FOOD PRESERVATIVES

Edited by N. J. Russell and G. W. Gould

FOOD PRESERVATIVES

Edited by

N.J. RUSSELL
Department of Biochemistry
University of Wales
Cardiff

and

G.W. GOULD Unilever Research Bedford

Blackie

Glasgow and London

Published in the USA by avi, an imprint of Van Nostrand Reinhold New York

Blackie and Son Ltd Bishopbriggs, Glasgow G64 2NZ and 7 Leicester Place, London WC2H 7BP

Published in the United States of America by AVI, an imprint of Van Nostrand Reinhold 115 Fifth Avenue New York, New York 10003

Distributed in Canada by Nelson Canada 1120 Birchmount Road Scarborough, Ontario M1K 5G4, Canada

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

© 1991 Blackie and Son Ltd First published 1991

All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means –

graphic, electronic or mechanical, including photocopying, recording, taping – without the written permission of the Publishers

British Library Cataloguing in Publication Data

Food preservatives.

1. Food. Preservation

I. Russell, N.J. (Nicholas J.) II. Gould, G.W. (Grahame Warwick) 664.028

ISBN 0-216-92910-5

Library of Congress Cataloging-in-Publication Data

Food preservatives / [edited by] N.J. Russell and G.W. Gould. p. cm.

Includes bibliographical references (p.).

ISBN 0-442-30280-0

1. Food preservatives. I. Russell, Nicholas J. II. Gould, G.W. (Grahame Warwick)
TP371.2.F67 1990
664'.028—dc20 89-29396

CIP

CII

Food Preservatives

Preface

Long ago man discovered how to treat his food so that it was less susceptible to spoilage during the long winter months when fresh food was difficult to find. Many different chemical preservatives developed through centuries of common usage and these are generally the ones, such as salt, sugar and vinegar and other acids derived by fermentation, with which everyone is familiar. In more recent times, chemically-synthesized compounds have been added to the growing list of substances which not only prolong the storage and shelf-life of commodities and improve safety, but may also enhance the organoleptic and other desirable qualities of food-stuffs.

During the past decade, eating habits have changed to become what is popularly termed 'more healthy'. As part of this trend, the general public is much more concerned with the effects that preservatives and other substances might have on their well-being. This concern has been matched by a reduction in the levels of preservatives that are used in a range of commodities. It is suspected in some quarters that this could have contributed to increases in the numbers of reported cases of food poisoning, but no clear proof of cause and effect has yet been provided.

It is against this background that the present book was conceived. We believe that it is particularly appropriate at this time because it not only provides a long overdue update on chemical methods of food preservation but also provides guidance on the effective use of food preservatives in industry. The authorship comes from industry, food research institutes and academia. Most chapters are written by two authors who discuss the usage and underlying modes of action, including effects on target micro-organisms, of the most important preservatives. This unique feature of the book is designed to rationalize many of the rules and guidelines for practical use and to make them more easily understood. Furthermore, improved understanding of the basic modes of action should assist industry in the search for compounds with improved qualities in relation to preservation and acceptability.

The book covers the major antimicrobial food additives, including the use of salts, sugars and other solutes to lower water activity; acidification and the use of specific acidulants; organic acids such as propionic, sorbic and benzoic; esters such as the parabens; sulphite; nitrite; antibiotics such as

vi PREFACE

nisin and pimaricin; ethanol; carbon dioxide; antimicrobial antioxidants and glycerol esters. Although not strictly chemical compounds, starter cultures are covered in a separate chapter.

Two introductory chapters provide basic information about the properties of the major types of food-poisoning and food-spoilage micro-organisms, and the main factors that affect their growth and survival. These are followed by chapters on individual preservative compounds or groups of compounds, covering their practical use, effects on micro-organisms, and current ideas about their mechanisms of action. Next comes a substantial chapter which summarizes trends in legislation worldwide, with particular attention to the EEC, UK and USA. The final chapter looks to the future, considering alternative preservation systems that many believe will soon find use, including the natural systems that operate so effectively in many plants and animals.

The book is directed at those companies involved in food production, distribution and sales, particularly their development and quality control departments. It should appeal to those in the various government and industrially-sponsored food research institutes and associations around the world. In addition, the book should be a useful teaching aid for degrees in food science, home economics, microbiology and other courses supporting food-related research in universities, polytechnics and colleges.

We should like to thank all the authors for their contributions and for their patience during the preparation of this manuscript. As editors we have tried to retain as much individuality of style whilst blending the individual contributions of co-authored chapters into a unified whole. We trust that this multi-faceted approach to the topic will make the book particularly useful to all those who are concerned with food preservatives.

NJR GWG

Contributors

- Dr R.G. Board School of Biological Sciences, University of Bath, Claverton Down, Bath BA2 7AY, UK
- **Dr I.R. Booth** Department of Molecular and Cell Biology, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, UK
- **Dr M.H. Brown** Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK
- **Dr R. Cammack** Department of Biochemistry, King's College London (KQC), Kensington Campus, Campden Hill Road, Kensington, London W8 7AH, UK
- **Dr R.G. Earnshaw** Campden Food and Drink Research Association, Chipping Campden, Glos. GL55 6LD, UK
- **Dr T. Eklund** Norwegian Dairies Association, P.O. Box 9051, Vaterland, N-0134 Oslo, Norway
- **Dr G.G. Fowler** Aplin and Barrett Ltd., North Street, Beaminster, Dorset DT8 3DZ, UK
- **Dr M.J. Gasson** AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK
- **Dr C.D. Gill** Agriculture Canada, Lacombe Research Station, Bag Service 5000, Lacombe, AB Alberta TOC 1SO, Canada
- **Professor G.W. Gould** Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK
- Dr J.J. Kabara Lauricidin, 414 Green Street, Glena, Illinois 61036, USA
- **Dr L. Leistner** Federal Centre for Meat Research, Oskar von Miller Strasse 20, D-8650 Kulmbach, Germany
- **Dr F.-K. Lücke** Microbiology Laboratory, Fachbereich Haushalt & Ernährung, Fachhochschule Fulda, P.O. Box 1269, D6400 Fulda, Germany
- **Dr G. Molin** Department of Applied Microbiology, Lund University, P.O. Box 124, 22100 Lund, Sweden

- **Dr M.J. Payne** Department of Biochemistry, King's College London (KQC), Kensington Campus, Campden Hill Road, Kensington, London W8 7AH, UK
- Miss J.A. Pollard Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, UK
- **Dr T.A. Roberts** AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT, UK
- **Dr N.J. Russell** Department of Biochemistry, University of Wales College of Cardiff, P.O. Box 903, Cardiff CF1 1ST, UK
- **Dr D.A.L. Seiler** Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts WD3 5SH, UK
- **Dr L.F.J. Woods** Harris Ipswich, Hadleigh Road, Ipswich, Suffolk IP2 0HQ, UK

Contents

1		jor food-poisoning and food-spoilage micro-organisms V. GOULD and N.J. RUSSELL	1
	1.1	Introduction	1
	1.2	Food-poisoning micro-organisms	1
		1.2.1 Salmonella	3
		1.2.2 Campylobacter	3
		1.2.3 Listeria	4
		1.2.4 Yersinia	4
		1.2.5 Vibrio	4 4 5 5 6 7
		1.2.6 Escherichia	4
		1.2.7 Staphylococcus	5
		1.2.8 Clostridium botulinum	5
		1.2.9 Clostridium perfringens	6
		1.2.10 Bacillus cereus	7
	1.3	Food-spoilage micro-organisms	7
		1.3.1 Gram-negative, catalase-positive, oxidase-positive rods	7
		1.3.2 Gram-negative, catalase-positive, oxidase-negative rods	9
		1.3.3 Gram-positive, catalase-positive, non-sporing rods	9
		1.3.4 Gram-positive, catalase-positive cocci	9
		1.3.5 Gram-positive, catalase-negative, non-sporing rods and cocci	10
		1.3.6 Gram-positive, catalase-positive, spore-forming rods	10
		1.3.7 Gram-positive, catalase-negative, spore-forming rods	10
		1.3.8 Yeasts and moulds	11
	1.4	Conclusions	11
	Refe	erences	11
2	Fac	ctors affecting growth and survival	13
		. RUSSELL and G.W. GOULD	
	2.1	Introduction	13
	2.2	Basis of food preservation	14
	2.3	Major food preservation techniques	14
		2.3.1 Low temperature	15
		2.3.2 Reduction in water activity	16
		2.3.3 Vacuum and modified-atmosphere packaging	17
		2.3.4 Acidification	18
		2.3.5 Use of preservatives	19
		2.3.6 Compartmentalization	19
		2.3.7 Heat	20
	2.4	Conclusions	20
	References		21

X CONTENTS

3	Aci	dulants and low pH	22	
	M.F	H. BROWN and I.R. BOOTH		
	3.1	Introduction	22	
	3.2	The effect of pH on growth and survival	23	
	3.2	3.2.1 Growth	23	
		3.2.2 Survival	24	
	3.3	Effects of acidification on different types of bacteria	25	
	5.5	3.3.1 Non-sporing bacteria	25	
		3.3.2 Sporing bacteria	25	
		3.3.3 Yeasts and moulds	26	
		3.3.4 Food-borne pathogens	26	
	3.4	Acidification of foods	26	
		3.4.1 Types of acid	27	
		3.4.2 The use of weak acids in food preservation	27	
		3.4.3 Methods of altering the pH of foods	28	
	3.5	Practical examples	29	
		3.5.1 Canning	29	
		3.5.2 Mayonnaise	30	
	_	3.5.3 Egg albumen	30	
	3.6	Mechanisms of action	31	
		3.6.1 General aspects	31	
		3.6.2 Mechanisms of the effects of external pH on growth and survival	32	
		3.6.3 Cytoplasmic pH and growth	35	
		3.6.4 pH homeostasis in micro-organisms 3.6.5 Sensing external and internal pH	36 39	
	Refe	erences	41	
	_			
4		ganic acids and esters	44	
	J.J.	KABARA and T. EKLUND		
	4.1	Introduction	44	
	4.2	Fatty acids as antimicrobial agents	47	
	4.3	Monoesters as antimicrobial agents	50	
	4.4	Phenolic esters as antimicrobial agents	53	
	4.5	Application of fatty acids and monoesters in food products	57	
	4.6	General mechanism of action	60	
	4.7	Specific modes of action	64	
		4.7.1 Acetic acid	64	
		4.7.2 Propionic acid	65	
		4.7.3 Lactic acid	65	
		4.7.4 Sorbic acid 4.7.5 Benzoic acid	65 67	
		4.7.6 Esters of <i>p</i> -hydroxybenzoic acid (parabens)	67	
	Refe	erences	69	
_	C1	LL:i.	72	
5		phite	72	
	G.V	G.W. GOULD and N.J. RUSSELL		
	5.1	Introduction	72	
	5.2	Uses in food	72	
	5.3	Mode of action	75	
		5.3.1 Chemistry of sulphite	75	
		5.3.2 Reactions of sulphite	77	
		5.3.3 Uptake of sulphite by micro-organisms	79	
		5.3.4 Metabolism of sulphite by micro-organisms	81	

		CONTENTS	хi
		5.3.5 Antimicrobial action of sulphite Postscript rences	83 86 87
6	Nitrite T.A. ROBERTS, L.F.J. WOODS, M.J. PAYNE and R. CAMMACK		
	6.1	Introduction	89
	0.1	6.1.1 Meat as a source of pathogenic bacteria	89
		6.1.2 Factors affecting growth	90
	6.2	Cured meats	91
		6.2.1 Raw cured meats 6.2.2 Comminuted cured meats	94 94
		6.2.3 Heated cured meats	95
	6.3	Nitrite in combination preservation	96
		Nitrosamines	97
		Nitrite alternatives	98 99
		Risk analysis Mechanisms of action	100
	0.7	6.7.1 Inhibition of bacteria by nitrite	100
		6.7.2 Nitrite in the preservation of meat products	101
		6.7.3 Inhibitory compounds produced from nitrite	102
	6.8	6.7.4 Electron spin resonance of iron-nitrosyl-thiol species Conclusions	105 108
		rences	108
7		utes and low water activity	111
	L. I	EISTNER and N.J. RUSSELL	
	7.1	Introduction	111
	7.2	Influence of water activity on micro-organisms	112
	,	7.2.1 Multiplication	112
		7.2.2 Metabolic activity 7.2.3 Resistance	113 114
		7.2.4 Survival	115
	7.3	Foods	115
		7.3.1 Adjustment	116
		7.3.2 Products 7.3.3 Traditional foods	116 117
		7.3.4 Novel foods	117
	7.4	Microbial response to low water activity: basic mechanisms	119
		7.4.1 Accumulation of compatible solutes	119
		7.4.2 Osmoregulation of compatible solute uptake	122
		7.4.3 Molecular properties of compatible solutes7.4.4 Gram-negative bacteria and osmoregulation of the periplasm	124 125
		7.4.5 Membrane lipid changes in response to low $a_{\rm w}$	127
	7.5	Combined factors	129
		7.5.1 Hurdle effect	130
	Refe	7.5.2 Practical applications	130 131
8		tibiotics—nisin	135
o		G. FOWLER and M.J. GASSON	133
	U. (, I O II LLIK GIIG III.J. GABBOTT	
	8.1 8.2	Introduction Background to the use of nisin in food preservation	135 135

xii CONTENTS

	8.3	Practical applications of nisin in food preservation 8.3.1 Definition of nisin activity and potency 8.3.2 Relationship between nisin concentration in food and numbers of	136 136
		potential spoilage bacteria and spores	137
		8.3.3 First uses of nisin	137
	8.4	8.3.4 Development of nisin applications Mode of action	138 146
	8.5	Structure and biosynthesis of nisin	146
	8.6	The genetic determinant for nisin biosynthesis	149
	Refe	erences	150
9	Eth	anol as a food preservative	153
	D.A	A.L. SEILER and N.J. RUSSELL	
	9.1	Introduction	153
	9.2	Early studies	153
	9.3	Studies with foods 9.3.1 Prevention of microbial growth	155 155
		9.3.2 Anti-staling effects	160
		9.3.3 Organoleptic effects	162
	9.4		163
		9.4.1 Background	163
		9.4.2 Pleiotropic effects of ethanol	163
		9.4.3 Ethanol action on membranes	164
	0.5	9.4.4 Are membranes the primary target for ethanol? Legal considerations	167 168
		Conclusions	169
		erences	169
10	Mo	odified atmospheres and vacuum packaging	172
	C.I	D. GILL and G. MOLIN	
		Introduction	172
	10.2	Practors other than packaging affecting spoilage development	173
		10.2.1 Initial contamination 10.2.2 Temperature	173 173
		10.2.2 Product composition	174
	10.3	Non-microbial product deterioration	175
		10.3.1 Colour	175
		10.3.2 Odour and flavour	176
		10.3.3 Tenderness and texture	176
		10.3.4 Exudate	177
		Packaging materials	177
	10.5	5 Preparation and use of preservative packagings 10.5.1 Vacuum packs	179 179
		10.5.2 High-oxygen modified-atmosphere packaging	180
		10.5.3 Low-oxygen modified-atmosphere packaging	182
		10.5.4 Controlled atmosphere packaging	183
	10.6	6 Mode of action	185
		10.6.1 Gas phase	185
		10.6.2 Biological effects	186
		10.6.3 Oxygen limitation of carbon dioxide inhibition? 10.6.4 Microbial ecology	189 191
	10.7	7 Conclusions	197
		erences	198
11	Ph	enols and chelators	200
		KABARA	_00
	11	1 Introduction	200

	CONTENTS	xiii
	 11.2 Phenolic antioxidants as antimicrobial agents 11.3 Application of antioxidants in foods 11.4 Chelating agents as potentiators for preservatives References 	200 203 206 212
12	Starter cultures FK. LÜCKE and R.G. EARNSHAW	215
	12.1 Introduction 12.2 Preservation of various food commodities with the aid of starter	215
	cultures	216
	12.2.1 Milk and dairy products	216
	12.2.2 Meat and meat products 12.2.3 Foods of plant origin	218 221
	12.3 Mechanisms of action	221
	12.3.1 Competition for nutrients and space	222
	12.3.2 Formation of organic acids	223
	12.3.3 Formation of other products of catabolism	224
	12.3.4 Bacteriocins	226
	12.3.5 Other antimicrobial products of starter cultures 12.4 Practical aspects of detecting and quantifying antimicrobial processes	229
	mediated by starter cultures 12.5 Culture development and strain improvement	230
	12.6 Conclusions	231
	References	231
13	Legislative aspects J.A. POLLARD	235
	J.A. I OLLARD	
	13.1 Introduction	235
	13.2 Role of preservatives	235
	13.3 Legislation of preservatives	238
	13.3.1 EEC legislation	238
	13.3.2 UK legislation	244 250
	13.3.3 Other European countries legislation 13.3.4 USA legislation	252
	13.3.5 Other major countries legislation	256
	13.3.6 Codex Alimentarius Commission	260
	13.4 Future trends in preservatives legislation	262
	References	265
14	Future prospects	267
	R.G. BOARD and G.W. GOULD	
	14.1 Introduction	267
	14.2 Food ecosystems	268
	14.3 Predictive mathematical modelling of microbial growth and survival	
	in foods	272
	14.4 Future prospects	275
	14.4.1 Principles	275
	14.4.2 Natural antimicrobial systems 14.5 Conclusions	277 282
	References	283
Ind	av.	285

1 Major food-poisoning and food-spoilage micro-organisms

G.W. GOULD and N.J. RUSSELL

1.1 Introduction

The various means available for combating the deleterious effects of microorganisms in foodstuffs include, firstly, the prevention of their access to foods; secondly, inactivation, should they nevertheless be present; and, thirdly, the slowing or inhibition of their growth should they have gained access and not been inactivated (see Chapter 2). Food preservatives act principally in the third of these modes, operating predominantly by inhibition, although some preservatives additionally inactivate the inhibited micro-organisms at some rate or other as well.

Food preservatives are employed principally to prevent the spoilage of foods during storage and throughout distribution, retailing and use by the consumer. In this way they help to ensure that desired shelf-lives are met, that food products are convenient to store in the home, and that economic losses are avoided. Therefore, the targets of food preservatives are primarily those micro-organisms which can multiply in, and spoil, the various categories of foodstuffs. However, they also have a role in preventing the growth of pathogenic and toxinogenic micro-organisms which may contaminate foods, so that their influence on these food-poisoning micro-organisms is of importance as well.

In this introductory chapter we briefly indicate the major groups of food-poisoning and food-spoilage micro-organisms that are the targets of food preservatives.

1.2 Food-poisoning micro-organisms

Notwithstanding the tremendous advances that have taken place in food technology, distribution control, hygienic standards and education during the last few decades, food poisoning continues to increase in most developed countries of the world. In the UK the number of reported cases doubled between 1987 and 1988. The types of micro-organism that cause most of

the sporadic cases and outbreaks are not numerous, and are summarized in Table 1.1 They include infectious organisms, such as Salmonella and Campylobacter species, which cause by far the majority of cases of food poisoning, and toxinogenic organisms, such as Staphylococcus aureus and Clostridium botulinum, which cause far fewer. Their temperature minima for growth range upwards from near zero degrees Celsius, so that some of them are capable of slow growth in refrigerated, chill-stored foods. Their heat resistances range greatly from the low resistance of the most sensitive vegetative forms, with decimal reduction values (D-values) of <1 minute at 60°C, to the most resistant spores, with D-values of nearly 1 minute at 120°C.

Table 1.1 Food-poisoning micro-organisms of major concern.

	Heat resistance		
Minimum growth	Low	High	
temperature	Vegetative cells	Spores	
Low	Listeria monocytogenes (INF)*	Clostridium botulinum E and non-proteolytic B (TOX)†	
	Yersinia enterocolitica (INF)		
	Vibrio parahaemolyticus (INF)	Bacillus cereus (TOX)	
Medium	Salmonella species (INF)	Bacillus subtilis and licheniformis (TOX)	
Medium	Escherichia coli entero- pathogenic strains (INF)	Clostridium perfringens (INF)	
	Staphylococcus aureus (TOX)	Clostridium botulinum A and proteolytic B (TOX)	
High	Campylobacter jejuni and C. coli (INF)		

^{*}INF, organisms that may contaminate foods, and may multiply in them, and which cause food poisoning by infection.

In addition to those organisms listed in Table 1.1, there are other, rarer, causative agents of food poisoning that are implicated, or are suspected of being implicated, in erratic problems: these organisms include Shigella species, Aeromonas hydrophila, Plesiomonas shigelloides, Enterococcus species, Pseudomonas aeruginosa, Edwardsiella tarda, Vibrio vulnificus, Clostridium difficile, Cryptosporidium parvum, viruses (e.g. 'small round' viruses and Norwalk virus) and mycotoxic fungi.

[†]TOX, organisms that may contaminate foods and multiply in them to form toxins that cause food poisoning by intoxication.

1.2.1 Salmonella

There are about 2000 different serotypes of Salmonella. Whilst some serotypes are more virulent than others, it is widely accepted that all are potentially infectious for man. Next to Campylobacter (see section 1.2.2) salmonellae cause the majority of food-poisoning cases in developed countries. Salmonellosis is substantially under-reported, so that estimations of the true incidence of the disease have to be made on the basis of assumptions derived from epidemiological data.

For example, it has been estimated that in the USA clinically significant infections may reach as many as 2 million per annum and, of these, up to about 2000 may be fatal (1, 2). By far the majority of human infections by salmonellae derive from man's association with animals. Most foods of animal origin can transmit salmonellae directly or indirectly to man and some, such as poultry, are particularly commonly implicated.

The organisms are Gram-negative, non-sporing rods which are not especially tolerant of any of the conventional food preservation techniques currently in use. Therefore, growth is possible in many non-acidified, high-water-activity foodstuffs if held above chill temperatures. Growth in foods is important because of its potential to raise numbers above the minimum infective dose, which ranges, depending on the stage of health and susceptibility of the host, from as low as about 10 cells for *S. typhi* to thousands of cells for the majority of salmonellae.

1.2.2 Campylobacter

Campylobacter jejuni and C. coli are common inhabitants of the intestinal tracts of a variety of animals, and consequently are found irregularly as contaminants on meat, and particularly on poultry (3). Raw milk is the vehicle most commonly implicated in large outbreaks. Whilst originally considered to be a pathogen solely of veterinary significance, causing abortion in sheep, Campylobacter is now considered to be responsible for more human cases of enteric disease than are salmonellae (4).

Campylobacters differ from most of the other food-poisoning microorganisms in having a particularly high minimum temperature for growth of about 30°C. In addition, they are microaerophilic and do not compete well with other micro-organisms, so that their *growth* in foods is not regarded to be a problem. Rather, their ability to *survive* is important, but here again they are relatively sensitive to environmental factors such as high partial pressures of oxygen, drying, freezing, etc., but this may be offset to some extent by the fact that the infective dose for causing disease is probably as low as a few hundred organisms.

1.2.3 Listeria

Like the campylobacters, Listeria monocytogenes was, until the early 1980s, thought to be mainly of veterinary significance, causing encephalitis and abortion in sheep and cattle. Only the haemolytic species (L. monocytogenes, L. seeligeri and L. ivanovii) are pathogenic and, of these, only L. monocytogenes is regularly so. The numbers of human cases are <1% those of salmonellosis, and very few of these have proven food origins. Most instances of food-borne listeriosis have involved ingestion of raw milk or soft cheese (5). However, the high virulence of the organism for immunocompromised and pregnant humans has led to an increase in concern about its occurrence and potential for growth in foods. In particular, the ability of L. monocytogenes to grow at temperatures near to 0°C has focused attention on its presence in some chill-stored foods.

1.2.4 Yersinia

Yersinia enterocolitica causes severe though rare human infections, most characteristically affecting children and causing appendicitis-like symptoms. Avirulent strains are commonly isolated from animals and are found in foods, but pigs most often yield virulent strains. Chocolate milk, pasteurized milk and unchlorinated spring water have been associated with outbreaks but, oddly, pork meat has not been directly implicated in major outbreaks of yersiniosis (6).

Like Listeria, Yersinia species are psychrotrophic and are capable of multiplying slowly at temperatures near to 0° C.

1.2.5 Vibrio

Vibrio cholerae outbreaks are principally water-borne, but, of course, the organism may contaminate foods. V. parahaemolyticus, however, is an important food-poisoning organism, particularly in Japan, where substantial amounts of raw sea-food are eaten, causing 50% of the cases of enteritis in that country (7). The association of V. parahaemolyticus with fish and fish products is encouraged by its salt tolerance, some strains growing in the presence of up to 10% sodium chloride.

1.2.6 Escherichia

Outbreaks of food-borne disease attributable to enteropathogenic strains of *Escherichia coli* have increasingly been recognized recently (8). There are