotic Pressure

Osmotic pressure is the hydrostatic pressure necessary to prevent solvent, usually water

flow, across a membrane that is permeable only to the solvent. But, even without a membrane or an osmometer, osmotic pressure is still considered a colligative property of a solution.[2,67] It is certainly the oldest used expression for available water with most of the fundamental concepts being developed before the turn of the century. Hammel and Scholander[74] present a delightful glance at the historical developments. The osmotic pressure, π, is related to the a_w by the equation

$$\pi = -(RT/V_w) \ln a_w \quad (8)$$

However, an experimental value of a_w is rarely used to calculate π and π is rarely used to calculate a_w. The π as defined by Equation 8 is the second term on the right side of Equation 7 and the first term, P, of Equation 7 is the turgor pressure. Under conditions where the water potential is zero, P is equal to negative π and there is no net flux of water across a membrane. The food microbiologists frequently use the term "osmotic pressure" in a descriptive qualitative sense but don't usually measure its value. Only a few microbiologists report the available water in terms of osmolarity or osmolality which is the molarity or molality of an ideal solution of nondissociating substance that exerts the same osmotic pressure as the solution under consideration.

C. Some Thermodynamic Considerations

If Scott[15] had more rigorously defined a_w as the ratio of the fugacity in the given state to that in some arbitrary standard state at the same temperature instead of by Equation 1, then the concept of a_w would probably have never gotten off the ground. Fugacity and activity were introduced by Lewis[79] in 1901 to simplify the treatment of cases in which the ideal gas and ideal solution laws, respectively, do not apply. Indeed, the activity as defined by Equation 1 is exactly what Lewis had in mind to correct when he originated the concept of thermodynamic activity. Reid,[70] while supporting the concept of a_w, has reminded us of its more rigorous origin and has further alerted us to the deviations that might occur by the substitution of vapor pressure for fugacity. He also points out, with excellent examples, that a measure of relative escaping tendency, a_w, does not infer an identical molecular state of water or water availability in two systems with identical values of a_w.[80]

D. Measurement of Water Sorption Isotherms

Water influences the microbial, enzymatic, and chemical activities of a food. The magnitude of the influence is determined by the two parameters of moisture content and a_w.[7,10,81] Neither moisture content nor a_w is by itself a completely satisfactory criterion for categorizing these processes.[80,82,83] The two are related in the water sorption isotherm, which is a plot of one against the other. At low a_w, approximately 0.2, the moisture content increases more rapidly than a_w, from a_w of 0.2 to 0.8 the moisture content is almost constant, and at a_w greater than 0.8 the moisture content increases almost exponentially with increased a_w.[10,35] The sorption isotherm can be produced by following the fate of either moisture added to a dry material, adsorption, or removal of moisture from a wet material, desorption. Depending upon the material, adsorption and desorption may produce a hysteresis loop with desorption giving the higher moisture content for a given equilibrium water activity.

Our concern is with the water available to microorganisms or that water which is not bound. Bound water is a complex subject, but generally can be considered as the moisture level at which significant discontinuity in water behavior is observed.[7] These discontinuities are found in solubility properties, NMR spectrum, sorption curves, binding energies, and freezing points.[84] Microbial activity and other rate processes, with the exceptions of the browning reaction and lipid oxidation, increases with increasing a_w.[47,85-87]

For the construction of a water sorption isotherm, no matter how it is plotted, it is necessary

to measure moisture content and a_w. Since the sorption isotherm, a major caution in any procedure is that the temperature be carefully controlled and recorded.

1. Measurement of Moisture Content

Pande[88] has written a four volume *Handbook of Moisture Determination and Control* that should provide any details one would want. Also, a concise short review of techniques is given by Karmas.[89] Direct methods generally measure water loss by weighing before and after treatment. The treatments include oven drying, extraction, distillation, and desiccation. Caution is suggested in using gravimetric methods on material with high concentrations of volatiles like glycerol-containing intermediate moisture foods.[90] The standard Karl Fischer titration and azeotropic distillation are also direct methods of measurement. The less time-consuming indirect measurements usually require calibration using direct methods. Indirect methods include a variety of electrical measurements, sonic absorption, or IR and NMR spectroscopy.

2. Measurement of a_w

From a practical standpoint, the a_w is defined by the experimental method which is used for its determination. This methodology has been widely reviewed.[24,41,91-94] Troller[94] discusses the historically significant and little used bithermal equilibration technique as the closest approach to a reference method. Next closest to the direct measurement of a_w is the vapor pressure manometer,[95] although it apparently is difficult to use. The equivalence of equilibrium relative humidity and a_w also makes the various psychrometric methods, the sling psychrometer, the dew point hydrometer, and the saturated salt dew point sensor fairly direct measurements of a_w. While the hair hygrometer and the Abbeon cup both require calibration they are simple, reliable, and inexpensive methods. A variety of electric hydrometers are available which are expensive and require calibration, but have good precision, are rapid, and are easy to use. The easiest way to establish a given a_w is to allow the test solution to equilibrate in an atmosphere which has its relative humidity established by a saturated salt solution.[96,97] Tables are available of the a_w values of these saturated solutions.[98-102] These same solutions are used in the various isopiestic techniques.

III. MICROBIAL ACTIVITY AND a_w

A reduction in the a_w of the microbial environment generally results in a reduction of microbial growth rate, metabolic activity, and resistance to inimical agents. Only a few microorganisms require a reduction in a_w for best growth and usually this results from a specific salt effect. The limit of growth is a summation of the interplay of a_w with temperature, chemical inhibitors, ionic strength, nature of the solute, oxidation-reduction potential, competitive microflora, and possibly illumination.[103-106] Such interplay should be kept in mind although not explicitly stated in discussing the a_w boundaries.

Scott[15] contended that the effect of a_w on microbial growth and activity was independent of the solute involved, but a host of examples confirm the contrary.[19,58,107-112] The role of the solute is particularly emphasized when comparing the action of one that can penetrate the cell, such as glycerol, and one that cannot, such as sodium chloride or sucrose.[113-117] This is visually rather dramatically demonstrated by viewing the curves of the effect of solutes on the heat resistance of *Salmonella typhimurium*, Figure 1.[118] The bunched linear plots are the nonpenetrating solutes, sucrose, glucose, fructose, and sorbitol, and the wiggly curve separated from the others is the penetrating solute, glycerol. Clearly, the penetrating solute is having quite a different effect from the nonpenetrating ones.

The penetrating solute also automatically reverses or minimizes plasmolysis resulting from a hypertonic growth medium. Such a solute balances itself across the cell membrane. While a high concentration of this solute inside the cell may be detrimental, the problem to the

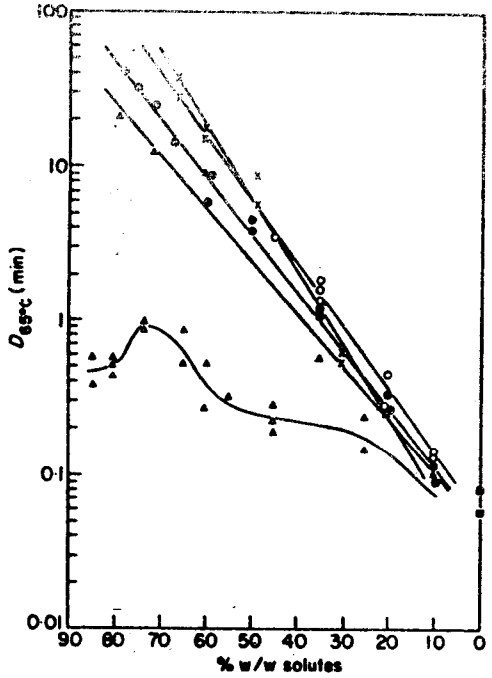

FIGURE 1. The effect of solute concentration on the heat resistance of *S. typhimurium* 7M 4987 in 0.1 *M* phosphate buffer at pH 6.5 with x, sucrose; ○, glucose; △, fructose; ●, sorbitol; ▲, glycerol; ■, no added sugar. (From Corry, J. E. L., *J. Appl. Bacteriol.*, 37, 31, 1974. With permission.)

Table 1
THE MINIMAL a_w LIMITS OF GROWTH OF MICROORGANISMS AND SOME BENCHMARKS

Minimal a_w	Microorganisms or benchmark
0.94	Gram-negative bacteria
	The most a_w-tolerant *Clostridium botulinum*
0.90	Bacteria
0.88	Yeasts
0.86	Ice at −15°C
0.85	Intermediate moisture foods — upper limit
0.83	The most a_w-tolerant nonhalophile *Staphylococcus aureus*
0.80	Molds
0.75	Halophilic bacteria
	Saturated solution of NaCl
0.60	Osmophilic yeasts
	Xerophilic molds
0.55	DNA instability

cell is not one of dealing with osmotic imbalance. Osmotic imbalance is a problem with a nonpenetrating solute and requires a strategy of osmoregulation.

A. Minimal a_w Supporting Growth

The general lower limits of growth for various microbial groups are indicated in Table 1. While Gram-positive bacteria are unlikely to have a minimum a_w below 0.90, they are

usually less sensitive to reduced a_w than the Gram-negative bacteria which are unlikely to grow below an a_w of 0.94.[114,118-120] An a_w of 0.94 is also the lowest reported for the growth of *Clostridium botulinum* type B,[121] which is considerably lower than type E that does not grow below a_w of 0.97.[122] *Staphylococcus aureus* is the most resistant nonhalophilic bacterium and when grown aerobically is usually reported to have a minimal a_w of 0.86[15,110,123,124] but has been reported to grow as low as an a_w of 0.83.[125] Anaerobically, it does not grow below an a_w of 0.91.[16]

The halophilic bacteria comprise a unique group. Not only can they grow in a saturated salt solution, but they require at least 2 *M* NaCl for growth and prefer 3 to 5 *M*. They also differ from nonhalophilic bacteria by not producing peptidoglycan, by producing ether-linked lipids rather than the esterified fatty acids linked to glycerol, and by producing a diether analog of phosphatidyl glycerol phosphate for phospholipids.[18]

Yeasts and molds are rarely inhibited at an a_w of 0.94. Unlike the bacteria, there is no clear demarcation between the osmophilic and nonosmophilic yeast or between the xerophilic and nonxerophilic mold. There appears to be a continuum and this requires an arbitrary definition to separate the two groups. While there are some small variations in the suggested a_w, the tolerant organisms are usually defined as those that can grow at or below an a_w of 0.85.[22,126-128] The above choice of definition was a convenient one since an intermediate moisture food is also arbitrarily defined as having an upper limit of an a_w of 0.85,[35,105,129,130] although some consider this a tad too high.[131] While most yeasts will not grow below an a_w of 0.88, there is still a handful of species able to grow at an a_w below 0.85. Most molds, on the other hand, have their minimal a_w for growth spread out between an a_w of 0.85 and 0.70 with still a half-dozen species growing below that. The lower limit, the marathon run for microbial growth, is an a_w of around 0.60. Falk et al.,[132] in a spectroscopic study of the effect of hydration on the structure of DNA, showed that between an a_w of 0.60 and 0.55 the DNA became reversibly disordered with an increase in the dichroic ratios and absorbance. These changes are similar to those observed when DNA is thermally denatured.

The general trend described above for yeasts, molds, and bacteria can be further observed in Table 2 which gives the approximate minimum a_w for the growth of individual species. Table 2 is a compilation of previous compilations by Corry,[133] Pitt,[126] Beuchat,[134] Rose,[135] Measures,[136] Measures and Gould,[137] Troller and Christian,[24] Brown,[18] Leistner et al.,[105,106] and Ben-Amotz[138] plus a few individual additions. Algae, which have not been considered before, have a water requirement profile similar to the bacteria. Most algae have a minimum a_w of 0.98, only a few can grow below an a_w of 0.90, and there is a large difference between the minimum a_w for the halophilic algae and the nonhalophilic species. The halophilic algae have the same lower limit of growth as the halophilic bacteria.

B. Physiology, Metabolism, and a_w

While microbial growth may be an overall measure of the physiology of a cell, individual metabolic events may be either more sensitive or more refractile to changes in environmental factors than growth. Formation of secondary metabolites which are independent of growth, might be expected to respond quite differently than growth to changes in a_w. Even the formation of primary metabolites, which is growth dependent, may be more or less limited by the a_w than growth.

1. Toxin Production

Staphylococcus aureus forms five enterotoxins, A, B, C, D, and E, of which enterotoxin A is the most commonly encountered in foods. Early workers showed that the progressive addition of NaCl to the growth medium caused a decrease in the growth rate of *S. aureus* and an even greater suppression of toxin production.[139-141] For example, at pH 6.9 growth was observed at 16% NaCl, while enterotoxin B was suppressed at greater than 10% NaCl. At pH 5.5 growth was observed up to 12% NaCl, while enterotoxin B was suppressed at

Table 2
THE MINIMAL A_W LIMITS OF GROWTH OF INDIVIDUAL SPECIES OF MICROORGANISMS

a_w	Bacteria	Yeasts	Molds	Algae
1.00				
0.99	*Bacillus cereus var. mycoides* *Spirillum serpens*			*Ochromonas malhamenis* *Klebsormidium marinum* *Chlorella emersonii*
0.98	*S. undula* *Enterobacter agglomerans*	*Schizosaccharomyces octosporus* *Saccharomyces pastori*		*Stichococcus bacillaris* *Cyclotella cryptica* *C. meneghinana* *Ascophyllum nodosum* *Colpomenia sinuosa* *Dictyota dichotoma* *Ecklonia radiata* *Scytosiphon lomentaria* *Monallantus salina* *Rhodymenia foliifera* *Centroceras clavulatum* *Iridophycus flaccidum* *Porphyra perforata*
0.97	*Clostridium botulinum* *Pseudomonas fluorescens* *P. aeruginosa*	*Hansenula suaveolens*		*Monochrysis luteri*
0.96	*Flavobacterium* sp. *Klebsiella* sp. *Shigella* sp.	*Kluyveromyces fragilis*	*Rhizoctonia solani*	*Synecococcus sp.*
0.95	*Escherichia coli* *Salmonella oranienburg* *S. newport* *Clostridium perfringens* *C. botulinum* A *Lactobacillus viridescens* *Bacillus cereus* *B. megaterium* *Vibrio parahaemolyticus* *Alcaligenes* sp. *Citrobacter* sp. *Propionibacter* sp. *Proteus* sp. *Serratia* sp.	*Saccharomyces microellipsoides*		*Platymonas subcordiformis* *P. suecica*
0.945	*Clostridium sporogenes* *Lactobacillus plantarum* *Bacillus megaterium*			

Table 2 (continued)
THE MINIMAL A_W LIMITS OF GROWTH OF INDIVIDUAL SPECIES OF MICROORGANISMS

a_w	Bacteria	Yeasts	Molds	Algae
	Serratia marcescens			
	Clostridium botulinum A			
0.94	*Klebsiella aerogenese*	*S. cerevisiae*	*Stachybotrys atra*	*Chlamydomonas* sp.
	Clostridium botulinum B	*Candida utilis*		*Pilayella littaoralis*
	Vibrio parahaemolyticus			*Porphyra purpurea*
	Microbacterium sp.			*Paraphysomonas vestita*
	Streptococcus faecalis			
	Lactobacillus viridescens			
	Pediococcus sp.			
	Microbacterium thermosphactum			
0.93	*Bacillus stearothermophilus*	*Candida pseudotropicalis*	*Botrytis cinerea*	
	Micrococcus lysodeikticus		*Mucor spinosus*	
	Enterobacter agglomerans		*Rhizopus nigricans*	
0.92	*Sarcina lutea*	*Saccharomyces cerevisiae*		
	Bacillus cereus	*Pichia* sp.		
	Salmonella typhimurium	*Rhodotorula*		
0.91	*Corynebacterium* sp.			
0.90	*Pediococcus* sp.	*Saccharomyces cerevisiae*	*Epicoccum nigrum*	*Botryococcus* sp.
	Staphylococcus aureus (anaerobic)	*Hansenula*	*Mucor circinelloides*	*Navieula* sp.
	Bacillus subtilis		*Phythium splendens*	
			Trichothecium roseum	
0.89				
0.88	*Staphylococcus albus*	*Aureobasidium pullulans*	*Cladosporium herbarum*	
		Hansenula anamala		
		Debaromyces		
		Hanseniaspora		
		Torulopsis		
0.87				*Porphyra umbilicalis*
0.86	*Vibrio costicolusbans*	*Saccharomyces rouxii*		
	Staphylococcus aureus (aerobically)			
0.85	*Micrococcus haldenitrificans*	*S. rouxii*	*Cladosporium cladosporioides*	*Aphanotheca halophytica*
			Alternaria tenuissima	
			Penicillium cyclopium	
			P. rugulosum	
			Aspergillus clavatus	
0.84		*Saccharomyces rouxii*	*Paecilomyces variotii*	
			Aspergillus niger	
			Alternaria citri	
			Aspergillus wentii	
			Byssochlamys nivea	

Table 2 (continued)
THE MINIMAL A_W LIMITS OF GROWTH OF INDIVIDUAL SPECIES OF MICROORGANISMS

a_w	Bacteria	Yeasts	Molds	Algae
0.83	*Staphylococcus aureus* (lowest)	*Debaryomyces hansenii*	*Penicillium islandicum*	*Asteromonas gracilis*
			P. expansum	
			P. patulum	
			P. cyclopium	
			P. martensii	
			Aspergillus niger	
			A. viridicatum	
			A. parasiticus	
			A. nidulans	
0.82			*A. fumigatus*	
0.81			*P. patulum*	
			P. puberulum	
			P. viridicatum	
			P. brevicompactum	
			P. cyclopium	
			P. frequentans	
			A. flavus	
			A. ochraceus	
0.80		*Saccharomyces bailii*	*P. citrinum*	
		S. cerevisiae	*P. satoryi*	
			P. fellutanum	
			P. spinulosum	
0.79			*P. martensii*	
			P. chrysogenum	
0.78			*A. flavus*	
			A. niger	
			A. tamarri	
			A. nidulans	
			A. terreus	
			A. versicolor	
			A. versicolor	
			A. sydowi	
0.77			*A. ochraceus*	
			A. niger	
			Eremascus tertilis	
0.76			*A. ochraceus*	
0.75	*Halobacterium salinarium*		*A. restrictus*	*Dunaliella* sps.
	Halococcus morrhuae		*A. candidus*	
	Actinospora halophila		*Aspergillus amstelodami*	
			Wallemia sebi	
0.74			*Eurotium carnoyi*	
			E. herbariorum	
0.73			*Aspergillus chevalieri*	
0.72				
0.71			*A. repens*	
			A. amstelodami	
			A. chevalieri	
			A. glaucus	
			Chrysosporium xerophilium	
0.70		*Saccharomyces bisporus* var. *mellis*	*A. rubrum*	

Table 2 (continued)
THE MINIMAL A_W LIMITS OF GROWTH OF INDIVIDUAL SPECIES OF MICROORGANISMS

a_w	Bacteria	Yeasts	Molds	Algae
			A. conicus	
			A. amstelodami	
			Eremascus albus	
0.69			*Chrysosporium fastidium*	
0.68				
0.67				
0.66				
0.65		*Torulopsis famata*		
		Saccharomyces rouxii		
0.64			*A. echinulatus*	
0.63				
0.62		*S. rouxii*		
0.61			*Xeromyces bisporus*	
0.605			*X. bisporus*	

greater than 8% NaCl.[142] The formation of enterotoxin A or B was also much more sensitive to a reduction of a_w than was growth,[143-145] as perhaps should be expected. In two food systems, the minimum a_w for toxin production was an a_w of 0.93 or 0.91 depending on the strain and enterotoxin,[146] which was somewhat higher than had been observed on laboratory medium. Lotter and Leistner[147] found the minimum a_w for growth of 0.867 was the same as that for enterotoxin A production at 30°C in a salt mixture broth. This is close to the a_w of intermediate moisture foods and may have implications upon their safety.[148] Although enterotoxin A, B, and C may be produced by nonreplicating cells,[149] this does not infer an extension of enterotoxin formation beyond conditions necessary for growth.

The minimum a_w for toxin production by *Clostridium botulinum* strains, while of great interest, is complicated by the interacting factors involved such as NaCl concentration and pH[140,150-152] oxidation-reduction potential,[153] proteolytic activity, trypsinization, and the behavior and magnitude of a tolerant subpopulation.[154,155] Regardless of the interplay of the variables of the growth environment, a few generalizations appear to be unchallenged; the formation of toxin by type E is more sensitive to reduced a_w than that of either type A or type B and neither growth nor toxin production has been reported at an a_w less than 0.93.

The minimum a_w for growth or toxin formation by *C. botulinum* type E was reported at 0.97.[121,122,156,157] This value may be revised upward depending on the solute used to adjust the a_w. For example, Emodi and Lechowich[122] found the minimum a_w with sucrose was from 0.980 to 0.977, with KCl or NaCl from 0.978 to 0.976, and with glucose from 0.976 to 0.971. For growth or toxin formation by *C. botulinum* type A or type B, the minimum a_w is usually set at 0.95,[121,158] although Ohye and Christian[121] describe a situation where type B showed toxin production at an a_w of 0.94.

Several reviews, Troller,[23] Troller and Christian,[24] and Leistner et al.,[106] summarize the water requirements of toxin-forming foodborne pathogens. Smith et al.[159] have reviewed staphylococcal enterotoxin synthesis.

Only a few investigations have been made on the effect of a_w on mycotoxin formation, but the series of papers, including concise reviews by Northolt and Bullerman[160] and by Northolt and co-workers are particularly impressive.[161-165] Also, the effect of a_w on mycotoxin production was briefly reviewed by Corry[133] and by Beuchat.[134] Mycotoxin formation is considerably more sensitive to a reduction in a_w than is the growth rate of the mold forming it. For example, the minimum a_w for patulin production by *Penicillium expansum, P. pa-*