

## PROCEEDINGS OF THE NUTRITION SOCIETY

*A Scientific Meeting (One Hundred and Eightieth Scottish Meeting) was held in conjunction with the Institute of Food Science and Technology at the Kelvin Conference Centre, Glasgow on 18 November 1988*

### Workshop on 'Nutrition and food processing'

#### Food preservatives and the microbiological consequences of their reduction or omission

BY T. A. ROBERTS\* AND P. J. MCCLURE

*AFRC Institute of Food Research, Bristol Laboratory, Langford, Bristol BS18 7DY*

At harvest or slaughter, agricultural products and animals carry a wide range of microbes that make up the 'primary contamination'. It varies from one commodity to another, with geographic region, and with production and harvesting methods. Microbes able to cause disease of the crop or animal are subject to stringent control measures because losses affect the producer. Other microbes constitute a hazard to man by being able to cause illness, either by infection, or by intoxication after they have grown in a food and produced a toxin. Even ancient food laws prohibited consumption of meat from diseased animals and from those that had died other than by slaughter, because some animal diseases are transmissible to man (e.g. bovine tuberculosis, brucellosis). Other animal diseases do not affect man but are important to the farmer because they reduce growth rate or feed conversion. A third category of microbes do not cause sickness in animals, but pass into the food chain and may cause illness in man, e.g. *Campylobacter* in milk, salmonellae in poultry. There is little incentive at production to control microbes that can later cause illness in man, unless they also cause sickness in animals or affect weight gain, or both.

The two major concerns for the food industry attributable directly to microbes are (a) losses before consumption due to spoilage, and (b) costs associated with food-borne disease. The true costs of spoilage are poorly documented, admitted, and rarely publicized. Few serious attempts to determine the full costs associated with food-borne illness have been made (Todd, 1985 *a, b*; Yule *et al.* 1986) and only relatively recently has it been recognized that some diarrhoeal diseases result in long-term sequelae, e.g. arthritis, cardiac problems, and food allergies (Archer & Kvenberg, 1985). Repeated episodes of food-borne disease may initiate or intensify malnutrition, or both.

At harvest or slaughter, care with particular procedures may limit, or even reduce, microbial contamination, but a point is reached when the costs of any additional measures outweigh the benefits. In the case of animals, the primary contamination is not under control to the extent that will assure freedom from many particular microbes of

\* Present address: AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT.

concern (e.g. *Salmonella*, *Clostridium botulinum*, *Cl. perfringens*, *Campylobacter*). With current technologies it seems unlikely ever to be so. Intensive production and high slaughter rates have sometimes markedly worsened the microbiological condition of the raw food (e.g. poultry). Hence food processors should assume that those microbes of concern will be present and consciously take measures to either kill them, or to ensure that they are unable to multiply in their products. Further processing (e.g. slicing and repacking) often recontaminate the products with bacteria of human origin, commonly *Staphylococcus aureus* and occasionally *Salmonella*.

To multiply, microbes need water, nutrients, and appropriate temperature and pH levels. Meats and meat products contain abundant nutrients, and are of pH values readily able to support microbial growth. The single most effective measure in limiting microbial growth is the application of appropriate temperatures. Monitoring should be applied to ensure intended temperatures are attained so that if the temperature drifts outside specification, whether a killing or a refrigeration temperature, effective corrective action can be taken immediately.

Although control of microbial growth is primarily by pH, water activity and storage temperature, additional factors such as the presence of preservatives, modified atmosphere packaging or heat treatment also contribute, e.g. in meats and meat products. In practice several of those factors act in combination, often at levels which singly would not control microbial growth.

During processing microbes are killed, inhibited, removed or otherwise excluded. Many food processes have developed empirically, e.g. chilling, freezing, pasteurizing, canning, drying, salting, sugaring, acidification, fermenting and using chemical preservatives such as curing salts. Nevertheless, the development of novel food processes has been greatly aided by research into the relevant properties of food-borne microbes, such as their response to heat and cold, water requirement, their sensitivity to acids and alkalis and antimicrobial substances.

Microbes differ greatly in their temperature requirements and their ability to withstand heat and cold. At a given lethal temperature the time taken to reduce the viable numbers of microbes tenfold is termed the decimal reduction value (D value). Representative values are given in Table 1. Inactivation occurs more rapidly with increasing temperature. The death rate of vegetative bacteria increases tenfold for every (approximately) 5° increase in temperature within the lethal range, and that of spores for every 10° increase. Bacteria that prefer low temperatures (psychrophiles) are unable to withstand even modest temperatures (40°) at which mesophiles multiply. Mesophilic vegetative bacteria are inactivated rapidly by temperatures above approximately 70° unless heating occurs at low water activities, e.g. as occurs in chocolate, when the heat resistance increases very substantially.

Bacterial spores (endospores) occur naturally in the environment, and are therefore found in all agricultural products and on meats. The most certain means of controlling spores in foods is to inactivate them by heating. Their resistance to heat varies considerably with the species and is affected by the environment in which they are heated. For example, products of low pH (acid products) can be rendered microbiologically stable and safe by lower heat processes than those of neutral pH, because many bacteria and spores are more sensitive to heat by increasing acidity, while others are unable to grow at very acid pH values (e.g. *Cl. botulinum* will not grow below pH approximately 4.5 except under unusual circumstances). Hence pH plays an

Table 1. Heat resistance of bacteria and bacterial spores

Organism	Temperature (°)	D value (min)	Source
<i>Brucella</i> spp.	at 65.5	0.1–0.2	Table 1.9 of ICMSF (1980)
<i>Salmonella senftenberg</i> 775W	at 65.5	0.8–1.0	
<i>Salmonella</i> spp.	at 65.5	0.02–0.25	
<i>Staphylococcus aureus</i>	at 65.5	0.2–2.0	
Yeasts and moulds and spoilage bacteria	at 65.5	0.5–3.0	
Spores of mesophilic aerobes			
<i>Bacillus cereus</i>	100	5.0	Table 1.8 of ICMSF (1980)
<i>B. subtilis</i>	100	11.0	
<i>B. polymyxa</i>	100	0.1–0.5	
Spores of mesophilic anaerobes			
<i>Clostridium butyricum</i>	100	0.1–0.5	Table 1.8 of ICMSF (1980)
<i>Cl. perfringens</i>	100	0.3–20.0	
<i>Cl. botulinum</i>	100		
Type A and type B proteolytic strains	100	50.0	
Type E and non-proteolytic types B and F strains	80	ca. 1.0	
Spores of thermophilic aerobes			
<i>Bacillus coagulans</i>	120	0.1	Table 1.8 of ICMSF (1980)
<i>B. stearothermophilus</i>	120	4.0–5.0	
Spores of thermophilic anaerobes			
<i>Cl. thermosaccharolyticum</i>	120	3–4	Table 1.8 of ICMSF (1980)
<i>Desulfotomaculum (Clostridium) nigrificans</i>	120	2–3	

D value, decimal reduction value (time taken to reduce viable numbers of microbes tenfold at a given lethal temperature); ICMSF, International Commission on Microbiological Specifications for Foods.

important role in food preservation, and numerous acids are used in food products to render them more stable and safer.

Similarly, temperature selects which microbes grow on a stored food. The effort put into defining the initial flora was, in part, misplaced because the storage conditions select those organisms best suited to it. Many of the bacteria present on carcasses are 'mesophiles', but many perishable foods are stored chilled and they are unable to multiply. Consequently microbes present initially as a small fraction of the total flora multiply and eventually become numerically dominant.

The microbial flora developing on a food is a function of the initial flora, any process that has been applied, particular properties of the food (e.g. substrates available for microbial growth) and the conditions under which it is stored. The microbial responses to pH, water activity ( $a_w$ ) and storage temperature, considered individually, are illustrated in Tables 2, 3 and 4. The microbial succession in foods is a response to those factors acting in combination, although the strong effects of pH are illustrated in Table 5 and those of  $a_w$  in Table 6. The impact of food handling and food processing on microbes is illustrated in Table 7.

Table 2. *The limits of pH allowing growth of various microbes in laboratory media adjusted with strong acid or alkali (from Table 5.3 (condensed) of International Commission on Microbiological Specifications for Foods (1980))*

Organism	Minimum pH	Maximum pH
Gram-negative bacteria		
<i>Escherichia coli</i>	4.4	9.0
<i>Pseudomonas aeruginosa</i>	5.6	8.0
<i>Salmonella paratyphi</i>	4.5	7.8
<i>Vibrio parahaemolyticus</i>	4.8	11.0
Gram-positive bacteria		
<i>Bacillus cereus</i>	4.9	9.3
<i>Clostridium botulinum</i>	4.7	8.5
<i>Lactobacillus</i> spp.	3.8-4.4	7.2
<i>Micrococcus</i> spp.	5.6	8.1
<i>Staphylococcus aureus</i>	4.0	9.8
<i>Streptococcus faecium</i>	4.4-4.7	9.2
Yeasts		
<i>Candida pseudotropicalis</i>	2.3	8.8
<i>Hansenula canadensis</i>	2.15	8.6
<i>Saccharomyces</i> spp.	2.1-2.4	8.6-9.0
Moulds		
<i>Aspergillus oryzae</i>	1.6	9.3
<i>Penicillium italicum</i>	1.9	9.3
<i>Penicillium variable</i>	1.6	11.1
<i>Fusarium oxysporum</i>	1.8	11.1

#### FRESH MEATS

When meats are stored, even under good refrigeration at about 0°, bacteria grow on the surface, eventually causing spoilage. In air the main spoilage organisms are *Pseudomonas* spp., with lower numbers of *Brochothrix thermosphacta*, Enterobacteriaceae and lactic acid bacteria. If meat is vacuum-packaged in gas-impermeable laminate (i.e. anaerobic conditions) spoilage is delayed considerably. Vacuum-packaging prevents access of oxygen, and carbon dioxide accumulates inside the package. Under these conditions lactic acid bacteria become dominant, with some growth of *B. thermosphacta* (Dainty *et al.* 1983) and Enterobacteriaceae but essentially no multiplication of *Pseudomonas* spp. The types of bacteria growing on chicken, turkey and duck stored in air or vacuum-packs are similar to those which grow on red meats. The microbiology of poultry spoilage has been reviewed by Mead (1982).

In countries where red meats are eaten raw, *Salmonella* is a serious problem. In the UK cooking before eating reduces considerably the risk of salmonellosis.

#### CURED MEATS

In the UK salt and nitrate/nitrite have long been used to cure meats, usually pork. In other countries cured beef is a traditional delicacy and in recent years cured poultry products have been developed. Cured meats have an excellent record of safety and are

Table 3. Approximate minimum levels of water activity ( $a_w$ ) permitting growth of micro-organisms at temperatures near optimal (from Table 4.3 (condensed) of International Commission on Microbiological Specifications for Foods (1980) and from Troller & Christian (1978))

Organism	$a_w$
<b>Moulds</b>	
<i>Aspergillus candidus</i>	0.75
<i>A. flavus</i>	0.78
<i>A. niger</i>	0.77
<i>Botrytis cinerea</i>	0.83
<i>Chrysosporium fastidium</i>	0.69
<i>Errotum (Aspergillus) amstelodami</i>	0.70
<i>E. chevalieri</i>	0.71
<i>E. echinulatum</i>	0.62
<i>Monascus (Xeromyces) bisporus</i>	0.61
<i>Mucor plumbeus</i>	0.93
<i>Penicillium chrysogenum</i>	0.79
<i>Rhizopus nigricans</i>	0.93
<b>Yeasts</b>	
<i>Debaryomyces hansenii</i>	0.83
<i>Saccharomyces bailii</i>	0.80
<i>S. cerevisiae</i>	0.90
<i>S. rouxii</i>	0.62
<b>Bacteria*</b>	
<i>Bacillus cereus</i>	0.95
<i>B. subtilis</i>	0.90
<i>Clostridium botulinum</i> type A	0.95
<i>Cl. botulinum</i> type B	0.94
<i>Cl. botulinum</i> type E	0.97
<i>Cl. perfringens</i>	0.95
<i>Escherichia coli</i>	0.95
<i>Halobacterium halobium</i>	0.75
<i>Brochothrix thermosphacta</i>	0.94
<i>Pseudomonas fragi</i>	0.97
<i>Salmonella</i> spp.	0.95
<i>Staphylococcus aureus</i>	0.86
<i>Vibrio parahaemolyticus</i>	0.94

\*  $a_w$  adjusted with salts.

relatively rarely involved in food poisoning (Tompkin, 1980), except when the curing process has been poorly controlled or when the product has been recontaminated by handling and subsequently stored without adequate refrigeration.

Traditional cured products were shelf-stable without refrigeration because they contained, by today's standards, a high salt concentration (which lowers  $a_w$ , thereby preventing growth of Gram-negative spoilage bacteria), and by today's standards, high levels of nitrite. Nitrite imparts to pork and poultry the characteristic pink cured colour (nitrosomyoglobin) and imparts the cured flavour. Equally important, salt and nitrite, together with the storage temperature and the pH of the meat, select the bacteria which can grow.

Table 4. *Minimum growth temperatures for bacteria causing food-borne illness*

Organism	Temperature (°C)
Enteropathogenic <i>Escherichia coli</i>	10
<i>Clostridium botulinum</i> (proteolytic)	10
<i>Cl. perfringens</i>	10
<i>Bacillus cereus</i>	8-10
<i>Staphylococcus aureus</i> (toxin production)	8-10
<i>Bacillus cereus</i>	8-10
<i>Salmonella</i>	6.7
<i>Staphylococcus aureus</i> (growth)	6.7
<i>Vibrio parahaemolyticus</i>	5
<i>Listeria monocytogenes</i>	1
<i>Cl. botulinum</i> (non-proteolytic)	3.3
<i>Streptococcus</i>	1
<i>Yersinia enterocolitica</i>	-2

#### BACON

The salt present in bacon prevents the pseudomonads from multiplying, but other salt-tolerant bacteria, such as lactobacilli, continue to grow, even under refrigeration and reach millions/g, but do not cause objectionable flavour changes. Reducing the salt level allows other microbes (e.g. proteolytic and lipolytic micrococci, *Vibrio* spp.) to grow, reducing the shelf-life, whether the bacon is stored in air or in vacuum-packs (Gardner, 1983).

Concern that nitrite may contribute to the formation of 'nitrosamines' led to reductions in permitted concentrations in foods in many countries. However, the occasional presence of very small amounts of N-nitrosated compounds in cured meats was judged to be of little consequence compared with in vivo nitrite formation in saliva or ingestion of nitrate in water or vegetables at many orders of magnitude above those in meat products. Although modern bacons with reduced levels of salt and nitrite tend to be less shelf-stable, they have not been associated with food poisoning.

Over the last 15-20 years pig-breeding programmes have succeeded in producing leaner pork, but this has increased the pH of certain muscles, particularly those in the collar (shoulder). Bacon from such high-pH muscle keeps less well than bacon from pork of normal pH values (Taylor & Shaw, 1975; Gardner, 1982, 1983).

#### PASTEURIZED HAMS

Small hams (up to about 1 kg) are heated quite severely, e.g. those in cans are heated virtually to 'commercial sterility'. Large hams are heated much less to avoid large 'cook-out' losses, but typical heat processes (e.g. raising the centre to 68° for 30 min) inactivates non-sporing pathogens, such as *Salmonella*, but does not kill bacterial spores. The most feared spore-forming bacterium is *Cl. botulinum* which occurs in soil, is an unavoidable contaminant of many agricultural products, including pork, and is

Table 5. Types of spoilage typically associated with foods (from Table 5.2 (condensed) of International Commission on Microbiological Specifications for Foods (1980))

pH range	Food	pH range	Additional factors	Spoilage microbes
Low acid 7.0-5.5	Milk	7.0-6.8	Natural antimicrobials	<i>Lactobacillus</i> spp., <i>Bacillus</i> spp., <i>Micrococcus</i> , coliforms
	Bacon	6.0-5.9	Sodium chloride Sodium nitrite	<i>Micrococcus</i> , <i>Vibrio</i>
	Carcass meat	7.0-5.4	Chill storage	<i>Acinetobacter-Moraxella</i> , <i>Pseudomonas</i>
	Red meat	6.2-5.4	Chilled, vacuum packed	<i>Lactobacillus</i> , <i>Brochothrix thermosphacta</i> , Enterobacteriaceae
	Canned vegetables	6.4-5.4	Heat	Flat-sour organisms, <i>Bacillus stearothermophilus</i>
Medium acid 5.3-4.5	Canned vegetable mixtures, soups, sauces	—	Heat	Thermophilic anaerobes, <i>Clostridium saccharolyticum</i> , putrefactive anaerobes
	Fermented vegetables	5.1-3.9	Weak organic acids	<i>Leuconostoc</i> , yeasts
	Pickled cucumber	7.0-4.5	Acetic acid	Yeasts, coliforms, lactic acid bacteria
	Cottage cheese	4.5	—	Gram-negative bacteria, moulds
Acid 4.5-3.7	Mayonnaise	4.1-3.0	—	Yeast, <i>Lactobacillus</i> <i>Clostridium pasteurianum</i> , <i>Bacillus coagulans</i>
	Tomatoes, fruits	4.0		
	Dried fruits	4.5	Low $a_w$ Sulphite	Moulds, yeasts
High acid <3.7	Canned pickles, fruit juice	3.9-3.5	Heat	Moulds, yeasts, lactic acid bacteria
	Sauerkraut		3.7-3.1	—
	Salad dressing	3.9-3.2	—	Yeasts, lactobacilli, <i>Bacillus</i> spp.
	Citrus fruits	3.5-3.0	—	<i>Penicillium</i> spp.

$a_w$ , water activity.

responsible for botulism. Therefore, hams will inevitably contain *Cl. botulinum* from time to time, and 'improperly cured' hams have often been responsible for botulism in man (e.g. in France during and after World War II). Nevertheless commercial hams have an excellent safety record (Tompkin, 1980), i.e. the curing and heating has prevented growth of *Cl. botulinum*.

Table 6. Water activity ( $a_w$ ) as it influences the microbial flora of food (from Table 1.5 of *International Commission on Microbiological Specifications for Foods (1988)*)

$a_w$	Foods	Micro-organisms
0.98 and above	Fresh meats and fish, vegetables, milk	Most food spoilage microbes and all food-borne pathogens grow
0.98–0.93	Evaporated milk, bread, cooked sausages	Enterobacteriaceae including <i>Salmonella</i> grow at upper end of range Spoilage flora, frequently lactic acid bacteria
0.93–0.85	Dried beef, sweetened condensed milk	<i>Staphylococcus aureus</i> and many mycotoxin-producing moulds grow Yeasts and mould cause spoilage
0.85–0.60	Flour, cereals, nuts	No pathogenic bacteria grow Spoilage by xerophilic, osmophilic and halophilic organisms
Below 0.60	Confectionery, noodles, biscuits, dried milk, dried eggs	Micro-organisms do not grow but can remain viable for long periods

Table 7. Effects of handling and processing on microbes

Operation	Food	Intended effect
Cleaning, washing	All raw foods	Reduces numbers of microbes
Antimicrobial dipping/ washing	Mostly fruits, vegetables	Kills selected microbes
Chilling (below 10°)	All foods	Prevents growth of most pathogenic bacteria; slows growth of spoilage microbes
Freezing (below –10°)	All foods	Prevents growth of all microbes
Pasteurizing (60–80°)	Milk, wines, etc.	Kills most non-sporing bacteria, yeasts and moulds
'Blanching' (95–110°)	Vegetables, shrimps	Kills vegetative bacteria, yeasts and moulds
Canning (above 100°)	Canned foods	'Commercially sterilizes' food; kills all pathogenic bacteria
Drying	Fruit, vegetables, meat, fish	Halts growth of all microbes when $a_w < 0.60$
Salting	Vegetables, meat, fish	Halts growth of many microbes at salt concentration of $\approx 100$ g/l
Syruping (sugars)	Fruits, jam, jellies	Halts growth when $a_w < 0.70$
Acidifying	Fermented dairy and vegetable products	Halts growth of most bacteria (effects depend on acid type)
Irradiating	Various	Inactivates vegetative bacteria or spores according to dose

 $a_w$ , water activity.



Table 8. Effect of reducing salt level on probability (%) of toxin production by *Clostridium botulinum*\* types A and B in pasteurized pork slurry†

Heat treatment‡ . . .		Low		High	
pH§ . . .		6.0	6.5	6.0	6.5
Sodium chloride (g/l)	Sodium nitrite (µg/g)				
45	100	9	71	9	37
35	100	35	92	27	73
25	100	76	96	59	86

\* Mixed spore inoculum five strains type A, five strains type B; 0.3 spores/g slurry (10 spores/28 g bottle).

† Stored at 20° for up to 6 months.

‡ Low, centre temperature to 70°; high, centre temperature maintained at 70° for 1 h.

§ Mean pH level.

#### PRESERVATIVES AND FOOD SAFETY

In recent years several developments have caused food microbiologists to question whether further reducing the levels of preservatives in cured meats is desirable. Salt levels have gradually fallen to meet consumer demands for milder cures; levels of nitrite have been reduced, and the pH value of some pork has increased. Hams often contain polyphosphate, incorporated by manufacturers to improve slicing, but some polyphosphates also increase the pH value by about 0.3 units. All these trends increase the likelihood of growth of *Cl. botulinum*. If the salt level is reduced, *Cl. botulinum* grows sooner and faster; if the level of nitrite is reduced, it grows sooner and faster; if the pH is increased it grows better. Experienced food microbiologists can estimate the effect of modifying one factor on the growth response of a microbe, but anticipating the outcome if all three happen at the same time is impossible if appropriate experiments have never been done.

Foreseeing the need for this type of information, we developed an experimental system to study bacteria relevant to food safety in order to define the combinations of conditions that permit or prevent growth (Gibson & Roberts, 1986 *a, b*).

Research over many years had identified several factors which were important in controlling growth of *Cl. botulinum* in cured meats, including salt, nitrite, heat treatment and incubation temperature, but the quantitative relationships of all those factors was unknown. Work at this laboratory in a model cured meat system established the relative contributions of the various factors acting alone and in combination to control growth of *Cl. botulinum* types A and B in pasteurized cured meats. The resultant mathematical model predicts the probability of toxin production (i.e. growth) of *Cl. botulinum* in the cured meat system at a range of salt and nitrite levels, following three heat treatments, in the presence or absence of iso-ascorbate, polyphosphate or nitrate, at incubation (storage) temperatures from 15 to 35° (Robinson *et al.* 1982; Roberts & Gibson, 1986).

Table 8 illustrates the effect of decreasing salt level when all other factors remain constant. At about pH 6.0, following high heat treatment (80°/7 min + 70°/1 h), the probability of toxin production increased from 9 to 59% when salt was reduced from 45

Table 9. *Effect of reducing input nitrite level on probability (%) of toxin production by Clostridium botulinum\* types A and B in pasteurized pork slurry†*

Heat treatment‡ . . .		Low		High	
pH§ . . .		6.0	6.5	6.0	6.5
Sodium chloride (g/l)	Sodium nitrite (µg/g)				
25	300	3	35	1	23
25	200	25	79	13	57
25	100	76	96	59	86

\* Mixed spore inoculum five strains type A, five strains type B; 0.3 spores/g slurry (10 spores/28 g bottle).

† Stored at 20° for up to 6 months.

‡ Low, centre temperature to 70°; high, centre temperature maintained at 70° for 1 h.

§ Mean pH level.

Table 10. *Effect of iso-ascorbate on probability (%) of toxin production by Clostridium botulinum\* types A and B in pasteurized pork slurry† containing 25 g sodium chloride/l, pH about 6.54*

Sodium nitrite (µg/g)	Iso-ascorbate (µg/g)	Heat treatment‡	
		Low	High
100	0	96	86
100	1000	26	8
200	0	79	57
200	1000	5	2
300	0	35	23
300	1000	1	0

\* Mixed spore inoculum five strains type A, five strains type B; 0.3 spores/g slurry (10 spores/28 g bottle).

† Stored at 20° for up to 6 months.

‡ Low, centre temperature to 70°; high, centre temperature maintained at 70° for 1 h.

to 25 g/l (salt on water). Table 9 shows that at 25 g salt/l, reducing nitrite from 300 to 100 µg/ml increased the probability of toxin production from 1 to 59%.

Ascorbate/iso-ascorbate is sometimes used in cured meat products to stabilize (fix) colour. It reacts with free nitrite and was seen as a possible means of reducing residual nitrite in products, i.e. reducing the nitrite ingested. In our experiments iso-ascorbate was a highly significant factor in preventing growth of *Cl. botulinum* (Table 10). Iso-ascorbate reduced the probability of toxin production from 96 to 26% in slurry containing 25 g salt/l following the low heat treatment, and 86–8% following the high heat treatment. Hence, if salt and nitrite must be kept low, iso-ascorbate is a useful additive. Iso-ascorbate is so effective that at 25 g salt/l and 100 µg nitrite/g the probability of toxin production was lower (8%) than if salt was increased from 25 to 45 g/l without iso-ascorbate (probability 37%, see Table 8).

Table 11. *Salmonellae* doubling time (h) in Tryptone Soya Broth (pH 5.9–6.2)

Sodium chloride (g/l) . . .	45		39		25		10		5	
	Obs	Pre	Obs	Pre	Obs	Pre	Obs	Pre	Obs	Pre
30	0.8	0.8	—	0.7	0.5	0.4	0.3	0	—	0.5
25	1.0	1.1	0.9	0.9	—	0.6	0.6	0.6	0.6	0.6
20	1.9	2.2	—	1.8	1.2	1.2	1.0	1.1	—	1.2
15	4.2	6.0	4.6	4.9	3.4	3.3	2.6	3.1	2.6	3.1
10	18.4	23.7	—	19.2	16.2	12.9	17.7	11.5	8.9	12.3

Obs, observed; Pre, predicted.

Because the naturally occurring level of contamination by *Cl. botulinum*, about 1 spore/kg meat (Roberts & Smart, 1977), is lower than the number we used, our probabilities overestimate the probability of toxin production in commercial products.

The serious microbiological consequences of indiscriminate removal of preservatives from food products is clearly evident from these predictions. The advantage of the model is that the consequences of manipulating several factors at once can be estimated.

A model which predicts the probability of toxin production is appropriate to *Cl. botulinum* and a great improvement on *ad hoc* experimentation using inoculated products. In other cases estimates of the rate and amount of growth of key microbes with respect to time, temperature and other factors would be particularly beneficial to the food industry.

The first step was to develop appropriate mathematical modelling. Growth responses of salmonellae in a laboratory medium at various pH values, concentrations of sodium chloride and incubation temperatures have now been modelled successfully Gibson *et al.* (1988). Table 11 illustrates the combined effect of  $a_w$  (salt concentration, g/l) and storage temperature at one pH level (about 6.0) on the growth of a mixed inoculum of three strains of salmonellae.

Much of the current concern over the continued use of food preservatives is misplaced. Food-borne illness is a major concern and much of it is preventable by applying our knowledge of hygiene. Salmonellosis and, since 1981, *Campylobacter* enteritis are the most common causes of food-borne illness. *Salmonella* is already endemic in poultry; recent studies in Scandinavia and N. America suggest infections with *Campylobacter* are due directly or indirectly to contaminated poultry (Galbraith *et al.* 1987). If levels of preservatives were reduced further, even greater reliance would be placed on the maintenance of lower storage temperatures and the application of our knowledge of hygiene at a time when the failure of one or the other has been identified repeatedly as a contributory factor in outbreaks of food poisoning (Roberts, 1982).

#### REFERENCES

- Archer, D. L. & Kvenberg, J. E. (1985). Incidence and cost of diarrhoeal disease in the United States. *Journal of Food Protection* **48**, 887–894.
- Dainty, R. H., Shaw, B. G. & Roberts, T. A. (1983). Microbial and chemical changes in chill-stored red meats. In *Food Microbiology: Advances and Prospects. Society for Applied Bacteriology Symposium Series* no. 1' pp. 151–178 [T. A. Roberts and F. A. Skinner, editors]. London: Academic Press.

- Galbraith, N. S., Barret, N. & Sockett, P. N. (1987). Surveillance of foodborne infections—the role of the Communicable Disease Surveillance Centre. *British Nutrition Foundation Bulletin* **12**, 21–31.
- Gardner, G. A. (1982). Microbiology of processing: bacon and ham. In *Meat Microbiology*, p. 129 [M. H. Brown, editor]. London: Applied Science Publishers.
- Gardner, G. A. (1983). Microbial spoilage of cured meats. In *Food Microbiology: Advances and Prospects*, Society for Applied Bacteriology Symposium Series no. 11, pp. 179–202 [T. A. Roberts and E. A. Skinner, editors]. London: Academic Press.
- Gibson, A. M., Bratchell, N. & Roberts, T. A. (1988). Predicting microbial growth: the effect of storage temperature, pH and sodium chloride on the growth of salmonellae in laboratory medium. *International Journal of Food Microbiology* **6**, 155–178.
- Gibson, A. M. & Roberts, T. A. (1986a). The effect of pH, water activity, sodium nitrite and storage temperature on the growth of enteropathogenic *Escherichia coli* and salmonellae in laboratory medium. *International Journal of Food Microbiology* **3**, 183–194.
- Gibson, A. M. & Roberts, T. A. (1986b). The effect of pH, sodium chloride, sodium nitrite and storage temperature on the growth of *Clostridium perfringens* and faecal streptococci in laboratory media. *International Journal of Food Microbiology* **3**, 195–210.
- International Commission on Microbiological Specifications for Foods (1980). *Microbial Ecology of Foods*, vol. 1. *Factors Affecting Life and Death of Microorganisms*. New York: Academic Press.
- International Commission on Microbiological Specifications for Foods (1988). *Micro-organisms in Foods*, vol. 4. *Applications of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality*. Oxford: Blackwell Scientific Publications.
- Mead, G. C. (1982). Microbiology of poultry and game birds. In *Meat Microbiology*, pp. 67–101 [M. H. Brown, editor]. London: Applied Science Publishers.
- Roberts, D. (1982). Bacteria of public health significance. In *Meat Microbiology*, pp. 319–386 [M. H. Brown, editor]. London: Applied Science Publishers.
- Roberts, T. A. & Gibson, A. M. (1986). Chemical methods for controlling *Clostridium botulinum* in processed meats. *Food Technology, Champaign* **40**, 163–171, 176.
- Roberts, T. A. & Smart, J. L. (1977). The occurrence of clostridia, particularly *Clostridium botulinum* in bacon and pork. In *Spores 1976*, vol. 2, pp. 911–915 [J. Wolf, A. N. Barker, D. J. Ellard, G. J. Dring and G. W. Gould, editors]. London: Academic Press.
- Robinson, A., Gibson, A. M. & Roberts, T. A. (1982). Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. V. Prediction of toxin production: non-linear effects of storage temperature and salt concentration. *Journal of Food Technology* **17**, 727–744.
- Taylor, A. A. & Shaw, B. G. (1975). Wiltshire curing with and without nitrate. I. Vacuum packed sliced bacon. *Journal of Food Technology* **10**, 157–167.
- Todd, E. C. D. (1985a). Economic loss from food-borne disease outbreaks associated with food service establishments. *Journal of Food Protection* **48**, 169–180.
- Todd, E. C. D. (1985b). Economic loss from food-borne disease and non-illness related recalls because of mishandling by food processors. *Journal of Food Protection* **48**, 621–633.
- Tompkin, R. B. (1980). Botulism from meat and poultry products—a historical perspective. *Food Technology, Champaign* **34**, 229–236, 257.
- Troller, J. & Christian, J. H. B. (1978). *Water Activity and Food*. New York: Academic Press.
- Yule, B. F., Forbes, G. I., Macleod, A. F. & Sharp, J. C. M. (1986). *The Costs and Benefits of Preventing Poultry-borne Salmonellosis in Scotland by Irradiation*. Aberdeen: Aberdeen University Health Economics Research Unit.