Behavior of *Salmonella* in Foods with Low Water Activity

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Understanding the Risk and Controlling the Process
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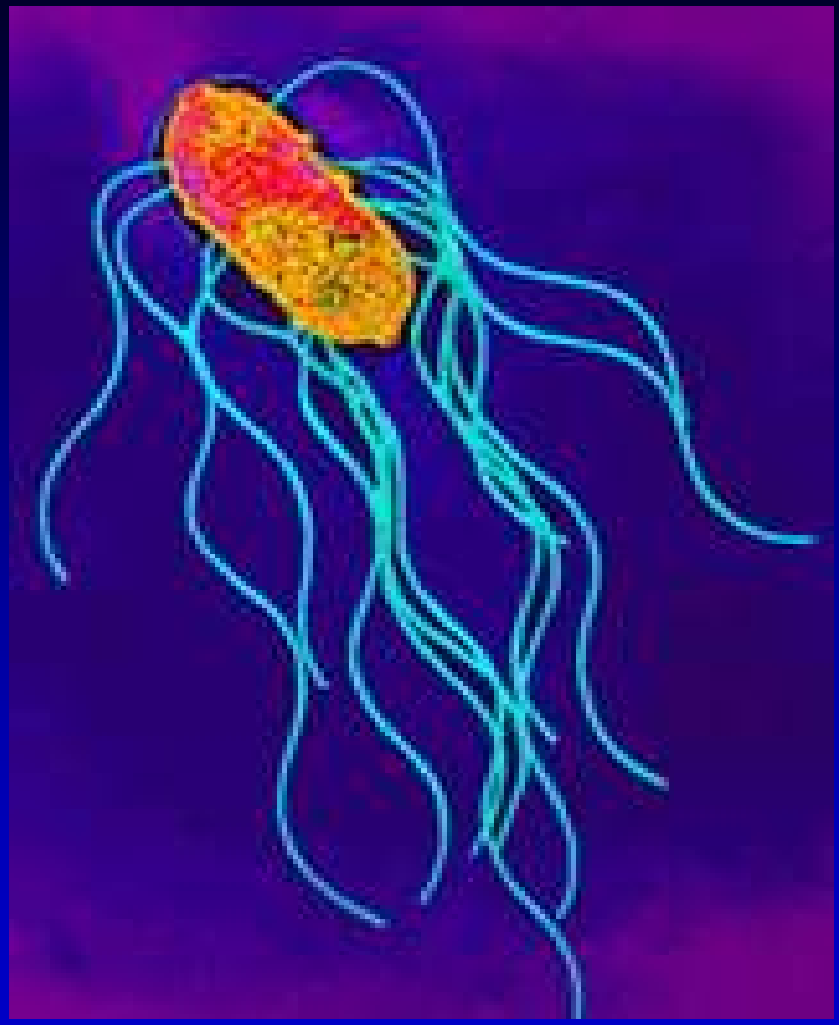
Overview

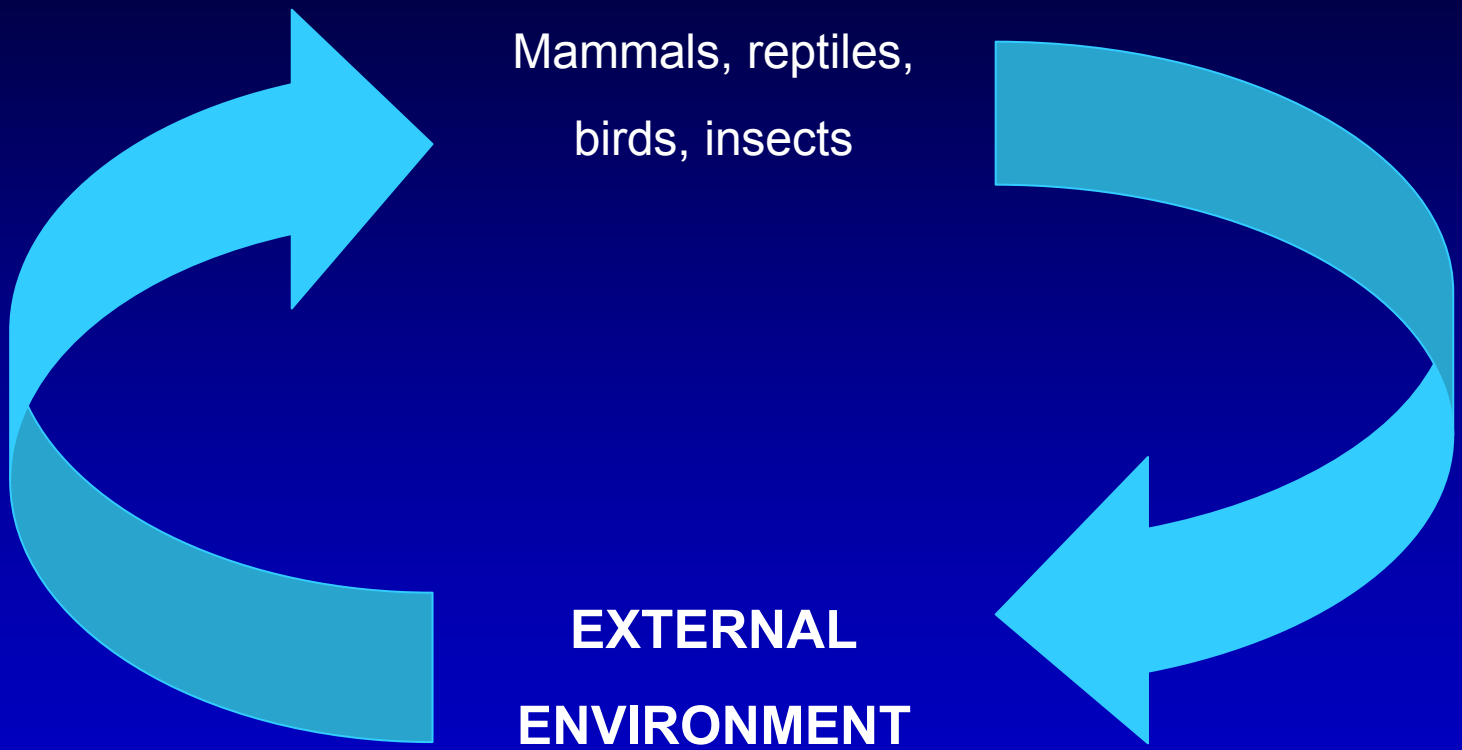
- General characteristics
- Salmonellosis associated with foods with reduced water activity (a_w)
- Water activity versus moisture relationship
- Survival in low-a_w foods
- Factors affecting heat tolerance
- Other microbiological concerns

Salmonella

- Two species
- 2500+ serotypes
- Found in mammals, reptiles, birds, insects
- Found in feces and in soil, dust, and water and on food contact surfaces contaminated with feces
- Illness can result from eating contaminated foods and beverages
- As few as one cell may cause illness



Life Cycle of *Salmonella*



Stresses include temperature fluctuation, desiccation, reduced nutrient availability, acidity, and ultraviolet light

Adapted from Winfield and Groisman, 2003.

Outbreaks Attributed to Post-process or Cross Contamination

Salmonella serotype	Product	Probable source or contamination route	Reference
Various serovars	Paprika on potato chips	Contaminated paprika powder	Lehmacher *et al*., 1995
Agona	Breakfast cereals	Environment, processing lines and equipment	Breuer, 1999
Berta	Soft cheese	Buckets for cheese ripening previously used for chicken carcasses	Ellis *et al*., 1998
Ealing	Infant formula	Environment, processing lines and equipment	Rowe *et al*., 1987

Outbreaks Attributed to Post-process or Cross Contamination

Salmonella serotype	Product	Probable source or contamination route	Reference
Enteritidis	Ice cream	Tanker use to transport ice cream previously used for raw eggs	Hennessy *et al*., 1996
Enteritidis	Pastry	Mixing bowl, cream piping bags and nozzles not cleaned adequately	Evans, *et al*., 1996
Typhimurium	Cooked sliced ham	Containers previously used for curing raw pork	Llewellyn *et al*., 1998

Outbreaks of Salmonellosis Associated with Peanut Products

Salmonella serotype	Product	Number of Cases	Location	Reference
Agona	Peanut flavored Kosher snack	2200	Israel, England, Wales, and U.S.	Killea *et al*., 1996; Shohat *et al*., 1996
Mbandaka	Peanut butter	15	South Australia	Scheill *et al*., 1998
Enteritidis	Peanut sauce	644	Japan	JMHW, 1999
Stanley, Newport	Inshell peanuts	109	Australia, Canada, U.K.	Kirk *et al*., 2004
Tennessee	Peanut butter	628	Multiple U.S. states	MMWR, 2007
Typhimurium	Peanut butter and peanut-containing products	529 (02/06/09)	Multiple U.S. states	MMWR, 2009

Factors Limiting Growth

- Salmonellae can grow at temperatures as low as 2ºC (35.6ºF) and as high as 54ºC (129.2ºF)
  - Optimum temperature for growth is 35 - 37ºC (95 – 98.6ºF)
- pH limits for growth are 3.99 – 4.05 to 9.50
  - Lower limit depends on the type of acidulant
  - Optimum is near pH 7
- Growth can occur at water activity (a_w) as low as 0.93
  - Lower limit depends on the type of water-binding components (ingredients) in the food

Definition

- Water activity (a_w): A measurement of the amount of water available for biological or chemical reactions

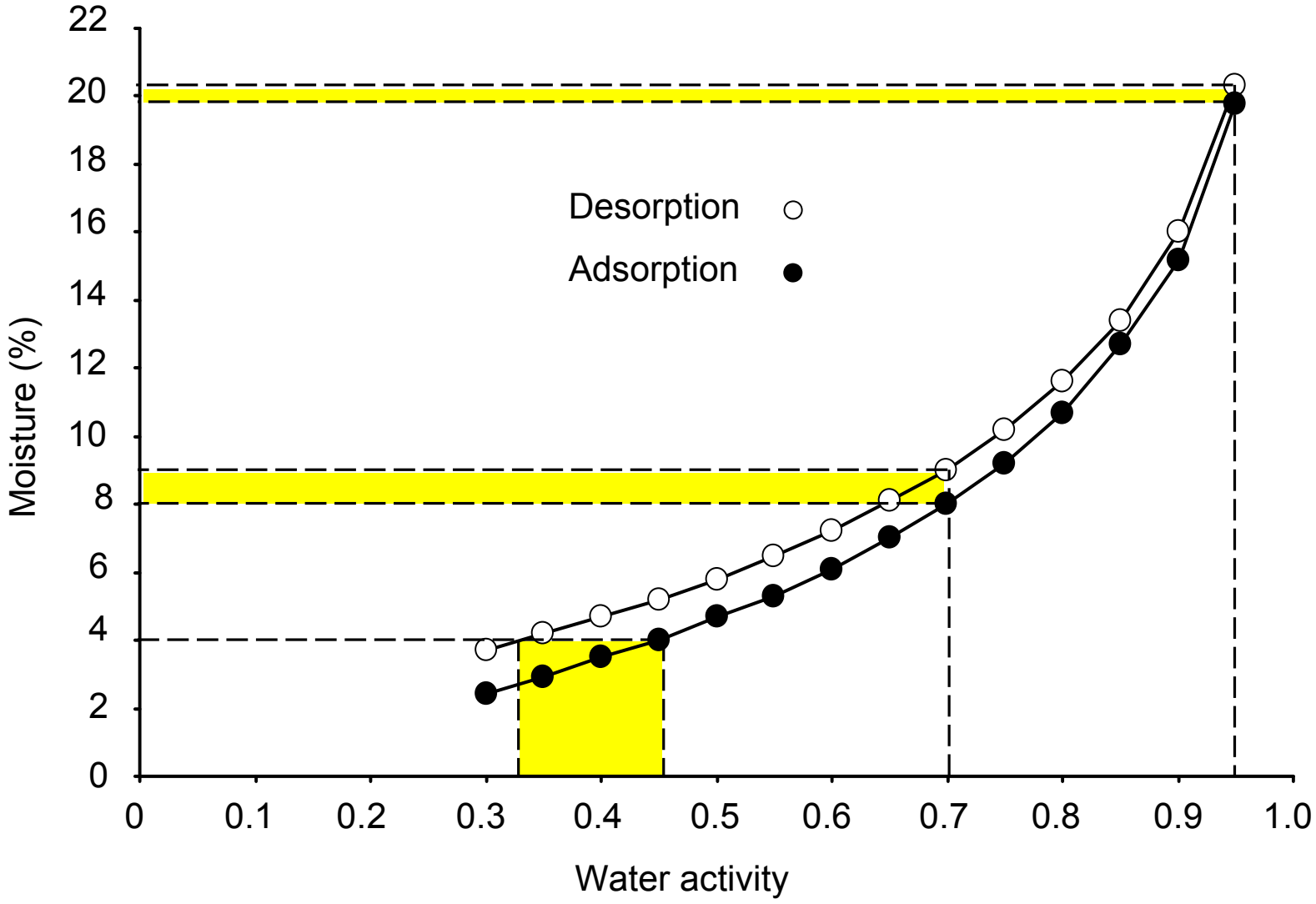
$$a_w = \frac{\text{Equilibrium relative humidity}}{100}$$

  - Depends largely on the amount of water-binding components (not necessarily the amount of water) in food

Approximate Oil and Moisture Content of Nuts at Water Activity 0.70

Nut	Oil	Moisture
Chestnut	2.5	12.1
Cashew	48	6.8
Pignolia	48	7.0
Peanut	**50**	**6.9**
Almond	52	6.6
Pistachio	57	6.4
Walnut (black)	59	6.1
Butternut	60	6.1
Filbert	62	5.9
Walnut (English)	67	4.7
Brazil	69	4.2
Pecan	70	4.5
Hickory	70	4.6
Macadamia	73	3.9

From Beuchat, J. Food Sci., 43:754-755, 1978.


Moisture (%)
22
20
18
16
14
12
10
8
6
4
2
0
Desorption
Adsorption
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Water activity

Factors Affecting Survival of *Salmonella*

- Salmonellae can adapt to extreme environmental conditions (desiccation, pH, temperature stress)
- Survival is enhanced at refrigeration and freezing temperatures
- Survival is enhanced at low a_w
- Thermal tolerance is enhanced at pH near 7 and at low a_w

Long-term Survival of *Salmonella* in Foods with Low a_w

Product	Serotype	Inoculum (log CFU/g)	a_w	Temp (ºC)	Length of survival	Reference
Milk chocolate	Eastbourne	5	0.38	20	9 mo	Tamminga *et al.*, 1976
		8	0.41	20	>9 mo	
Halva	Enteritidis	7	0.18	6, 8-20	>8 mo	Kotzekidou, 1998
Black pepper	Rubislaw	5	0.66	5, 25, 35	>15 d	Restori *et al.*, 2007
Almonds	Enteritidis	7		-20, 4	>550 d	Uesugi *et al.*, 2006
				23	161 d	
Pecans	Agona, Enteritidis, Oranienberg, Sundsvall, Tennessee	2.5-4.5	0.53-0.63	-20, 4, 21, 37	>36 wk	Beuchat (not published)

Persistence in Environment

- *S.* Infantis
  - Seven strains isolated from broilers in Japan between 1993 and 1998 have antimicrobial resistance susceptibilities and PFGE profiles similar to 22 strains isolated between 2001 and 2003 (Asui *et al.*, 2007)
- *S.* Enteritidis PT30
  - Persists in almond orchard soil for at least 5 years (Uesugi *et al.*, 2007)

Reduction in *Salmonella* in Peanut Butter and Peanut Spread Stored at 5°C (41°F) for up to 24 weeks

Product	a_w	Reduction (log CFU/g)[a]		
		2 wk	6 wk	24 wk
Peanut butter				
Traditional 1	0.29	2.0	2.5	2.9
Traditional 2	0.22	2.1	2.7	3.5
Natural	0.20	3.3	3.7	4.8
Reduced sugar, low Na 1	0.33	2.4	2.5	2.9
Reduced sugar, low Na 2	0.25	2.6	2.7	3.4
Peanut spread				
Reduced fat 1	0.28	1.7	2.2	2.9
Reduced fat 2	0.22	1.7	2.3	2.9

[a]Initial population was 5.7 log CFU/g.

From Burnett *et al*., J. Appl. Microbiol., 89:472-477, 2000.

Reduction in *Salmonella* in Peanut Butter and Peanut Spread Stored at 21°C (69.8°F) for up to 24 weeks

Product	a_w	Reduction (log CFU/g)[a] 2 wk	6 wk	24 wk
Peanut butter				
Traditional 1	0.29	2.7	2.9	4.4
Traditional 2	0.22	2.6	3.3	4.5
Natural	0.20	4.0	4.9	>5.7
Reduced sugar, low Na 1	0.33	2.7	3.4	4.5
Reduced sugar, low Na 2	0.25	3.2	3.5	4.4
Peanut spread				
Reduced fat 1	0.28	2.4	3.0	4.1
Reduced fat 2	0.22	2.3	3.1	4.4

[a]Initial population was 5.7 log CFU/g.

From Burnett *et al*., J. Appl. Microbiol., 89:472-477, 2000.

Reduction in *Salmonella* in Peanut Butter and Peanut Spread Stored at 5°C (41°F) and 21°C (69.8°F) for up to 36 Hours

Product	a_w	Reduction (log CFU/g)[a]			
		5°C		21°C	
		1 h	36 h	1 h	24 h
Peanut butter					
Traditional 1	0.29	1.4	1.7	1.5	2.3
Natural	0.20	2.1	2.5	2.0	2.6
Reduced sugar, low Na 1	0.33	1.1	1.6	1.0	2.3
Peanut spread					
Reduced fat 2	0.22	0.9	1.3	0.9	1.7

[a]Initial population was 5.7 log CFU/g.

From Burnett *et al*., J. Appl. Microbiol., 89:472-477, 2000.

Detection of *Salmonella* in Peanut Butter and Peanut Spread Stored at 5°C (41°F) for up to 24 weeks

Product	a_w	Number of positive samples out of three analyzed		
		2 wk	6 wk	24 wk
Peanut butter				
Traditional 1	0.29	3	3	3
Traditional 2	0.22	3	3	3
Natural	0.20	3	1	0
Reduced sugar, low Na 1	0.33	3	3	3
Reduced sugar, low Na 2	0.25	3	3	1
Peanut spread				
Reduced fat 1	0.28	3	3	3
Reduced fat 2	0.22	3	3	3

[a]Initial population was 1.5 log CFU/g (32 CFU/g).
From Burnett *et al*., J. Appl. Microbiol., 89:472-477, 2000.

Detection of *Salmonella* in Peanut Butter and Peanut Spread Stored at 21°C (69.8°F) for up to 24 weeks

Product	a_w	Number of positive samples out of three analyzed		
		2 wk	6 wk	24 wk
Peanut butter				
Traditional 1	0.29	3	2	0
Traditional 2	0.22	3	2	0
Natural	0.20	1	0	0
Reduced sugar, low Na 1	0.33	3	3	0
Reduced sugar, low Na 2	0.25	2	2	0
Peanut spread				
Reduced fat 1	0.28	3	3	1
Reduced fat 2	0.22	3	3	0

[a]Initial population was 1.5 log CFU/g (32 CFU/g).
From Burnett *et al*., J. Appl. Microbiol., 89:472-477, 2000.

Order of Survival of *Salmonella* in Peanut Butter and Peanut Spread

Peanut butter spreads >

Traditional and reduced-sugar, low-sodium peanut butters >

Natural peanut butter

Reduction in *S.* Tennessee in Peanut Butter Stored at 4ºC (39.2ºF) up to 14 days[a]

	Reduction (log CFU/g)[b]		
Brand	3 days	7 days	14 days
A	0.1	0.2	0.3
B	0.2	0.4	0.4
C	0	0.1	0.2
D	0.2	0.1	0.1
E	0.1	0.1	0.4

[a] a_w of peanut butters was 0.17 – 0.25.
[b] Initial population was 6.1 – 6.5 log CFU/g.

From Park *et al.*, J. Food Sci., 73:M82-M86, 2008.

Reduction in *S.* Tennessee in Peanut Butter Stored at 22ºC (71.6ºF) up to 14 days[a]

	Reduction (log CFU/g)[b]		
Brand	3 days	7 days	14 days
A	0.6	0.8	1.3
B	0.5	0.8	1.3
C	0.1	0.3	0.3
D	0.4	0.5	0.5
E	0.2	0.3	0.3

[a]a_w of peanut butters was 0.17 – 0.25.
[b]Initial population was 6.1 – 6.5 log CFU/g.

From Park *et al.*, J. Food Sci., 73:M82-M86, 2008.

Reduction in *Salmonella* and *E. coli* O157:H7 in Peanut Butter Stored at 4ºC and 25ºC

Pathogen	Storage temp (ºC)	Reduction (log CFU/g)	
		9 wk	15 wk
Salmonella	4	1.0	
	25		2.6
E. coli O157:H7	4	4.4	
	25		2.9

From Kilonzo-Nthenge *et al.*, (Presented at IAFP Mtg., Columbus, OH, 2008).

Factors Affecting Heat Tolerance

- Affected by prior growth conditions
  - Stationary phase cells are more resistant than log phase cells
  - Starved cells are more resistant than those grown in carbohydrate-rich media
- Affected by composition of food
  - Habituation at reduced a_w increases tolerance to heat
  - Highest resistance in low-a_w foods and high-fat foods
  - Resistance may be affected by location of cells in the food (surface vs. internal)

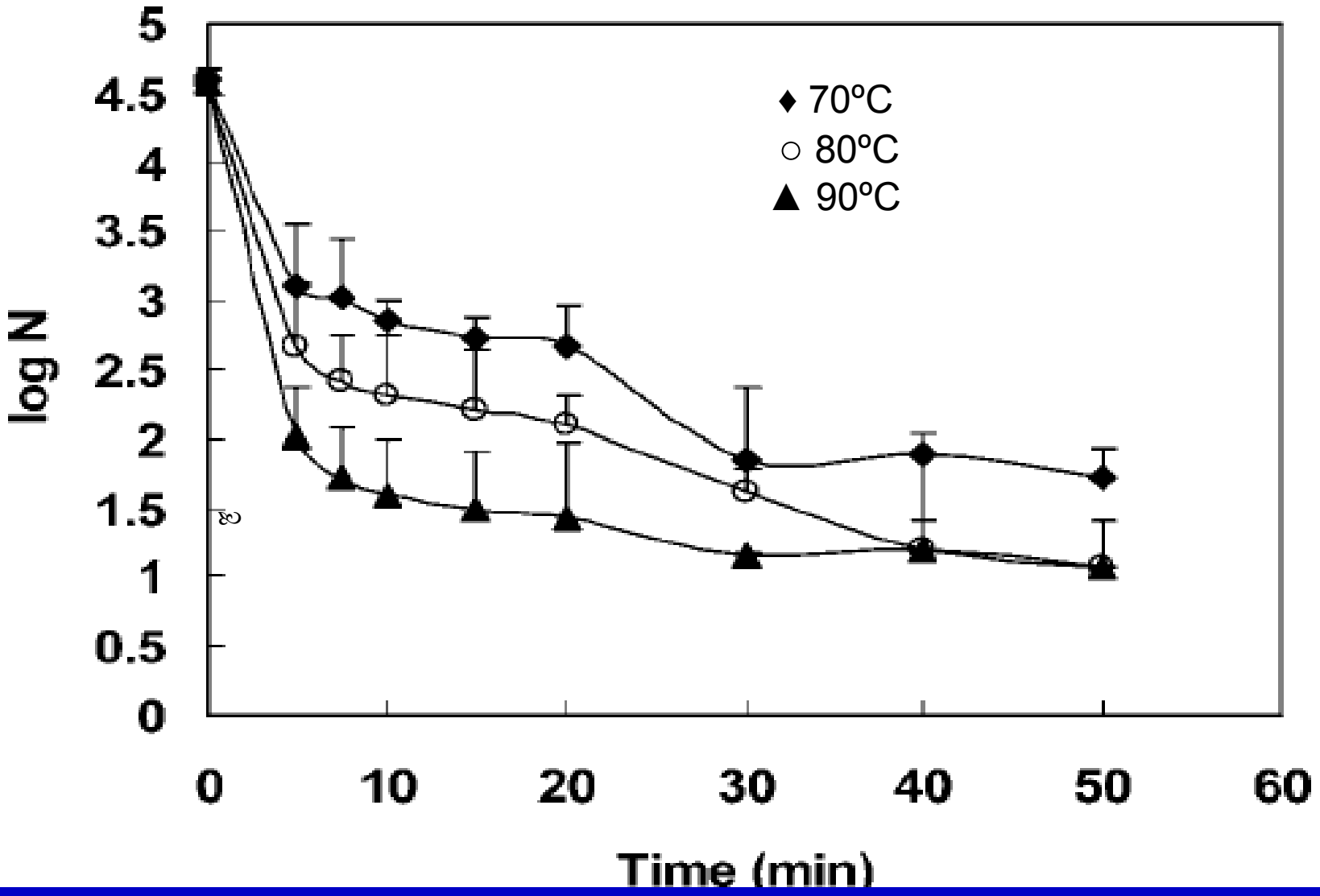
Factors Affecting Heat Tolerance

- Tolerance is enhanced when *Salmonella* is grown at elevated temperatures
- Varies among serotypes
  - *S*. Senftenberg is exceptionally heat resistant
- Tailing effect
  - Cells in a given population vary in level of tolerance to heat

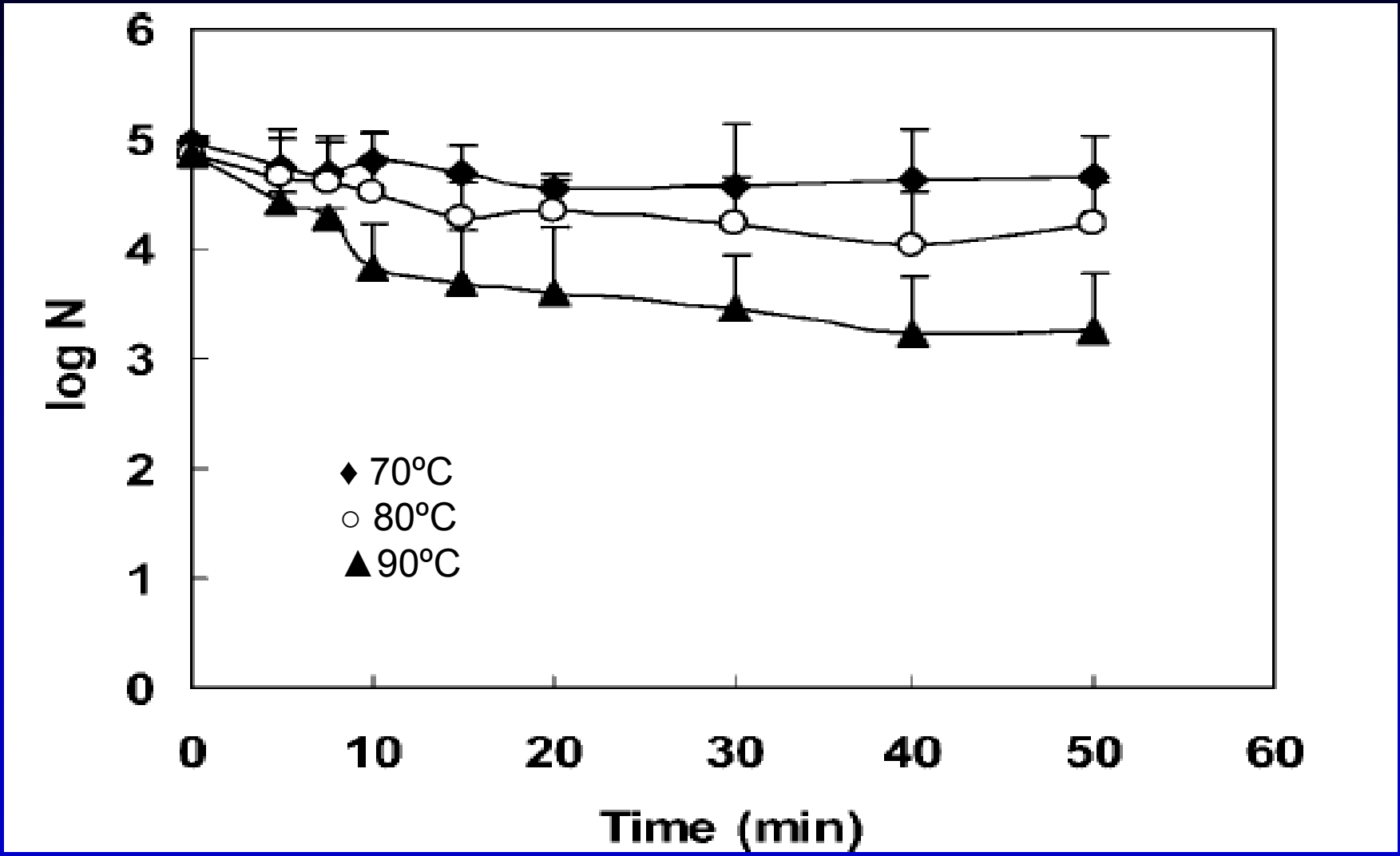
Heat Tolerance in Peanut Butter

- *S.* Agona, *S.* Enteritidis, and *S.* Typhimurium in peanut butter
  - Reductions at 70, 80, and 90°C (158, 176, and 194ºF, respectively) were 1.8 – 3.0 log/g after heating for 20 min
  - Cells in fatty, hydrophobic microenvironment were probably protected against inactivation

(Shachar and Yaron, 2006).



From Shachar and Yaron, J. Food Prot., 69:2687-2691, 2006.



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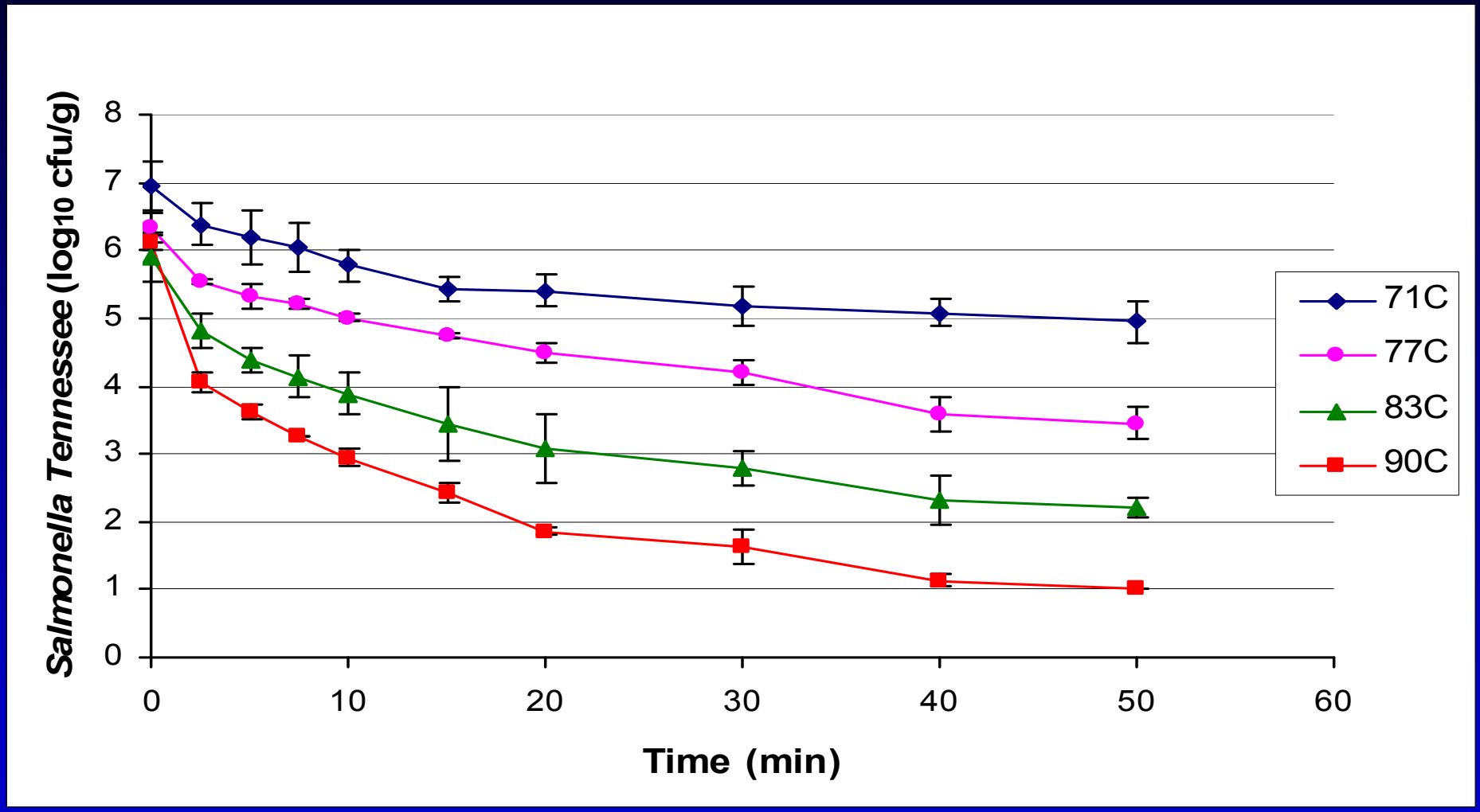

From Ma *et al*. (Presented at IFT Mtg., New Orleans, LA, 2008).

Time (min) for Thermal Inactivation of *S.* Tennessee in Peanut Butter

Temp (ºC)	Calculated time (min) for log reduction			
	1 log	3 log	5 log	7 log
71 (160ºF)	8.41 (0.97)	103 (16)	328 (61)	706 (141)
77 (170ºF)	5.20 (1.51)	62 (22)	197 (75)	423 (174)
83 (181ºF)	1.92 (0.28)	27 (3)	93 (9)	210 (25)
90 (194ºF)	0.26 (0.01)	9 (1)	42 (8)	120 (29)

From Ma *et al.* (Presented at IAFP Mtg., Columbus, OH, 2008).

Time (min) for Thermal Inactivation of *S.* Typhimurium in Peanut Butter[a]

Temp (ºC)	Time (min) to obtain a 3-log reduction
55 (131ºF)	98
65 (149ºF)	24
74 (167ºF)	6

[a] a_w of peanut butter was 0.50

From Mattick *et al.*, Appl. Environ. Microbiol., 67:4128-4136, 2001.

Other Microbiological Concerns

- Foodborne bacterial pathogens other than *Salmonella*
  - *E. coli* O157:H7, *Shigella*, *Listeria monocytogenes*, *Clostridium botulinum*
    - May survive on inshell and shelled nuts for several months
    - *C. botulinum* can grow in peanut spread at a_w 0.96 – 0.98 at 30ºC (86ºF) and produce toxin (Clavero *et al*., 2000)
    - *L. monocytogenes* survives in peanut butter and chocolate-peanut spread for 16 – 24 weeks at 20ºC (68ºF) (Kenney and Beuchat, 2004)

Survival of *Listeria monocytogenes*

Product	a_w	$D_{60°C}$ value
Whole-fat milk	0.99	3.3
Chocolate-peanut spread	0.46	37.5
Peanut butter	0.32	26.0

From Kenney and Beuchat, J. Food Prot., 67:2205, 2004.

Other Microbiological Concerns

- Mycotoxigenic molds
  - *Aspergillus flavus, A. parasiticus,* and *A. nomius* produce aflatoxins
    - Aflatoxins can be produced at 12 - 40ºC (53.6 - 104ºF) and a_w 0.85 - 0.99
  - *Aspergillus ochraceus, A. carbonarius*, and *A. niger* produce ochratoxin
  - Other *Aspergillus* species produce cyclopiazonic acid, sterigmatocystin, fumitremorgens, territrems, cytochalasins, and tryptoquivalines
  - Some *Penicillium* species can produce ochratoxin and tremorgins in nuts

Summary

- *Salmonella* can remain viable in peanut butter and peanut spreads for extended/periods of time, particularly when products are stored at refrigeration temperature
- Heat resistance of *Salmonella* is enhanced in low-a_w, high-fat foods such as peanut butter
- Survival of *Salmonella* in peanut butter-containing products can be enhanced by ingredients other than peanuts


THANK YOU