WATER ACTIVITY AND MICROBIAL STABILITY

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Ecology of Foodborne Microorganisms

Large variety of initial microflora

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Selective pressure of ecological factors

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Characteristic microbial community for each type of food

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Specific associations are influenced by a_w

Factors Influencing Growth

- Temperature
- Water activity (a_w) (0.61 - 0.999)
- Oxygen
- Nutrients
- Acidity and pH
- Natural and added inhibitors

Water content of foods at a_w 0.70

Food	Water Content (%, w/w)
Nuts	4 - 9
Whole milk powder	7
Cocoa	7 - 10
Soybeans	9 - 13
Dried whole egg	10
Skim milk powder	10
Dried lean meat and fish	10
Rolled oats	11
Rice	12 - 15
Pulses	12 - 15
Dried vegetables	12 - 22
Wheat flour, pasta	13 - 15
Dried soup mixes	13 - 21
Dried fruits	18 - 25

Adapted from Mossel, Crit. Rev. Environ. Control, 5:1-139 (1975)

Lower limits of moisture for growth of molds on cereal grains and oilseeds

Mold	Moisture (%, w/w)		
	Corn, wheat, sorghum, rice	Soybeans	Peanuts, sunflower seeds, copra
Aspergillus candidus	15.0 - 15.5	14.5 - 15.0	10.0 - 11.0
A. flavus	18.0 - 18.5	17.0 - 17.5	11.0 - 12.0
A. glaucus	14.0 - 14.5	12.5 - 13.0	8.0 - 9.0
A. halophilicus	13.5 - 14.5	12.0 - 12.5	9.0 - 10.0
A. ochraceus	15.0 - 15.5	14.5 - 15.0	10.0 - 11.0
A. restrictus	13.5 - 14.5	12.0 - 12.5	9.0 - 10.0
Penicillium spp.	16.5 - 19.0	16.0 - 18.5	11.0 - 13.0
Wallemia sebi	13.5 - 14.5	12.0 - 12.5	9.0 - 10.0

Adapted from Christensen, Food and Beverage Mycology, p. 211-232 (1987)

Definition and Measurement

- Proportion of water available for biological (biochemical) and chemical reactions

- For an ideal solution:

$$a_w = \frac{n_1}{n_1 + n_2} = \frac{p}{p_0} = \frac{ERH}{100}$$

Where n_1 = moles of solvents (water)
n_2 = moles of solute
p = vapor pressure of solution
p_0 = vapor pressure of solvent

Water Activity Affects the Behavior of Microorganisms

- Growth
- Sporulation
- Toxin production, stability
- Survival during processing, storage
- Recovery on agar media

Minimum a_w for Growth Depends on Other Factors

- Temperature
- pH
- Nutrient availability
- Antimicrobial substances
- Procedure for formulation

Xerophilic Fungi

- Growth characteristics
  - Xerotolerance depends on genus
  - Range for growth is a_w 0.61 - 0.99
  - Spoilage of foods with $a_w < 0.85$ usually involves xerotolerant yeasts or molds
  - Lower a_w limit for growth depends on availability of nutrients, pH, type of solute, temperature

Minimum a_w for growth of microorganisms

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
1.00 - 0.95	*Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens,* some yeasts	Highly perishable (fresh) foods and canned fruits, vegetables, meat, fish, and milk; cooked sausages and breads, foods containing up to approximately 40% (w/w) sucrose or 7% NaCl

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.95 - 0.91	*Salmonella, V. parahaemolyticus, C. botulinum, Serratia, Lactobacillus, Pediococcus,* some molds, yeasts (*Rhodotorula, Pichia)*	Some cheese (Cheddar, Swiss, Muenster, Provolone), cured meat (ham), some fruit juice concentrates, foods containing 55% (w/w) sucrose or 12% NaCl

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.91 - 0.87	Many yeasts (*Candida, Torulopsis, Hansenula), Micrococcus*	Fermented sausage (salami), sponge cake, dry cheeses, margarine, foods containing 65% (w/w) sucrose (saturated) or 15% NaCl

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.87 - 0.80	Most molds (mycotoxigenic penicillia), *Staphylococcus aureus,* most S*accharomyces, Debaryomyces*	Most fruit juice concentrates, sweetened condensed milk, chocolate syrup, maple and fruit syrups, flour, rice, pulses containing 15-17% moisture, fruit cake, country-style ham, fondants, high-ratio cakes

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.80 - 0.75	Most halophilic bacteria, mycotoxigenic aspergilli	Jam, marmalade, marzipan, glacé fruits, some marshmallows
0.75 - 0.65	Xerophilic molds (*Aspergillus chevalieri, A. candidus, Wallemia sebi),* *Saccharomyces bisporus*	Rolled oats containing approximately 10% moisture, grained nougats, fudge, marshmallows, jelly, molasses, raw cane sugar, some dried fruits, nuts

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.65 - 0.61	Osmophilic yeasts (*Zygosaccharomyces rouxii),* few molds (*Aspergillus echinulatus, Monascus bisporus)*	Dried fruits containing 15-20% moisture, some toffees and caramels, honey

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.30	No microbial proliferation	Cookies, some crackers, bread crusts, etc. containing 3-5% moisture
0.20	No microbial proliferation	Whole milk powder containing 2-3% moisture, dried vegetables containing approximately 5% moisture, corn flakes containing approximately 5% moisture, some crackers

Range of a_w	Microorganisms inhibited by lowest a_w in this range	Foods generally within this range
0.50	No microbial proliferation	Pasta containing approx-imately 12% moisture, spices containing approximately 10% moisture
0.40	No microbial proliferation	Whole egg powder con-taining approximately 5% moisture

Adaptation to Reduced a_w

- Plasmolysis occurs when cells are subjected to lower a_w (higher osmotic pressure)

- “Compatible” solutes are synthesized or taken up from the environment to increase internal osmotic pressure
  - Bacteria: free amino acid pool increases, with glycine betaine (trimethyl glycine) predominating. Sucrose, trehalose, glycerol, and/or glutamate and K^+ may also accumulate
  - Yeasts and molds: mannitol, glycerol, arabitol, sorbitol, erytritol, and K^+ are most often accumulated. Glucose, fructose, sucrose, and proline may also increase within cells
  - Compatible solutes can also serve as energy reserves

Solutes accumulated by fungi to response to reduced a_w

Fungus	A_w-Controlling solute	Solute(s) accumulated
Saccharomyces cerevisiae	NaCl	Glycerol
Zygosaccharomyces rouxii	NaCl	Glycerol
Debaryomyces hansenii	NaCl	Glycerol, arabitol, K^+
Penicillium ochro-chloron	NaCl	Glycerol
Penicillium chrysogenum	KCl	Glycerol and K^+
	Glucose	Glycerol, glucose, K^+
Chrysosporium fastidium	Glucose	Glucose and glycerol
	KCl	Glucose and glycerol
Xeromyces, bisporus, Wallemia sebi, Eurotium chevalieri, Penicillium digitatum	NaCl	Glycerol
	Sorbitol	Sorbitol and glycerol
	Glucose and fructose	Glucose, fructose, and glycerol
Mucor hiemalis, Pythium debaryanum	KCl	K^+ and proline
	Sucrose	Sucrose or glucose and fructose, proline

Spore Production and Germination

- When the lower a_w limit for growth is approached, sporulation often occurs
- Minimum a_w for germination is usually higher than the minimum a_w for sporulation

Effects of pH and solute on minimum a_w for germination of fungal spores at 25° C

Solute	pH	*Aspergillus ochraceus* Minimum a_w	*Aspergillus ochraceus* Germ. time (d)	*Wallemia sebi* Minimum a_w	*Wallemia sebi* Germ. time (d)
NaCl	4.0	0.839	6	0.805	5.8
	6.5	0.805	69	0.751	12
Glucose/fructose	4.0	0.792	12	0.691	38
	6.5	0.787	21	0.711	42

Adapted from Pitt and Hocking, J. Gen. Microbiol., 101:35 (1977)

Minimum a_w for growth and toxin production by bacteria

	Minimal a_w	
Bacteria	Growth	Toxin Production
Staphylococcus aureus	0.86	0.87 (enterotoxin A) 0.97 (enterotoxin B)
Salmonella	0.92-0.95	
Vibrio parahaemolyticus	0.94	
Clostridium botulinum	0.93 (A, B) 0.95 (E)	0.94 (A, B) 0.97 (E)
Clostridium perfringens	0.93-0.95	
Bacillus cereus	0.93-0.95	

Minimum a_w for growth and mycotoxin production

Mycotoxin	Mold	Minimum a_w: Growth	Minimum a_w: Toxin production
Aflatoxin	*Aspergillus flavus*	0.78 - 0.84	0.84
			0.83 - 0.87
Ochratoxin	*A. parasiticus*	0.82	0.87
	A. ochraceous	0.77- 0.81	0.83 - 0.87
Penicillic acid	*Penicillium cyclopium*	0.82 - 0.85	0.87 - 0.90
	P. viridicatum	0.80 - 0.81	0.83 - 0.86
	A. ochraceous	0.77	0.80 - 0.88
Patulin	*P. cyclopium*	0.82 - 0.85	0.97
	P. marensii	0.79	0.99
	P. patulum	0.81 - 0.85	0.95
Stachybotryn	*P. expansum*	0.82 - 0.84	0.99
	Stachybotrys atra		
		0.94	0.94

Estimates of half-lives of patulin and citrinin for various grain-a_w combinations at 25° C

		Half-life (days)	
Grain	a_w	Patulin	Citrinin
Barley	0.70	12.7	7.8
	0.90	6.8	1.8
Corn	0.70	4.4	15.5
	0.90	2.4	10.4
Wheat	0.70	4.4	11.9
	0.90	1.9	3.0

Adapted from Harwig et al., J. Food Sci., 42:1225 (1997)

Heat Resistance

- Heat resistance increases as the a_w of the environment decreases
- At a given a_w, the type of solute can influence the rate of thermal inactivation

Influence of a_w and type of solute on D values of mold conidia

Fungus	a_w	Temp. (°C)	Solute	D value (min)
Aspergillus flavus	0.99	55	None	3
	0.90		NaCl	70
	0.90		Sucrose	66
	0.85		Glucose	66
Aspergillus parasiticus	0.99	55	None	8
	0.90		NaCl	230
	0.90		Sucrose	199
	0.85		Glucose	214
Aspergillus niger	1.00	55	None	6
	0.60		None	100
	0.30		None	216
	0.00		None	100
Penicillium puberulum	0.99	48	None	31
	0.89		Sucrose	30
	0.93		NaCl	30

Influence of a_w and type of solute on D values of fungal ascospores

Fungus	a_w	Temp. (°C)	Solute	D value (min)
Byssochlamys nivea	0.98	75	Sucrose	60
	0.92		Sucrose	260
	0.84		Sucrose	470
	0.99	80	Control	39
	0.93		NaCl	48
	0.89		Sucrose	49
Zygosaccharomyces bailii	0.999	80	Control	8.5
				10
	0.975		NaCl	9.4
				20
	0.975		Sucrose	11
				16
	0.95		Sucrose	21
				20
Kluyveromyces marxianus	0.999	60	Control	24
	0.975		NaCl	30
	0.95		Sucrose	40
			NaCl	36
			Sucrose	54

Influence of a_w and type of solute on D values of vegetative cells of yeasts

Yeast	a_w	Temp. (°C)	Solute	D value (min)
Geotrichum candidum	0.99	52	None	30
	0.97		NaCl	21
	0.93		NaCl	10
	0.97		Sucrose	57
	0.89		Sucrose	59
Saccharomyces cerevisiae	0.99	51	None	21
	0.97		NaCl	24
	0.93		NaCl	13
	0.97		Sucrose	49
	0.89		Sucrose	53
Debaryomyces hansenii	0.99	48	None	12
	0.97		NaCl	17
	0.93		NaCl	18
	0.97		Sucrose	40
	0.89		Sucrose	43

Heat resistance of yeasts as affected by solute at a_w 0.95

Solute	*S. pombé*	*Z. rouxii*
48% Sucrose	1.48	1.99
35% Sorbitol	0.73	0.81
35% Glucose	0.41	0.55
35% Fructose	0.27	0.58
25% Glycerol	0.21	0.28

Adapted from Corry, J. Appl. Bacteriol., 40:269-276 (1976)

Heat resistance of *Bacillus subtilis* spores as affected by a_w and solute

Solute	a_w	$D_{95°C}$ value (min)
None	1.00	9.1
NaCl	0.93	11
	0.78	22
Glucose	0.96	3.5
	0.82	5.6
Glycerol	0.96	9.8
	0.89	12
	0.80	28
	0.66	220

Fungicidal activity of propylene oxide at various relative humidities

Mold	D value (h, room temp.) at various RH (%)				
	0	33	53	75	93
Aspergillus niger	8.2	6.0	4.2	3.3	2.15
Penicillium thomii		16.0	7.2	5.0	2.80

Adapted from Tawaratani and Shibasaki, J. Ferment. Technol., 50:349 (1972)

Considerations When Designing Challenge Studies to Determine the Minimum a_w for Growth of Microorganisms

- Use food system
- Select formula and processing conditions
- Inoculate with test microorganism(s)
- Select appropriate incubation conditions
- Select appropriate detection and enumeration procedures

Control of a_w in Foods

- Equilibrate with atmosphere of lower or higher equilibrium relative humidity (ERH)
- Remove or add water
- Add solute(s)
- Change temperature

Solutes and Humectants

- Sodium chloride
- Sugars (sucrose, glucose, fructose)
- Sorbitol
- Glycerol
- Propylene glycol

Detection/Enumeration of Xerotolerant Fungi

- Diluent - Add 20-30% sucrose, glucose, or glycerol
- Agar Media - Various, supplemented with solute
    - Czapek or potato dextrose agar with up to 50% sucrose
    - DG18 agar
    - Malt extract yeast extract glucose (50 - 70%) agar
    - Malt salt agar