Microbial Toxins in Dairy Products

Edited by Adnan Y. Tamime

Staphylococcus aureus
Staphylococcal enterotoxins (SEs)

Aspergillus niger
Aflatoxin B₁

WILEY Blackwell

Society of Dairy Technology
Microbial Toxins in Dairy Products
Society of Dairy Technology Series

Series Editor(s): A.Y. Tamime.

The Society of Dairy Technology has joined with Wiley-Blackwell to produce a series of technical dairy-related handbooks providing an invaluable resource for all those involved in the dairy industry; from practitioners to technologists working in both traditional and modern large-scale dairy operations.

Biofilms in the Dairy Industry
by Koon Hoong Teh, Steve Flint, John Brooks, and Geoff Knight (Editors)

Milk and Dairy Products as Functional Foods
by Ara Kanekanian (Editor)

Membrane Processing: Dairy and Beverage Applications
by Adnan Y. Tamime (Editor)

Processed Cheese and Analogues
by Adnan Y. Tamime (Editor)

Technology of Cheesemaking, 2nd Edition
by Barry A. Law and Adnan Y. Tamime (Editors)

Dairy Fats and Related Products
by Adnan Y. Tamime (Editor)

Dairy Powders and Concentrated Products
by Adnan Y. Tamime (Editor)

Milk Processing and Quality Management
by Adnan Y. Tamime (Editor)

Cleaning-in-Place: Dairy, Food and Beverage Operations, 3rd Edition
by Adnan Y. Tamime (Editor)

Structure of Dairy Products
by Adnan Y. Tamime (Editor)

Brined Cheeses
by Adnan Y. Tamime (Editor)

Fermented Milks
by Adnan Y. Tamime (Editor)

Probiotic Dairy Products
by Adnan Y. Tamime (Editor)
Microbial Toxins in Dairy Products

Edited by

Adnan Y. Tamime
Consultant in Dairy Science and Technology, Ayr, UK

WILEY Blackwell
Contents

List of Contributors xi
Preface to the Technical Series xv
Preface xvi

1 Microbial Toxins – An Overview 1
R. Early and A.Y. Tamime

1.1 Introduction 1
1.2 Microbial toxins: modes of action 2
1.3 Bacterial toxins 4
  1.3.1 Staphylococcal enterotoxins (SEs) 4
  1.3.2 Bacillus cereus group enterotoxins 6
  1.3.3 Clostridium botulinum neurotoxin 6
1.4 Mycotoxins 7
  1.4.1 Background 7
  1.4.2 General aspects of mycotoxins 7
  1.4.3 Postscript on mycotoxins 13
1.5 Biogenic amines (BAs) 14
1.6 Conclusions 14
References 16

2 Incidences of Mould and Bacterial Toxins in Dairy Products 19
M.L.Y. Wan, N.P. Shah and H.I. El-Nezami

2.1 Background 19
2.2 Bacterial toxins 19
  2.2.1 Emetic toxin produced by Bacillus cereus 20
  2.2.2 Enterotoxins produced by Staphylococcus aureus 24
  2.2.3 Botulinum neurotoxins produced by Clostridium botulinum 26
2.3 Mould toxins (mycotoxins) 33
  2.3.1 Aflatoxins B$_1$ and M$_1$ 34
  2.3.2 Sterigmatocystin 42
  2.3.3 Ochratoxin A 43
2.4 Other mycotoxins 47
2.5 Conclusion 49
References 50
3 Bacterial Toxins – Structure, Properties and Mode of Action 71
J.W. Austin
3.1 Background 71
3.2 Bacillus cereus toxins 72
3.2.1 Bacillus cereus emetic toxin 73
3.2.2 Bacillus cereus enterotoxins 74
3.2.3 Bacillus cereus haemolysin BL (Hbl) 76
3.2.4 Bacillus cereus non-haemolytic enterotoxin (Nhe) 76
3.2.5 Cytotoxin K (Cyt K) 77
3.3 Botulinum neurotoxin 77
3.3.1 Outbreaks of botulism caused by dairy products 78
3.3.2 Structure of botulinum neurotoxin 79
3.3.3 Mode of action of BoNTs 80
3.4 Staphylococcus aureus enterotoxin 80
3.5 Conclusions 83
References 83

4 Biogenic Amines in Dairy Products 94
V. Ladero, D.M. Linares, M. Pérez, B. del Rio, M. Fernández and M.A. Alvarez
4.1 Introduction 94
4.2 Biochemistry: biosynthesis pathways, enzymes and transporters 98
4.2.1 Tyramine 98
4.2.2 Histamine 99
4.2.3 Putrescine 100
4.2.4 Cadaverine, β-phenylethylamine, tryptamine 101
4.3 Biogenic amine-producing micro-organisms 101
4.3.1 Genes involved in the biosynthesis of biogenic amines 103
4.3.2 Is the production of BAs a strain- or species-dependent characteristic? 105
4.3.3 Physiological functions of BAs biosynthesis 106
4.4 Toxicological effects 107
4.4.1 Tyramine 108
4.4.2 Histamine 109
4.4.3 Putrescine and polyamines 110
4.4.4 Cadaverine, tryptamine and β-phenylethylamine 111
4.4.5 Recommended limits of BAs 111
4.5 Factors affecting BAs accumulation in dairy products 113
4.5.1 Presence of BAs-producing bacteria 113
4.5.2 Physiochemical factors 114
4.5.3 Technological factors 117
4.6 Other preventive methods 119
4.7 Conclusions 119
Acknowledgements 120
References 120
## 5 Contamination of Raw Milk: Sources and Routes Up to the Farm Gate

R. Early

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction</td>
<td>132</td>
</tr>
<tr>
<td>5.2 The concept of contamination</td>
<td>132</td>
</tr>
<tr>
<td>5.2.1 What does contamination mean to the concept of food?</td>
<td>134</td>
</tr>
<tr>
<td>5.2.2 Contamination and cow health</td>
<td>135</td>
</tr>
<tr>
<td>5.3 Sources of contamination</td>
<td>136</td>
</tr>
<tr>
<td>5.3.1 Biological contamination</td>
<td>136</td>
</tr>
<tr>
<td>5.3.2 Chemical contamination</td>
<td>142</td>
</tr>
<tr>
<td>5.3.3 Mycotoxins</td>
<td>147</td>
</tr>
<tr>
<td>5.3.4 Physical contamination</td>
<td>148</td>
</tr>
<tr>
<td>5.4 Conclusion</td>
<td>150</td>
</tr>
</tbody>
</table>

References | 150 |

## 6 Milk Product Contamination After the Farm Gate

R. Early

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 Introduction</td>
<td>154</td>
</tr>
<tr>
<td>6.2 The significance of microbial contamination</td>
<td>154</td>
</tr>
<tr>
<td>6.2.1 Product spoilage</td>
<td>154</td>
</tr>
<tr>
<td>6.2.2 Food-borne illness</td>
<td>155</td>
</tr>
<tr>
<td>6.2.3 Microbial toxins</td>
<td>155</td>
</tr>
<tr>
<td>6.3 Factories, processes and people</td>
<td>157</td>
</tr>
<tr>
<td>6.4 Raw milk handling</td>
<td>158</td>
</tr>
<tr>
<td>6.5 Milk-processing and dairy products manufacture</td>
<td>160</td>
</tr>
<tr>
<td>6.5.1 Liquid milk and cream processing</td>
<td>161</td>
</tr>
<tr>
<td>6.5.2 Packing, storage, distribution and the retail environment</td>
<td>162</td>
</tr>
<tr>
<td>6.6 Buttermaking</td>
<td>164</td>
</tr>
<tr>
<td>6.7 Cheesemaking</td>
<td>165</td>
</tr>
<tr>
<td>6.8 Yoghurt</td>
<td>168</td>
</tr>
<tr>
<td>6.9 Milk powders</td>
<td>170</td>
</tr>
<tr>
<td>6.10 Evaporated milk and sweetened condensed milk</td>
<td>172</td>
</tr>
<tr>
<td>6.10.1 Evaporated milk</td>
<td>172</td>
</tr>
<tr>
<td>6.10.2 Sweetened condensed milk</td>
<td>175</td>
</tr>
<tr>
<td>6.11 Ice-cream</td>
<td>175</td>
</tr>
<tr>
<td>6.12 Hygiene, food safety management and cleaning-in-place (CIP)</td>
<td>177</td>
</tr>
<tr>
<td>6.13 Packaging, storage, distribution and the retail environment</td>
<td>178</td>
</tr>
<tr>
<td>6.14 Conclusions</td>
<td>180</td>
</tr>
</tbody>
</table>

References | 180 |

## 7 Techniques for Detection, Quantification and Control of Bacterial Toxins

L. Ramchandran, A. Warnakulasuriya, O. Donkor and T. Vasiljevic

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 Introduction</td>
<td>183</td>
</tr>
<tr>
<td>7.2 Bacterial toxins</td>
<td>184</td>
</tr>
</tbody>
</table>
7.3 Control of toxins 186
7.4 Methods for identification and detection of microbial toxins 187
  7.4.1 Traditional biological assays 189
  7.4.2 Antibody and immunoassay 191
7.5 Conclusion 196
References 196

8 Techniques for Detection, Quantification and Control of Mycotoxins in Dairy Products 201
O. Donkor, L. Ramchandran and T. Vasiljevic

8.1 Introduction 201
8.2 Methods for detection and quantification of mycotoxins 203
  8.2.1 Sample pre-treatment method 203
  8.2.2 Liquid-liquid extraction 204
  8.2.3 Supercritical fluid extraction 204
  8.2.4 Solid phase extraction 204
8.3 Separation methods 206
  8.3.1 Thin layer chromatography 206
  8.3.2 High pressure liquid chromatography (HPLC) 208
  8.3.3 Gas chromatography (GC) 210
  8.3.4 Capillary electrophoresis (CE) 210
  8.3.5 Biosensors 210
  8.3.6 Enzyme-linked immunosorbent assay (ELISA) method 212
  8.3.7 Electrochemical immunoassay 214
  8.3.8 Polymerase chain reaction (PCR)-based detection and quantification 215
8.4 Mathematical model (exposure assessment of mycotoxins in dairy milk) 216
8.5 Control of mycotoxin 217
  8.5.1 Physical methods 218
  8.5.2 Chemical methods 218
  8.5.3 Biological methods 219
  8.5.4 Activated carbon (AC) 219
8.6 Conclusion 220
References 220

9 Approaches to Assess the Risks/Modelling of Microbial Growth and Toxin Production 229
N. Murru, R. Mercogliano, M.-L. Cortesi, F. Leroy, R. Condoleo and M.F. Peruzy

9.1 Background on risk analysis 229
9.2 Focus on cheese risk assessment 231
  9.2.1 Source of milk 231
  9.2.2 Raw and/or heat-treated milk cheeses 231
  9.2.3 Level of moisture in cheese 232
10.2.3 Milk and dairy powders 290
10.2.4 Cheeses (natural and processed) and fermented milks 291
10.3 Bacterial toxins 292
10.3.1 Staphylococcal enterotoxins 292
10.3.2 Bacillus cereus toxins 293
10.3.3 Clostridium botulinum toxins 294
10.4 Regulatory provisions on bacterial toxins in milk and milk products 297
10.4.1 European regulations on food hygiene and food safety 297
10.4.2 US milk hygiene and food safety standards 303
10.4.3 International perspective on food hygiene and safety – Codex Alimentarius 307
10.5 Mycotoxins 310
10.5.1 Aflatoxins 311
10.5.2 Other mycotoxins 311
10.5.3 Aflatoxins M₁ and B₁ and their regulatory provisions 312
10.5.4 EU legislations on aflatoxins in milk, milk products and animal feed 313
10.5.5 Regulations of aflatoxins of importance in milk and milk products in the USA 314
10.5.6 Regulations of aflatoxins of importance in milk and milk products in Canada 314
10.5.7 Regulation of aflatoxins in Australia and New Zealand 315
10.5.8 MERCOSUR standard on aflatoxins 315
10.6 Conclusions 316
References 316

Index 323
List of Contributors

Editor

Dr A.Y. Tamime
Dairy Science & Technology Consultant
Ayr
Scotland - United Kingdom
Tel. +44 (0)1292 265498
Fax +44 (0)1292 265498
Mobile +44 (0)7980 278950
E-mail: adnan@tamime.fsnet.co.uk

Contributors

Dr M.A. Alvarez
Spanish National Research Council (CSIC)
Dairy Research Institute (IPLA-CSIC)
Head of the Department of Biotechnology of Dairy Products
Villaviciosa
Asturias
Spain
Tel. +34 985893418
Fax +34 985892233
E-mail: maag@ipla.csic.es.

Dr J.W. Austin
Health Canada
Food Directorate
Health Products and Food Branch
Ottawa
Ontario
Canada
Tel. +1 613 957-0902
Fax +1 613 941-0280
E-mail: John.Austin@hc-sc.gc.ca

Dr R. Condoleo
Istituto Zooprofilattico Sperimentale Lazio e Toscana
M. Aleandri
Roma
Italy
Tel. +39 06 79099360
Fax +39 06 79099312
E-mail: roberto.condoleo@izslt.it

Dr M.-L. Cortesi
Research Group of Food Inspection of Department of Veterinary Medicine and Animal Production
Napoli
Italy
Tel. +39 081 2536469
Fax +39 081 458683
E-mail: cortesi@unina.it

Dr O. Donkor
Victoria University
Advanced Food Systems Research Unit
College of Health and Biomedicine
Werribee Campus
Melbourne
Victoria
Australia
Tel. + 61 (0)3 9919 8062
Fax + 61 (0)3 9919 8284
E-mail: osaana.donkor@vu.edu.au
Dr R. Early
Harper Adams University
Department of Food Science and Agri-Food Supply Chain Management
Newport
Shropshire
England – United Kingdom
Tel. +44 (0) 1952 815365
Mobile +44 (0) 7792 453319
Fax +44 (0) 1952 814783
E-mail: rearly@harper-adams.ac.uk & early818@btinternet.com

Dr M. Fernández
Spanish National Research Council (CSIC)
Dairy Research Institute (IPLA-CSIC)
Department of Biotechnology of Dairy Products
Villaviciosa
Asturias
Spain
Tel. +34 985893352
Fax +34 985892233
E-mail: mfernandez@ipla.csic.es

M. Hickey
Michael Hickey Associates Food Consultancy, Derryreigh
Creggane
Charleville
Co. Cork
Ireland
Tel. +353 (0)63 89392
Mobile +353 (0)87 2385653
E-mail: hickeymfg@eircom.net

Dr V. Ladero
Spanish National Research Council (CSIC)
Dairy Research Institute (IPLA-CSIC)
Department of Biotechnology of Dairy Products
Villaviciosa
Asturias
Spain
Tel. +34 985892131
Fax +34 985892233
E-mail: ladero@ipla.csic.es

Dr F. Leroy
Vrije Universiteit
Research Group of Industrial Microbiology and Food Biotechnology (IMDO)
Faculty of Sciences and Bio-engineering Sciences
Brussels
Belgium
Tel. +32 (0)2 6293612
Fax +32 (0)2 6292720
E-mail: fleroy@vub.ac.be

Dr D.M. Linares
Spanish National Research Council (CSIC)
Dairy Research Institute (IPLA-CSIC)
Department of Biotechnology of Dairy Products
Villaviciosa
Asturias
Spain
Tel. +34 985892131
Fax +34 985892233
E-mail: daniml@ipla.csic.es

Dr R. Mercogliano
Research Group of Food Inspection of Department of Veterinary Medicine and Animal Production
Napoli
Italy
Tel. +39 081 2536062
Fax +39 081 458683
E-mail: raffaella.mercogliano@unina.it

Dr N. Murru
Research Group of Food Inspection of Department of Veterinary Medicine and Animal Production
Napoli
Italy
Dr H.I. El‐Nezami  
University of Hong Kong  
5S‐13 Kadoorie Biological Sciences Building  
Hong Kong  
Tel. +852 2299 0835  
Fax +852 22990364  
E-mail: elnezami@hku.hk

Dr M.F. Peruzy  
Research Group of Food Inspection of  
Department of Veterinary Medicine and Animal Production  
Naples  
Italy  
Tel. + 0039 03 391884980  
E-mail: mariafrancesca.peruzy@gmail.com

Dr M. Pérez  
Spanish National Research Council (CSIC)  
Dairy Research Institute (IPLA-CSIC)  
Department of Biotechnology of Dairy Products  
Villaviciosa  
Asturias  
Spain  
Tel. +34 985892131  
Fax +34 985892233  
E-mail: mpgarcia@ipla.csic.es

Dr B. del Rio  
Spanish National Research Council (CSIC)  
Dairy Research Institute (IPLA-CSIC)  
Department of Biotechnology of Dairy Products  
Villaviciosa  
Asturias  
Spain  
Tel. +34 985892131  
Fax +34 985892233  
E-mail: beadelrio@ipla.csic.es

Dr N.P. Shah  
University of Hong Kong  
6N-08, Kadoorie Biological Sciences Building  
Dairy and Probiotic Unit  
Food and Nutritional Science Programme  
Hong Kong  
Tel. +852 2299 0836  
Fax +852 2559 9114  
E-mail: npshah@hku.hk

Dr T. Vasiljevic  
Victoria University  
Advanced Food Systems Research Unit  
College of Health and Biomedicine  
Werribee Campus  
Melbourne  
Victoria  
Australia  
Tel. + 61 (0)3 9919 8062  
Fax + 61 (0)3 9919 8284  
E-mail: todor.vasiljevic@vu.edu.au

Dr M.L.Y. Wan  
University of Hong Kong  
5S-12 Kadoorie Biological Sciences Building  
Hong Kong  
Tel. +852 2299 0835  
Fax +852 2299 0364  
E-mail: murphyly@connect.hku.hk

Dr L. Ramchandran  
Victoria University  
Advanced Food Systems Research Unit  
College of Health and Biomedicine  
Werribee Campus  
Melbourne  
Victoria  
Australia  
Tel. + 61 (0)3 9919 8062  
Fax + 61 (0)3 9919 8284  
E-mail: lata.ramchandran@vu.edu.au
Dr A. Warnakulasuriya
Victoria University
Advanced Food Systems Research Unit
College of Health and Biomedicine
Werribee Campus
Melbourne

Victoria
Australia
Tel. +61 (0)3 9919 8062
Fax +61 (0)3 9919 8284
E-mail: asalangika.warnakulasuriya@vu.edu.au
For more than 60 years, the Society of Dairy Technology (SDT) has sought to provide education and training in the dairy field, disseminating knowledge and fostering personal development through symposia, conferences, residential courses, publications and its journal, the *International Journal of Dairy Technology* (previously published as the *Journal of the Society of Dairy Technology*).

Over the last 150 years there has been an enormous gain in our understanding of the role of the microbial flora in food preservation, spoilage and the threats to our health. At the same time, improvements in process technology have been accompanied by massive changes in the scale of many milk processing operations, and the manufacture a wide range of dairy and other related products.

The Society has embarked on a project with Wiley-Blackwell to produce a technical series of dairy-related books to provide an invaluable source of information for practicing dairy scientists and technologists, covering the range from small enterprises to modern large-scale operation. This, the fourteenth volume in the series, on Microbial Toxins in Dairy Products, provides a timely and comprehensive update of the potential and possible routes for contamination, techniques for detection and how this knowledge can be used to reduce the risks to the consumer.

Andrew Wilbey
Chairman of the Publications Committee - SDT
October 2015
Food-borne diseases, including dairy products, have been recognised as major threats to human health and can affect the national economies of both industrialised and developing countries worldwide. It can be argued that some of the causes associated with dairy food-borne diseases are the use of raw milk in the manufacture of dairy products, faulty processing conditions during the heat treatment of milk, contamination of products after post-processing, failure in due-diligence (i.e. adding processed food to dairy products, for example, the case of botulism in hazel nut yoghurt in the United Kingdom), in-adequate clean water supply, and so on. Primarily, dairy food-borne diseases affecting human health are associated with certain strains of bacteria (e.g. belonging to the genera of *Clostridium*, *Bacillus*, *Escherichia*, *Staphylococcus* and *Listeria*) that are capable of producing toxins, plus moulds that are similarly capable of producing mycotoxins, such as aflatoxins, sterigmatocytin and ochratoxin. In addition, biogenic amines can accumulate in dairy products through microbial activity (e.g. starter cultures in cheeses), where the ingestion of high concentrations can be dangerous to human health.

Incidences of human illnesses associated with dairy products are relatively low compared with foodborne illnesses in general. The purpose of this book, which is written by a team of well-known international scientists, is to review the latest scientific knowledge/developments in these fields, such as surveying the incidences of human illnesses caused by the consumption of dairy products, updating the analytical techniques required to examine bacterial and mould toxins, and the potential for contamination of milk as it passes along the food chain (i.e. from ‘farm-to-fork’). This is complemented by a review of current approaches used to model microbial growth and toxin production plus the associated risks, and the regulatory measures available in different countries for control of microbial toxins in dairy products. It is anticipated that this up-dated information will help to further minimise the incidences of dairy food-borne illnesses in humans.

I would like to acknowledge the time and effort that the expert contributors have given to make this edition possible. Although some overlap of scientific data have occurred in some chapters, I have felt justified in allowing this overlap because it has resulted in making the chapter(s) easier to read and understand.

Adnan Y. Tamime

October 2015
1 Microbial Toxins – An Overview

R. Early and A.Y. Tamime

1.1 Introduction

Microbial toxins can be and often are troublesome to human health and well-being. History records numerous occurrences of death and suffering caused by disease organisms, such as *Yersinia pestis*, of bubonic plague or Black Death fame, *Corynebacterium diptheriae* and *Vibrio cholerae*, the causative organism of diptheria and cholera respectively, as their names suggest, *Bordetella pertussis*, which causes whooping cough, and *Salmonella enterica* subsp. *enterica* serotype Typhi, which causes typhoid. Although these bacterial pathogens exhibit very different aetiologies in terms of vectors and modes of infection, they are all similar in that they cause disease by means of toxins.

The word 'toxin' is derived from the ancient Greek language, and refers to poison produced by living cells or organisms; although today it has a wider application, and can apply to synthetic compounds.

Modern medicine, linked with improvements in sanitation and other public health measures, has reduced the incidence of many bacterially mediated diseases, particularly in technologically developed societies. Since the nineteenth century, our scientific understanding of the mechanisms by which these organisms proliferated and caused disease has increased greatly. Our ability to treat the diseases by means of vaccines and antibiotics has meant that for many people today, the spectre of the once common illnesses and death that the organisms represented no longer hovers close to society. This is not to say, however, that illness and disease caused by micro-organisms and their toxins are no longer problematic. While diseases, such as diptheria, cholera and typhoid are relatively uncommon today, modern consumers all too often encounter the actions and consequences of microbial toxins, particularly bacterial toxins. When they consume food products that have been contaminated and/or mismanaged, often during production, a consequence can be affliction with food-borne disease, commonly referred to as food poisoning.

All food businesses engaged in the production, processing, manufacture, distribution and sale of food products to consumers have to be concerned about food safety and the problem of food-borne disease. The dairy industry is no exception. Although dairy products are amongst the safest of food products, because of the exceptionally high
standards of general management, and specifically hygiene and food safety management, employed throughout the dairy chain, from farm to supermarket, the potential exists for dairy products to be involved in the occurrence of food-borne disease. The food safety management strategies of dairy farmers, milk processors and milk products manufacturers are designed and implemented precisely to safeguard consumers. An important part of the strategies concerns understanding the nature of the food-borne disease organisms that may be associated with dairy products, and how to minimise the risk of their occurrence in products.

The purpose of this chapter is to review the issue of toxins associated with dairy products and specifically those of microbial origin, thereby setting the scene for the rest of the book.

### 1.2 Microbial toxins: modes of action

Toxigenesis is the ability to produce toxins and the sources of microbial toxins that may be associated with dairy products are two-fold: (a) those produced by bacteria, and (b) those produced by fungi (or moulds). Bacterial toxigenesis is very significantly of greater concern to the dairy industry and consumers of milk products than are toxins produced by fungi, although the latter are not unimportant.

Bacterial toxins are commonly designated as endotoxins and exotoxins. Most Gram-negative bacteria and all Enterobacteriacea produce endotoxins (Lüderitz et al., 1966). These toxins are commonly components of bacterial cell walls in the form of lipopolysaccharides, and are compounds composed of three units: (a) the lipid A component, which is hydrophobic in nature, (b) the core oligosaccharide (consisting of an inner and outer core), the structure of which varies diversely according to bacterial species and subspecies, and (c) the O polysaccharide, or O antigen as it is also known. The somatic antigen is located in the cell wall of both Gram-positive and Gram-negative bacteria. However, the somatic O antigen is exhibited by many organisms, including salmonellas and the well-known *Escherichia coli* O157:H7, of which the letter O is at times erroneously reproduced as the numeral zero. Like the core oligosaccharide, the structure of the O antigen also varies widely according to bacterial species and subspecies.

As biologically active, heat stable endotoxins, lipopolysaccharides are commonly associated with the infections and variety of symptoms caused by Gram-negative bacteria. The lipopolysaccharide structures are released from the cell walls of pathogenic bacteria as they break down, or autolyse, following the normal path of death and decay, or experience externally mediated lysis such as when attacked by the immune system of the host organism. Lipid A is the toxic component of the lipopolysaccharide, considered by some to be the most potent element, although Bishop (2005) noted that other inflammatory compounds may be derived from bacteria, such as the diacylglycerolcysteine component of bacterial lipoproteins and nucleic acids amongst others. Because of its hydrophobic nature, lipid A attaches to and becomes embedded in the cell wall of the host cell, interfering with the normal transport and cell regulation processes undertaken by the cell wall. This has the consequence of causing inflammatory and other responses, including in some instances toxic shock, to the host organism.
Exotoxins, in contrast to endotoxins, are toxic compounds released by bacterial cells. They are commonly enzymes, although some are polypeptides. In some few cases, lipopolysaccharides may be secreted as exotoxins. Exotoxins are proteinaceous compounds secreted by bacterial cells locally to the point of action or at some distance from the tissue sites that they affect. For example, amongst the range of toxins produced by the Gram-positive organism *Staphylococcus aureus*, one is a protein enterotoxin that may be secreted onto food. Although the *Staph. aureus* organism may be killed by heat treatment, the toxin, which is quite heat stable, may remain unaffected and cause subsequent foodborne illness and the commonly exhibited emetic food poisoning symptoms. In contrast, *Clostridium botulinum*, the most heat resistant food-related pathogen of concern, produces an exotoxin, which is readily denatured by heating. Terms, such as enterotoxin and neurotoxin associated with, in this instance, *Staph. aureus* and the also Gram-positive *C. botulinum*, describe either the mode of action of the toxin, or the target tissues. Some bacterial toxins have specific cytotoxic activity, such as *C. botulinum* neurotoxin (BoNT), of which there are seven antigenic types labelled BoNT A to G, affecting only nerve tissues and preventing the proper functioning of neurotransmitters. Dembek *et al.* (2007) reported that human botulism was caused by BoNT A, B, E and F, and that the different neurotoxins exhibit different toxicities and persistence in cells. Other bacteria, such as *Bacillus cereus*, have a broad, non-specific cytotoxic activity, affecting various cells and tissue types causing non-specific cellular necrosis. Bacterial protein toxins exhibit strongly antigenic properties, although in some instances antitoxins can be used to neutralise their toxicity. In the fight against bacterial disease, toxoids can be produced from bacterial protein toxins by exposing the compounds to a combination of reagents, such as formalin and organic acids, and moderate heat. The toxoids can then be used to provide artificial immunisation against diseases, such as diphtheria. Such immunisation is desirable where infectious bacterial diseases are concerned, but is not normally practiced in relation to food-borne disease organisms of the kind associated with food poisoning.

Mycotoxins are a class of toxic compound produced by fungi. In contrast to bacterial toxins, they are of lesser concern to the dairy industry as mycotoxin producing fungi do not commonly grow on dairy products. Fungi normally associated with dairy products tend to be limited to organisms, such as *Penicillium roqueforti* used to produce mould ripened blue cheeses, for example, Stilton and Roquefort, and *Geotrichum candidum* used in the manufacture of white mould ripened soft cheeses, for example, Neufchatel, Camembert and Brie. The mycotoxins of concern to food safety are secondary metabolites, released from fungal cells during growth. They include aflatoxins (AFs), produced by *Aspergillus* species, such as *Aspergillus flavus* and *Aspergillus parasiticus*, *Fusarium* spp. mould toxins, ochratoxin produced by various *Aspergillus* spp. and *Penicillium* spp., patulin, produced by *Penicillium expansum*, amongst other species, and ergot, the cause of ergotism and produced by the fungus *Claviceps purpurea*. Mycotoxins are generally associated with the spoilage of commodity crops, such as cereals spoiled in the field by, for example, *Fusarium* spp. or *Clav. purpurea*, and harvested seeds and nuts kept in store, such as cereal grains, peanuts, and so on, contaminated with aflatoxins from the growth of *Aspergillus* spp.

From the perspective of the dairy industry, perhaps the main cause of concern with mycotoxins is the possibility of the transmission of these toxic compounds from contaminated animal feed through the cow (or other milk producing animal) into milk. The
risk then exists that mycotoxin residues in milk may be carried into dairy products, affecting consumers. As stated by the International Dairy Federation (IDF, 2012), the ability of a mycotoxin or its metabolite to be excreted in milk will depend on the ability of the compound to pass the blood-milk barrier. The IDF records that the only mycotoxin which has been shown to possess this ability to any significance is AF B_1, which is excreted in milk as AF M_1. Aflatoxin M_1 is presumed to be carcinogenic, affecting the liver, but is considerably less so than AF B_1, by a factor of 10.

1.3 Bacterial toxins

It is a well established fact that bacterial pathogens can cause food-borne diseases in humans, and the possible routes of infection with relevance to dairy products are: (a) ingestion of already produced toxin(s) (i.e. sensu stricto), and (b) ingestion of a pathogenic bacterium that is capable of producing toxins in the gastrointestinal (GI) tract (in situ). The micro-organisms that have been identified to cause food-borne illness via the consumption of dairy products belong to the genera of *Staphylococcus* (i.e. production of staphylococcal enterotoxin – SE), *Clostridium* (production of BoNT) and *Bacillus* (emetic type). These micro-organisms including their toxin production are reviewed extensively in Chapter 3; however, readers are referred to the following selected references for complete discussion of food-borne diseases including dairy products (Cary et al., 2000; Hui et al., 2001; Labbe & Garcia, 2001; De Buyser et al., 2001; de Leon et al., 2003; Jay et al., 2005; Granum, 2006; Heidinger et al., 2009; Argudín et al., 2010; Hale, 2012; Claeyss et al., 2013, 2014; Hadrya et al., 2013).

1.3.1 Staphylococcal enterotoxins (SEs)

According to Paulin et al. (2012), some characteristics of SEs in milk and dairy products are summarised as follows:

- The SEs pass through the stomach into the intestinal tract where they stimulate emesis and diarrhoea.
- Common symptoms are nausea, vomiting, retching, abdominal cramping and diarrhoea.
- Symptoms start 1–6 h after consuming food containing SEs and resolve within 1–3 d without the need for treatment.
- Food poisoning containing SEs is not usually fatal, but some fatalities can occur in very young or old people.
- Dairy-borne outbreaks in many countries are associated with consumption of dairy products made from raw milk that cause SEs intoxications.
- Pasteurisation of milk inactivates *Staph. aureus*, whilst cheeses made from raw milk do not have such an elimination step; thus, safety is not guaranteed.
- In general, staphylococci are inactivated by D_{60°C} of 6 min and the presence of lactoperoxidase in milk enhances the inactivation of *Staph. aureus*, decreasing its D value 15-fold; however, SEs are heat-stable at 121°C for 15 min.
• At present, 23 SE serotypes have been identified, and the potential for enterotoxin to occur in cheese can be defined as: (a) the initial concentration of *Staph. aureus* in milk prior to cheesemaking must be sufficient, (b) the genes in *Staph. aureus* must be able to encode SE production in cheese milk, (c) the environmental conditions of pH, temperature and other factors must be suitable to permit bacterial growth and enterotoxin production, and (d) subsequent treatments, such as scalding the curd and brining may inactivate *Staph. aureus*, but any enterotoxin which has been formed is unlikely to be destroyed in the cheese.

It is of interest to note that some strains of staphylococci are used as secondary starter cultures for the manufacture of certain cheese varieties, and according to Bockelmann (2010), “*Staphylococcus xylosus* and *Staphylococcus carnosus* are used in certain varieties of cheese to optimise the texture and aroma development. They are used as cheese adjuncts in starter cultures, or can be brushed or sprayed onto the cheese surface. These strains exhibit medium proteolytic and low lipolytic and aminopeptidase activities.”

“*Staphylococcus equorum* is ubiquitous in cheese brines. It became available as starter culture only recently, and it has similar technological properties as *Staph. xylosus*, which is used to optimise the texture and aroma development in the cheese. In combination with *Debaromyces hansenii*, *Staph. equorum* supports the growth of other smear type bacteria when the ripening of the cheese starts, and it has a mould-inhibiting effect. In addition, it can contribute to colour development when pigmented strains are used. Although the common consensus is that coagulase-negative staphylococci (CNS), such as *Staph. equorum* do not represent a concern with respect to food-borne disease, Irlinger et al. (2012) suggested that this may be changing and that CNS may produce enterotoxins harmful to humans.”

In dairy-mediated staphylococcal food poisoning, cheese has been the most frequently incriminated. In France, it accounted for about 90% of the staphylococcal-mediated outbreaks with raw-milk cheese representing 96.2% of the recorded cheese-borne staphylococcal intoxications. Also, the high incidence of SE-producing *Staph. aureus* in cheese compared to other dairy products appears to be a general tendency, probably because this product provides an optimal medium for the growth of enterotoxigenic *Staph. aureus*, which thrives in media rich in protein and with a high salt content (Singh et al., 2012), as is the case for many types of cheeses. Also, the commonly applied mesophilic temperature (25–37°C) during fermentation allows the pathogen to grow rapidly and produce enterotoxins before conditions are no longer favourable (active development of lactic acid by lactic acid bacteria (LAB) and consequent pH drop).

“*Staphylococcus* spp. are salt- and acid-tolerant micro-organisms, which can grow at the early stages of cheese ripening when the pH is still below 6, and they are found in all kinds of surface ripened cheeses. Like the yeasts, *Staph. equorum* is found in the cheese brine, sometimes at high cell counts (max. \(10^5\) colony forming units (cfu) mL\(^{-1}\)). When the cheese brine is pasteurised, frequently to reduce the yeast counts (a practice adopted by many soft cheese producers), no or very low concentrations of staphylococci are present. Species most frequently observed on smear cheeses are *Staph. equorum* (natural flora), *Staph. xylosus* (cultural flora), and the nonfood-grade *Staphylococcus saprophyticus* (natural contamination).”
“Staphylococcus equorum and *Staph. xylosus* seem to be the typical, naturally occurring species in cheese brines and on most smear cheeses. In a different study, all 150 cocci of a smeared Gouda cheese and a Bergkaese isolated from organic farmhouse cheese producer in Northern Germany were classified as *Staph. equorum* by amplified ribosomal deoxyribonucleic acid (DNA) restriction analysis (ARDRA) method. This was confirmed when staphylococci, which were isolated from French smeared soft cheeses of three different producers, were identified by species and strain level. The *Staph. equorum* flora consisted of a variety of strains, typical of a house flora, whereas all *Staph. xylosus* isolates showed identical DNA restriction patterns in pulsed-field gel-electrophoresis, which matched the pattern of a commercial *Staph. xylosus* strain, indicating that this organism was added as a starter culture.”

“*Staphylococcus saprophyticus* is a nonfood-grade species, and it is repeatedly isolated in low numbers from smear-ripened cheeses and brine. Acid curd cheese (Harzer) seems to be an exception, where *Staph. saprophyticus* can be predominant in the staphylococcal surface flora, and can grow to high counts (e.g. 10⁹ cfu cm⁻²)” – (Source: Technology of Cheesemaking, reproduced with permission of Wiley-Blackwell).

### 1.3.2 Bacillus cereus group enterotoxins

A wide range of enterotoxins are produced by *B. cereus* (see Chapter 3), and some characteristics of these toxins include: (a) the identified toxins are: emetic toxins (heat-stable at 126°C for 90 min), haemolysin BL (Bbl) (heat-labile and inactivated at 56°C for 30 min, for details refer to Chapter 3), non-haemolytic enterotoxin (Nhe) isoforms A, B and C (heat-labile and inactivated at 56°C for 30 min), and cytotoxic toxin (Cyt) isoforms K₁ and K₂ (heat-labile and inactivated at 56°C for 30 min), (b) the symptoms of food- or dairy-borne illnesses include nausea, abdominal cramps, watery diarrhoea and/or vomiting, and (c) the food-poisoning symptoms are caused by intoxication with the peptide cereulide, and the diarrheal form of *B. cereus* food poisoning is caused by enterotoxins produced by growth of *B. cereus* in the small intestine after ingestion of viable cells or spores.

### 1.3.3 Clostridium botulinum neurotoxin

The symptoms of botulism are caused by the ingestion of highly soluble exotoxin produced by *C. botulinum* while growing in foods or dairy products compared to the food poisoning of *Clostridium perfringens* where large numbers of viable cells must be ingested (for more details, refer to Chapter 3). The toxin produced is known as BoNT, and seven serotypes have been identified, for example, BoNT A to G; only types A, B, E, F and G cause diseases in humans (Jay et al., 2005). The thermal D values of endospores of *C. botulinum* (i.e. BoNT A to G) are: D₁₁₀°C of 2.7-2.9; D₁₁⁰°C of 1.3-1.7-2.9; not reported; D₈⁰°C of 0.8; D₈⁰°C of 1.6-1.8; D₈⁰°C of 0.3-0.8; and D₈⁰°C of 0.5, respectively. Also, all BoNTs are produced as single polypeptides (Jay et al., 2005). The chemical formula of the toxin is C₆₇₆₀H₁₀₄₄₇N₁₇₄₃O₂₀₁₀S₃₂ (http://en.wikipedia.org/wiki/Botulinum_toxin) – accessed on 22nd April 2015, and the structure of the BoNT is reported by Silvaggi et al. (2007).
1.4 Mycotoxins

1.4.1 Background

Fungal metabolites, which are toxic to humans and animals, are known as mycotoxins and consist of aflatoxins (AF – also known as A. flavus toxin – A-fla-toxin), ochratoxins (OTs), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, trichothecenes and ergot alkaloids (Zain, 2011). The International Agency for Research on Cancer (IARC, 2002a, 2002b) of the World Health Organisation (WHO) classified the carcinogenicity of mycotoxins to humans as follows: (a) AF is carcinogenic (Group 1), (b) OT and F are possibly carcinogenic (Group 2B), and (c) trichothecenes and ZEN are not carcinogenic to humans (Group 3) (IARC, 1993a, 2002a, 2002b; see also www.afro.who.int/des). The most likely predominant genera of fungi to produce mycotoxins in dairy products are Penicillium and Aspergillus (Frazier & Westhoff, 1988; Yousef & Juneja, 2003; Jay et al., 2005). The former organism could originate in milk due to unhygienic milk production (i.e. cheesemaking using raw milk), or the use of secondary starter cultures (e.g. Penicillium roqueforti) for the manufacture of Blue Veined cheeses (Roquefort, Stilton, Gorgonzola, Blue d’Auvergne, Cabrales, Blauschimmelkase, Tulum and Danablue) and white mould cheeses (Penicillium camemberti), such as Camembert, Brie and Gammelost. Although some penicilia spp. have been reported in old dairy books (Penicillium caseiocolum, Penicillium caseicola, Penicillium candidum and Penicillium album), these are now considered biotypes of, or synonyms for, P. camemberti (Tamime, 2002). However, Aspergillus spp. can contaminate animal feed (e.g. aflatoxins - AF), which can be excreted into the milks after being consumed by lactating cows. Another mould specie, Byssoclamys fulva, has been found in milk and can produce toxins (e.g. byssotoxin A, byssochlamic acid, patulin, fumitremorgin A and C, verruculogen, fischerin and eupenifeldin) (Tournas, 1994), but none have been implicated in dairy-borne products outbreaks, and they will not be reviewed in this chapter; however, more detailed information of incidences of mycotoxins in dairy products that can be implicated in human health risks are reviewed in Chapter 2.

1.4.2 General aspects of mycotoxins

Aflatoxin

According to Frazier & Westhoff (1988), IARC (2002a, 2002b), Yousef & Juneja (2003) and Jay et al. (2005), AF B₁ and B₂ are produced by A. flavus, whilst A. parasiticus produces AF B₁, B₂, G₁ and G₂. However, some aspergilla strains (Aspergillus nminus, Aspergillus bombycis, Aspergillus pseudotamari and Aspergillus ochraceoroseus) and Emericella venezuelensis are also AF-producers, but they are encountered less frequently especially in dairy products, and will not be reviewed in this book. Aflatoxin B₁ is produced by all aflatoxin mould-producers, which is the most potent form of all. Some AF serotypes (AF L, LH₁, Q₁ and P₁) are derived from AF B₁, and some of the main AF characteristics are shown in Table (AF 1.1). The potent toxicity of the main six AF in descending order is as follows: AF B₁ (blue)>M₁ (blue/violet)>G₁ (green)>B₂
(blue) > M₂ (violet) and G₂ (green/blue) – data in parenthesis illustrate the fluorescence noted when viewed under ultraviolet (UV) light, where the colours designate the AF serotype (IARC, 2002a, 2002b; Jay et al., 2005). Aflatoxins cross the human placenta, and exposure has been associated with growth impairment in young children. In general, AF production by moulds occurs in a growth environment of water activity (a_w) of 0.85 and at a temperature of 25–40°C.

**Ochratoxin**

Also, the genera of Aspergillus (e.g. A. ochraceus, A. westerdijkiae, A. niger, A. carbonarius, A. taeicoffeatus and A. sclerotioniger) and Penicillium (P. verrucosum and P. nordicum), which consist of many filamentous fungi, produce mycotoxin known as ochratoxin (OT) (el Khoury & Atoui, 2010; Sorrenti et al., 2013). The metabolite, which has first identified, is known as OT A, and its related metabolites, such as OT B (i.e. dechloro analogue of OT A) and OT C (i.e. the isocoumaric derivative of OT A) (see Table 1.1). The International Union of Pure and Applied Chemistry (IUPAC) names of OT A to C are as follows: N-[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-isochromen-7-yl]carbonyl]-L-phenylalanine, (2S)-2-[(3R)-8-hydroxy-3-methyl-1-oxo-3,4-dihydroisochromene-7-carbonyl]amino]-3-phenylpropanoic acid and ethyl (2S)-2-[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydroisochromene-7-carbonyl] amino]-3-phenylpropanoate, respectively. However, at present another 16 related metabolites of OT A have been identified, and for detailed information refer to the review by el Khoury and Atoui (2010). In addition to OT A to C, another sixteen OT A related derived metabolites have been also identified (el Khoury & Atoui, 2010), and some examples are OT α, OT β, OT A methyl-ester, OT B methyl-ester, OT B ethyl-ester, 4-R-hydroxy-OT A, 4-s-hydroxy-OT A, and 10-hydroxy-OT A.

The florescence colour noted viewed under UV light for OT A is greenish, whilst OT B emits blueish (Jay et al., 2005). According to Frazier & Westhoff (1988), although the effects of OTs to human beings are unknown or possibly slightly toxic, the significance of OTs in food is of interest for the following aspects:

- OTs are toxic to certain animals;
- Some OTs are heat resistant, and are not destroyed after prolonged autoclaving;
- Many OTs-producing moulds are able to grow and produce mycotoxin at temperatures below 10°C; and
- Ochratoxins have been isolated from many foods.

**Citrinin**

Mould organisms belonging to the following genera: Aspergillus (A. niveus, A. ochraceus, A. oryzae and A. terreus), Monascus (M. ruber and M. purpureus) and Penicillium (P. citrinum and P. viridicatum and P. camemberti) have been reported to produce mycotoxin known as citrinin (Jay et al., 2005; http://en.wikipedia.org/wiki/Citrinin). Citrinin is also known as antimycin and, according to the IUPAC, it is known as (3R,4S)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3H-isochromene-7-carboxylic acid.
Table 1.1  Some general characteristics of mycotoxins detected in dairy products.

<table>
<thead>
<tr>
<th>Name of mycotoxin</th>
<th>Chemical formula</th>
<th>Comments</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins (AF) B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Catalysing 3-hydroxylation of AF B&lt;sub&gt;1&lt;/sub&gt; to yield the AF Q&lt;sub&gt;1&lt;/sub&gt; metabolite Carcinogenic, immunosuppressive, hepatocarcinogens, genotoxic Binds covalently to live mitochondrial deoxynucleic acid</td>
<td></td>
</tr>
<tr>
<td>AF B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>It is the 2,3-dihydroform of AF B&lt;sub&gt;1&lt;/sub&gt;, which reduces the mutagenicity by 200- to 500-fold Carcinogenic, hepatocarcinogens, mutagenic, teratogenic, and causes immunosuppression</td>
<td></td>
</tr>
<tr>
<td>AF G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Carcinogenic, hepatocarcinogens</td>
<td></td>
</tr>
<tr>
<td>Name of mycotoxin</td>
<td>Chemical formula</td>
<td>Comments</td>
<td>Structure</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
</tbody>
</table>
| AF G₂             | C₁₇H₁₄O₇        | It is the 2,3-dihydroform of AF G₁  
Carcinogenic, hepatocarcinogens | ![AF G₂ Structure](image) |
| AF M₁             | C₁₇H₁₂O₇        | Hydroxylated product of AF B₁  
Hepatotoxic, mutagenic (the C₂─C₃ double bond in the dihydrofurofuran moiety),  
carcinogenic, immunotoxic, teratogenic,  
Less toxic than the parent compound AF B₁ | ![AF M₁ Structure](image) |
| AF M₂             |                  | It is a 4-hydroxy AF B₂  
Less toxic than the parent compound AF B₂ | ![AF M₂ Structure](image) |
| Ochratoxin (OT) A | C₂₀H₁₈ClNO₆     | It is hepatotoxic, nephrotoxic, neurotoxic, teratogenic and immunotoxic  
Carcinogenic to humans (Group 2B), and weakly mutagenic  
It is neurotoxic and cause immunosuppression and immunotoxicity in animals  
Can cause Balkan endemic nephropathy | ![Ochratoxin (OT) A Structure](image) |
OT B  \( \text{C}_{20}\text{H}_{19}\text{NO}_8 \)  Most likely it has similar properties as above, but not toxic

OT C  \( \text{C}_{22}\text{H}_{22}\text{CINO}_6 \)  Most likely it has similar properties as above

Citrinin (also known as antimycin)  \( \text{C}_{13}\text{H}_{14}\text{O}_5 \)  It is a nephrotoxin, and can permeate through the human skin. Although no significant health risk is expected after dermal contact in agricultural or residential environments but, nevertheless, it should be limited.
Under long-wave UV light → fluoresces lemon yellow
Compound produces nephritis in mice
Possess antimicrobial activity
Its chemical nature is quinonemethine

Patulin  \( \text{C}_{7}\text{H}_6\text{O}_4 \)  Toxicity - neurotoxic, hepatotoxic, nephrotoxic, genotoxic/teratogenotoxic, pulmonary congestion and edema
Demonstrated as carcinogen
It is toxic primarily through affinity to sulfhydryl groups (SH)
Its chemical nature is polyketide lactone

Roquefortine C  \( \text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_2 \)  Neurotoxin
Its chemical nature is indole alkaloid

(Continued)
<table>
<thead>
<tr>
<th>Name of mycotoxin</th>
<th>Chemical formula</th>
<th>Comments</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopiazonic acid (CPA)</td>
<td>C_{20}H_{20}N_{2}O_{3}</td>
<td>Appears to be toxic in high concentrations</td>
<td></td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>C_{18}H_{12}O_{6}</td>
<td>Toxic and it is related to dermatoxin Closely related to AF B_{1}, which is a potent liver carcinogenic, mutagenic and teratogenic</td>
<td></td>
</tr>
<tr>
<td>Penicillic acid</td>
<td>C_{7}H_{10}O_{4}</td>
<td>Demonstrated some toxic effects on laboratory animals</td>
<td></td>
</tr>
</tbody>
</table>

*Note:* The reported incidences of mycotoxins in dairy products are detailed in Chapter 2.