

# WATER ACTIVITY AND MICROBIOLOGICAL ASPECTS OF FOODS A KNOWLEDGE BASE

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## Introduction

Water activity,  $a_w$ , is a physical property that has a direct implication for microbiological safety of food. Water activity also influences the storage stability of foods as some deteriorative processes in foods are mediated by water. Storage life of dry foods such as biscuits is generally longer than of moist foods such as meat at the same temperature. In this connection freezing of foods is equivalent to drying – the water is removed from the food matrix although it is still in the food as ice.

Because of this strong association between the physical property,  $a_w$ , and the chemical and microbiological properties of food, it is appropriate to include these aspects in the form of a Knowledge Base on this website.

## Definitions of Water Activity

From the physicist's point of view, water activity is defined in terms of thermodynamic concepts such as the chemical potential and is related to the osmotic pressure of an aqueous solution. When a substance such as salt (sodium chloride) is dissolved in water, the water activity is reduced. This is why salting is an ancient way of preserving foods.

The  $a_w$  of a food or solution is the ratio of the water vapour pressure of the food or solution ( $p$ ) to that of pure water ( $p_0$ ) at the same temperature: -

$$a_w = p/p_0$$

The  $a_w$  is related to the boiling and freezing points, equilibrium relative humidity (ERH; see above equation), and osmotic pressure. The  $a_w$  of a solution is a 'colligative property', i.e. dependent upon the number of 'particles' (molecules or ions) in solution. Increases in solute concentration decreases  $a_w$ . Microorganisms require water for solution of cell contents and metabolic processes. The cell membrane is 'semi-permeable' (or more correctly selectively permeable), and decreases in the  $a_w$  of the suspending medium below a certain maximum value (dependent upon the specific organism) will withdraw water from the cell, concentrating the cellular contents until the internal and external  $a_w$  values are in balance. This concentrating effect slows metabolic processes until at a limiting value, growth ceases. Many microorganisms under osmotic stress (low  $a_w$ ) can accumulate or synthesize 'compatible solutes' to relieve the stress. These solutes generally

interfere little with the metabolic functions of the cell, and may be accumulation of  $K^+$  ions, accumulation or synthesis of proline, glutamine, betaine, certain sugars or sugar alcohols (e.g. trehalose in yeasts), etc. However, this activity also requires energy, diverting some of the metabolic activities from growth to accumulation of solutes, and resulting in lowering of growth rates.

## Microbiological definitions

Halophiles, halotolerant organisms, xerophiles,

Micro-organisms generally grow best between  $a_w$  values 0.995-0.980, while most microbes cease growth at  $a_w < 0.900$ . However, halophiles ('salt-loving') are unable to grow in salt-free media and often have an obligatory requirement for substantial concentrations of salt (NaCl). For example *Halobacterium halobium* will not grow in salt concentrations below *ca* 14% w/w,  $a_w$  *ca* 0.89. Halotolerant organisms, while capable of growth at low  $a_w$  / high salt concentrations, grow best at high  $a_w$  values, e.g. *Staphylococcus aureus* will grow at  $a_w$  0.90 at *ca* 10% of the maximum rate at  $a_w$  *ca* 0.98.

Xerophilic organisms grow best at low  $a_w$  values adjusted with sugars, for example the mould *Xeromyces bisporus* grows best at  $a_w$  *ca* 0.92, although is capable of growth at  $a_w$  0.70 (*ca* 10% of maximum rate), but ceases growth at  $a_w$  *ca* 0.96, when  $a_w$  is adjusted with sucrose.

Microorganisms react not only to  $a_w$  *per se*, but also to the solute adjusting the  $a_w$ . Minimum  $a_w$  values for growth are often very different for different solutes.

## Food poisoning, food-borne infections

Food poisoning is the result of ingesting a pre-formed toxin in food. These toxins may result in vomiting (e.g. *Staph. aureus* or *Bacillus cereus* enterotoxins), or other systemic effects, e.g. botulinal neurotoxin paralysis of the nerve-muscle junction. Food-borne infections result from ingesting an organism capable of surviving the acidic environment of the stomach and growing in the intestinal tract, e.g. *Salmonella* spp. Gastro-intestinal symptoms, e.g. diarrhoea, result from toxic metabolites produced in the gut.

## Spoilage

Microbial spoilage of foods results from changes in the food composition, and/or appearance or structure as a result of the growth and metabolism of microorganisms. Commonly the evolution of obnoxious odours is the cause for rejection of foods, e.g. fresh meats, although the appearance of mould colonies on semi-dry foods, e.g. bread, cheeses, is also common. A wide range of organisms can be responsible for spoilage, and therefore a wide range of changes in foods may be regarded as 'spoilage'. Certain 'controlled spoilage' by micro-organisms is used to produce a different food from the starting ingredients, e.g. yoghurt or cheese from milk, fermented sausages from raw meats, sauerkraut from shredded cabbage.

## Solute Effects on Microbial Growth and/or Death Cell Membrane Phenomena

The cell membrane is semi-permeable, or rather selectively permeable. Thus glycerol penetrates the membrane readily, glucose penetrates poorly, sucrose very poorly, and NaCl is almost non-penetrating. When an organism is grown or exposed to low  $a_w$  conditions, the cells may accumulate from the environment or synthesize 'compatible solutes', e.g. glutamine, proline, betaine in bacteria, trehalose in yeasts. These internal solutes interfere little with the metabolism of the cell, although metabolic energy must be diverted for synthesis, but increase resistance to low external  $a_w$  conditions, and also increases resistance to other injurious treatments, e.g. heat. This effect differs with different external solutes, e.g. *Staph. aureus* synthesizes compatible solutes at high NaCl levels, but not in the presence of sugar.

If the partially dehydrated cell is exposed to a high temperature, then the microorganism displays a greater thermal resistance than when grown at a higher  $a_w$ . Proteins and other essential cellular components are more resistant to thermal damage in the partially dehydrated state. Water activity plays an important role in the heat resistance of microbes (see Table 3). Death curves are not always linear and interpolation of D-values (and z-values) into application of thermal processes may not always be safe. Similarly, the ratio of effects of the different solutes on D-values (as in Table 3) differs for each organism. Thus D-values in low  $a_w$  solutions or foods must always take into account the actual solute controlling the  $a_w$ , and if necessary be determined in that solute.

### Interactions with other physico-chemical parameters

Since extra energy is required to combat the inimical effects of low  $a_w$ , any other conditions also requiring expenditure of extra energy, e.g. low pH, presence of preservatives, will result in an additive or synergistic effect in limiting microbial growth. Thus even moderate reductions in  $a_w$  in combination with low levels of preservative or pH values, can be sufficient to inhibit growth. One good example is that of inhibition of *Clostridium botulinum*. Under ideal conditions 10% NaCl is required to inhibit the proteolytic species; at the pH values typical of meats (ca. pH 5.4 – 5.8) and in the presence of ca 100ppm nitrite, only 3.5% NaCl is required to produce botulinal-stable cured meats.

## TABLES

Table 1. Aw values of sugar solutions

Aw	Glucose %w/w	Glucose Syrups %w/w		
		DE 42.0	DE 55.0	DE 83.4
0.99	8.90	3.34	3.15	2.73
0.96	28.51	13.36	12.59	10.90
0.90	48.54	33.39	31.49	27.25
0.88	53.05	40.07	37.78	32.70
0.85	61.84	50.09	47.23	40.88

DE, Dextrose equivalent, i.e. the concentration (%w/w) of glucose in syrup.

Table 2. Minimum\* aw values for microbial growth

Food poisoning organisms		Food-borne infectious organisms	
Name	Min a <sub>w</sub> for growth*	Name	Min a <sub>w</sub> for growth
<i>Bacillus cereus</i>	0.95	<i>Clostridium perfringens</i>	0.95
<i>Campylobacter coli</i>	0.97	<i>Escherichia coli</i>	0.95
<i>C. jejuni</i>	ca. 0.98	<i>Salmonella</i> spp.	0.95
<i>Clostridium botulinum</i> :		<i>Vibrio parahaemolyticus</i>	0.94
Type A	0.95	<i>Yersinia enterocolitica</i>	0.96
Type B	0.94		
Type E	0.97		
<i>Listeria monocytogenes</i>	0.92		
<i>Staphylococcus aureus</i>	0.86		

\*, the minimum a<sub>w</sub> for growth of bacteria is generally by addition of salt. Minimum a<sub>w</sub> for growth with other solutes may be different. For toxin production minimum a<sub>w</sub> values may be rather higher.

Table 3. Effect of solutes on D-values of *Salmonella* spp.

Solute	%w/w	D <sub>65</sub> -values (mins)	
		<i>S. typhimurium</i>	<i>S. senftenberg</i>
Sucrose	30	0.7	1.4
	70	53	43
Glucose	30	0.9	2.0
	70	42	17
Fructose	30	0.5	1.1
	70	12	8.5
Glycerol	30	0.2	0.95
	70	0.9	0.7

## Mathematical Models describing microbial growth/death.

### Predictive modelling

To assess the microbiological safety or shelf life of foods the traditional methods were to inoculate foods with the organisms of concern, incubate under conditions appropriate to the food and enumerate the organisms or assess spoilage/acceptability. When the parameters / recipes of the foods changed the exercise had to be repeated with the new conditions, and no prediction could be made regarding the behaviour of the organisms in the food. Predictive microbiology is concerned with collecting growth/survival data for relevant organisms in simple media systems under a wide range of physico-chemical conditions, e.g.  $a_w$ , pH, temperature, gas atmospheres, preservatives, etc. From this data, mathematical equations (models) describing the growth/survival/death of the organisms over the range of conditions studied are constructed. These are then validated in real food systems for which the physico-chemical conditions are known. If good agreement is achieved between the predicted and observed values then the model may be used to predict, within the range of conditions studied, growth, survival or death of those organisms under conditions for which data has not been collected. Several equations have been suggested to fit growth/death curves, most of which are empirical models. Response surface models are generally used to represent the interrelationships between the physico-chemical conditions and growth/death although neural networks are also being investigated for this purpose. Some of the most common models are:

- Ratkowsky - originally derived for the effects of temperature on spoilage of fish (initially developed at Torry Research Station, Aberdeen, UK and expanded more recently at Hobart, Tasmania) square root plots relate microbial growth with square roots of parameters)
- Baranyi (Institute of Food Research, Reading, UK) – modelling of the initial lag after inoculation as well as subsequent growth and saturation, in terms of an autonomous differential equation. This model is capable of accommodating the effect of changing ambient temperature and is considered for the next major upgrade of the UK MAFF Food Micromodel.
- Buchanan (Pathogen Modelling Program, USA)
- Gompertz – based on the logistic sigmoidal curve and exists in various modified versions.

The Buchanan and Gompertz models have been utilised in a commercial software Food Micromodel. This has been developed using funding from the UK Ministry of Agriculture, Fisheries and Food in the 1990s. The software runs on PCs and is distributed by Oxoid Ltd, England.

### FIGURES

Growth rate vs  $a_w$ .

FMM growth predictions for different  $a_w$ /solute.