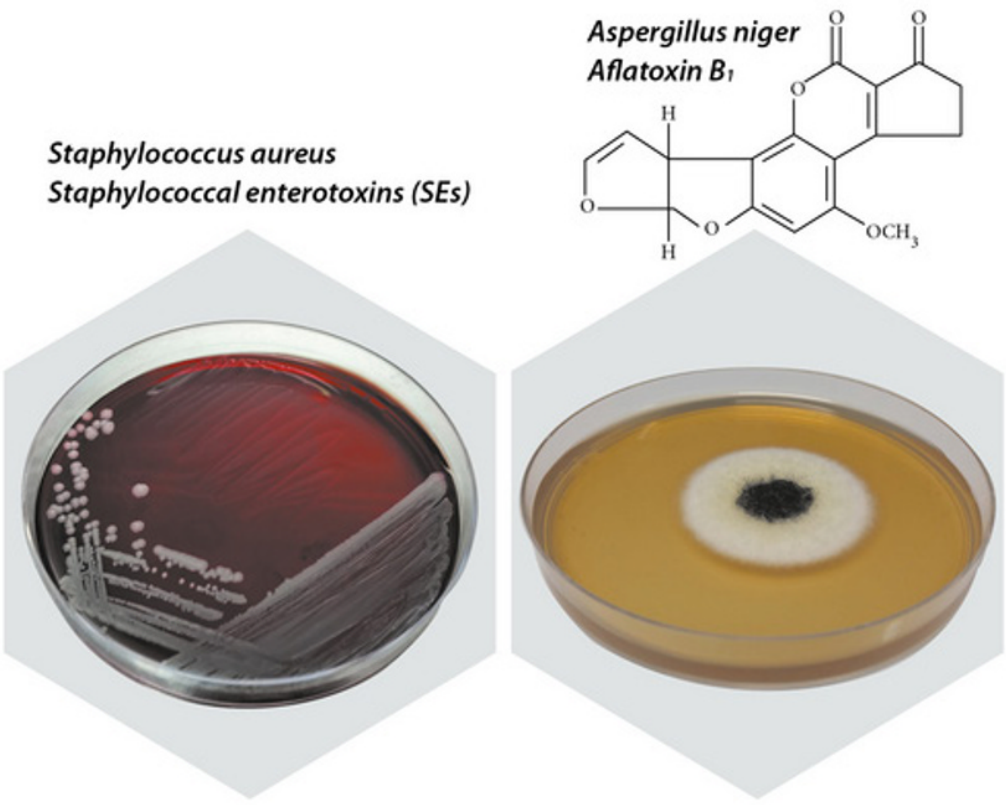
Microbial Toxins in Dairy Products

Edited by Adnan Y. Tamime



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Microbial Toxins in Dairy Products

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Registered Office
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Library of Congress Cataloging-in-Publication Data

Names: Tamime, A. Y., editor.
Title: Microbial toxins in dairy products / edited by Adnan Y. Tamime.
Other titles: Society of Dairy Technology series.
Description: Chichester, West Sussex, UK ; Hoboken, NJ : John Wiley & Sons, Inc., 2017. | Series: Society of Dairy Technology series | Includes bibliographical references and index.
Identifiers: LCCN 2016032565 (print) | LCCN 2016033156 (ebook) | ISBN 9781118756430 (cloth) | ISBN 9781118823651 (pdf) | ISBN 9781118823149 (epub)
Subjects: | MESH: Dairy Products–microbiology | Bacterial Toxins–toxicity | Dairy Products–toxicity
Classification: LCC QR121 (print) | LCC QR121 (ebook) | NLM QW 85 | DDC 579.3/7–dc23
LC record available at https://lccn.loc.gov/2016032565

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover image: Dr O. Donkor

Set in 10/12.5pt Times by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

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Preface to the Technical Series

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

Preface

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

Microbial Toxins in Dairy Products, First Edition. Edited by Adnan Y. Tamime.

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 diacylglycerylcysteine 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 food-borne 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; Claeys *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. 10^9 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 K_1 and K_2 (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 *nerotoxin*

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: $D_{110°C}$ of 2.7-2.9; $D_{110°C}$ of 1.3-1.7-2.9; not reported; $D_{80°C}$ of 0.8; $D_{110°C}$ of 1.6-1.8; $D_{80°C}$ of 0.3-0.8; and $D_{110°C}$ of 0.5, respectively. Also, all BoNTs are produced as single polypeptides (Jay *et al*., 2005). The chemical formula of the toxin is $C_{6760}H_{10447}N_{1743}O_{2010}S_{32}$ (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 caseiocola, 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, *Byssochlamys 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 infromation of incidences mycotoxins in dairy products that can be implicated in human health risk 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 B_1 and B_2 are produced by *A. flavus*, whilst *A. parasiticus* produces AF B_1, B_2, G_1 and G_2. However, some aspergilla strains (*Aspergillus nominus*, *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 B_1 is produced by all aflatoxin mould-producers, which is the most potent form of all. Some AF serotypes (AF L, LH_1, Q_1 and P_1) are derived from AF B_1, 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 B_1 (blue) > M_1 (blue/violet) > G_1 (green) > B_2

(blue) > M_2 (violet) and G_2 (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. lacticoffeatus* 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*-{[(3*R*)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1*H*-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.

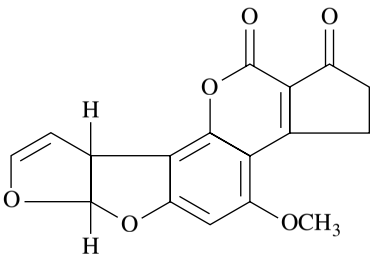
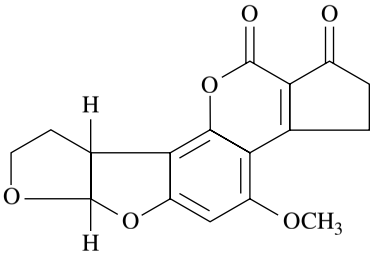
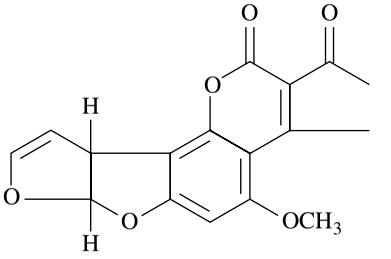
The florescence colour noted viewed under UV light for OT A is greenish, whilst OT B emits blueish (Jay *et al*., 2005). According to Frazier & Westhoff (1988), although the effects of OTs to human beings are unknown or possibly slightly toxic, the significance of OTs in food is of interest for the following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

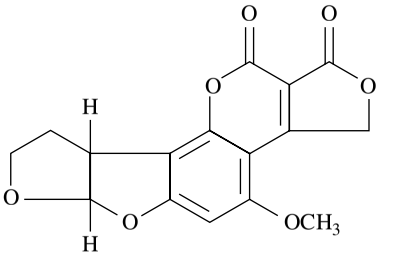
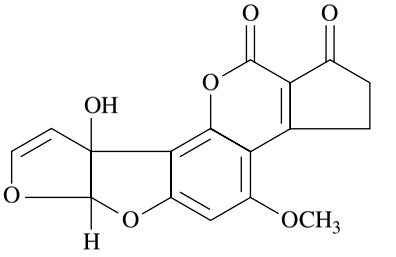
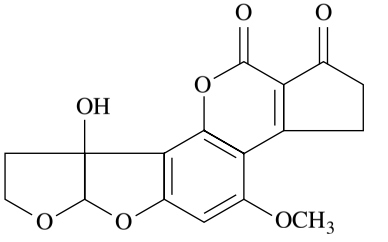
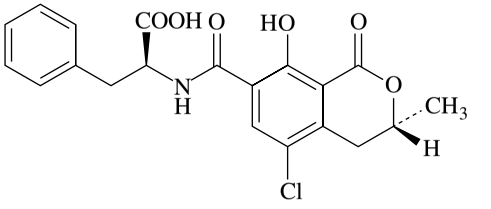
Mould organisms belonging to the following genera: *Aspergillus* (*A. niveus, A. ochraceus, A. oryzae* and *A. terreus*), *Monascus* (*M. ruber* and *M. purpureus*) and *Penicillium* (*P. citrinum* and *P. viridicatum* and *P. camemberti*) have been reported to produce mycotoxin known as citrinin (Jay *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.

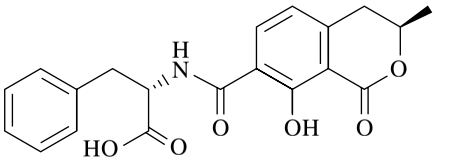
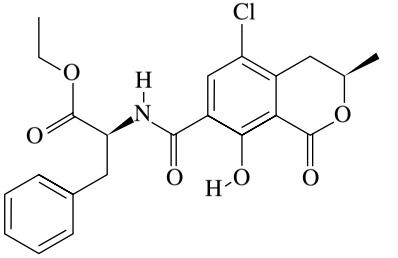
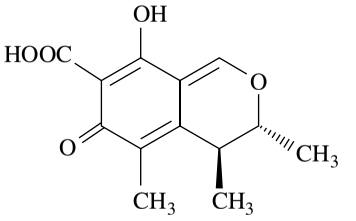
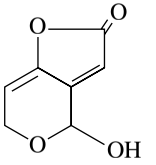
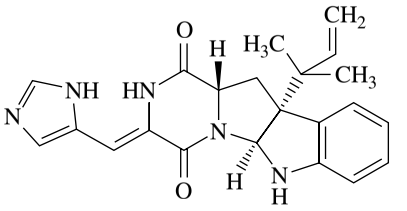
Table 1.1 Some general characteristics of mycotoxins detected in dairy products.

Name of mycotoxin	Chemical formula	Comments	Structure
Aflatoxins (AF) B_1	$C_{17}H_{12}O_6$	Catalysing 3-hydroxylation of AF B_1 to yield the AF Q_1 metabolite Carcinogenic, immunosuppressive, hepatocarcinogens, genotoxic Binds covalently to live mitochondrial deoxynucleic acid	
AF B_2	$C_{17}H_{12}O_6$	It is the 2,3-dihydroform of AF B_1, which reduces the mutagenicity by 200- to 500-fold Carcinogenic, hepatocarcinogens, mutagenic, teratogenic, and causes immunosuppression	
AF G_1	$C_{17}H_{12}O_7$	Carcinogenic, hepatocarcinogens	

(*Continued*)

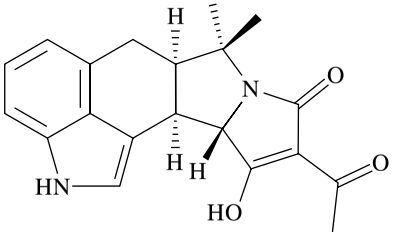
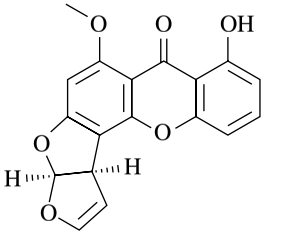
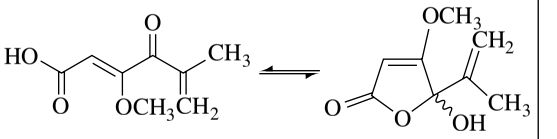
Table 1.1 (Continued)

Name of mycotoxin	Chemical formula	Comments	Structure
AF G_2	$C_{17}H_{14}O_7$	It is the 2,3-dihydroform of AF G_1 Carcinogenic, hepatocarcinogens	
AF M_1	$C_{17}H_{12}O_7$	Hydroxylated product of AF B_1 Hepatotoxic, mutagenic (the C_2-C_3 double bond in the dihydrofurofurane moiety), carcinogenic, immunotoxic, teratogenic, Less toxic than the parent compound AF B_1	
AF M_2		It is a 4-hydroxy AF B_2 Less toxic than the parent compound AF B_2	
Ochratoxin (OT) A	$C_{20}H_{18}ClNO_6$	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	

OT B	$C_{20}H_{19}NO_8$	Most likely it has similar properties as above, but not toxic	
OT C	$C_{22}H_{22}ClNO_6$	Most likely it has similar properties as above	
Citrinin (also known as antimycin)	$C_{13}H_{14}O_5$	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	$C_7H_6O_4$	Toxicity - neurotoxic, hepathotoxic, 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	$C_{22}H_{23}N_5O_2$	Neurotoxin Its chemical nature is indole alkaloid	

(*Continued*)

Table 1.1 (Continued)

Name of mycotoxin	Chemical formula	Comments	Structure
Cyclopiazonic acid (CPA)	$C_{20}H_{20}N_2O_3$	Appears to be toxic in high concentrations	
Sterigmatocystin	$C_{18}H_{12}O_6$	Toxic and it is related to dermatoxin Closely related to AF B_1, which is a potent liver carcinogenic, mutagenic and teratogenic	
Penicillic acid	$C_8H_{10}O_4$	Demonstrated some toxic effects on laboratory animals	

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

Data compiled from ICMSF (1978), Frazier & Westhoff (1988), IARC (2002a, 2002b), Yousef & Juneja (2003) and Jay *et al*. (2005); see also www.bing.com, http://wwww.fermentek.co.il/aflatoxin_M2.htm, http://en.wikipedia.org, http://pubchem.ncbi.nlm.nih.gov/compound/20997#section=2D-Structure

Patulin

Penicilla species (*Penicillium claviform, Penicillim expansum, Penicillium patulum, P. roqueforti, Penicillium clavigerum, Penicillium griseofulvum, Penicillium crustosum* and *P. enicilliumpaneum*), aspergilla species (e.g. *Aspergillus clavatus, Aspergillus terreus* and others), and *Byss. fulva* and *Byssochlamys nivea* produce mycotoxin known as patulin. When first discovered, it was described as an antibiotic and its chemical structure is synonymous with penicidin, clavatin, claviformin, calvicin, mycoin C, expansin, and gigantic acid (Frazier and Westhoff, 1988), and the IUPC name is 4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one.

Miscellaneous mycotoxins

A wide range of mycotoxins have been found in dairy products, mainly cheeses (see Chapter 2) and, in brief, their characteristics are as follows:

- Roquefortine - Excessive growth of *P. roqueforti* in Blue Vein cheese (Roquefort, Stilton, Danablue, etc.) during the maturation period results in the production of a toxin known as Roquefortine C. It acts as a neurotoxin to animals (e.g. mice) when injected into the body leading to convulsive seizers (Frazier & Westhoff, 1988).
- Cyclopiazonic acid (CPA) – Chemically, it is known as an indole tetramic acid, and is isolated from the *Penicillium cyclopium, Penicillium griseofulvum, P. camemberti, Penicillium commune, A. flavus*, and *Aspergillus versicolor*. The IUPAC name is (6a*R*,11a*S*,11b*R*)-10-Acetyl-11-hydroxy-7,7-dimethyl-2,6,6a,7,11a,11b-hexahydro-9*H*-pyrrolo[1',2':2,3]isoindolo[4,5,6-*cd*]indol-9-one, (Holzapfel, 1968 ; http://en.wikipedia.org/wiki/Cyclopiazonic_acid), accessed on 21st April 2015.
- Sterigmatocystin – This mycotoxin is isolated from the crust of hard cheeses. The IUPAC name is (3a*R*,12c*S*)-8-hydroxy-6-methoxy-3a,12c-dihydro-7*H*-furo[3',2':4,5]furo[2,3-*c*]xanthen-7-one, and is produced by moulds of the genera *Aspergillus*, and the classification by the IARC of sterigmatocystin is Group 2B (http://en.wikipedia.org/wiki/Sterigmatocystin), accessed on 21st April 2015.
- Penicillic acid – This mycotoxin is produced by *Penicillium roqueforti, Penicillium* spp. and *Aspergillus* spp. This toxin was found in hard cheeses and Roquefort. The IUPAC name is 5-hydroxy-5-isopropenyl-4-methoxy-furan-2-one (http://en.wikipedia.org/wiki/Penicillic_acid), accessed on 21st April 2015.
- Andrastin A-D and isofumigaclavines A and B – They are secondary metabolites of *P. roqueforti*, and the latter mycotoxin is considered neurotoxic. As can be expected, their occurrences have been in Blue Vein cheeses.

1.4.3 *Postscript on mycotoxins*

It is evident that different mycotoxins have been found in dairy products which may pose a slight or more severe health risks to humans depending on the ingested amounts of these toxin. However, some emerging mycotoxins (i.e. the masked, bound and or conjugated types) may pose potential health risk to humans if they appear in dairy products, but further studies are required to categorise their toxicity. Some of

these masked mycotoxins are produced by *Fusarium* spp., such as deoxynivalenol, zearalenone, fumonisins, nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, and fusaric acid) (Berthiller *et al*., 2013; see also Scott, 1989; Murphy *et al*., 2006; Zinedine *et al*., 2007; Tsakalidou, 2011; Singh *et al*., 2012; Todd *et al*., 2014), including some bound and conjugated mycotoxins (enniatins, beauvericin and fusaproliferin) should not be overlooked. Last, but least, *Cladosporium* spp. have been isolated from Cheddar cheese (Hocking & Faedo, 1992; Basilico *et al*., 2003; Panelli *et al*., 2014), and the descriptive term used of the contaminant as 'Thread Mould'. The source of the *Cladosporium* spp. contamination in Cheddar cheese made using the Block-Forming unit was the factory environment including the compressed air system (A.Y. Tamime, unpublished data); however, the problem was contained by installation of microbiological filters in the compressed air-line entering the cheesemaking area. Nevertheless, more work is required to establish the toxicity of such mould metabolite in humans and animals.

1.5 Biogenic amines (BAs)

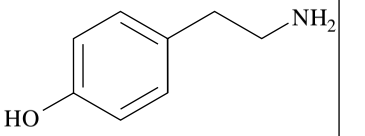
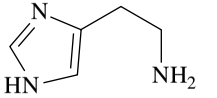
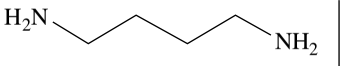
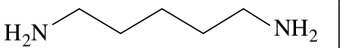
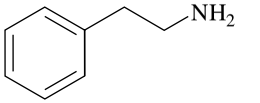
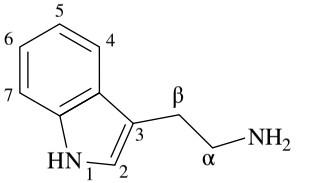
Biogenic amines are nitrogenous substances with one or more amine groups, which are formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. In the present context, BAs are only found in cheeses, and they are formed by the metabolic activity/catabolism of the starter culture and their enzymes including any other bacteria that could be present in the milk prior to cheesemaking, and coagulating enzymes. The catabolism by bacteria and possibly moulds of raw material can produce BAs in milk, or they are generated by microbial decarboxylation of amino acids.

It is well established that BAs play an important role as source of nitrogen and precursor for the synthesis of hormones, alkaloids, nucleic acids, proteins, amines and food aroma compounds. However, dairy products (e.g. cheeses) containing high amounts of BAs may have toxicological effects, and some characteristics of BAs are summarised in Table 1.2.

1.6 Conclusions

This chapter has proposed through its argument and illustration that toxin-producing micro-organisms represent an important source of food-borne hazard to consumers, which must therefore be managed. The control of endotoxin and exotoxin producing organisms must be an aim of all food processors and manufacturers, not just those involved in the manufacture of dairy products. However, as with the manufacture of any food product, the targets set for levels of toxin producing micro-organisms in dairy products must be tempered with knowledge and common sense. For instance, the infective dose level of *E. coli* O157:H7 is low at 10–100 cfu g^{-1}, whereas the requirement for the formation of *Staph. aureus* exotoxin sufficient to cause illness is much higher at some 10^6 cfu g^{-1}. While every measure must be taken to prevent or eliminate *E. coli* O157:H7 contamination of dairy products, it must also be recognised that in the case of some dairy products, hand-made, specialist cheeses for instance, saleable product

Table 1.2 Some physical characteristics of biogenic amine (BAs) that have been identified in dairy products, mainly cheeses that are potential health risks to humans.

Name of BAs	Chemical formula	IUPAC[1]	Classification and other names applied	Structure
Tyramine	$C_8H_{11}NO$	4-(2-aminoethyl) phenol	Cyclic aromatic amine, monoamine and trace of amine derived from the amino acid tyrosine It is also known as 4- hydroxyphenethylamine, para-tyramine, mydrial or uteramin	
Histamine	$C_5H_9N_3$	2-(1*H*-imidazol-4-yl) ethanamine	Heterocyclic amine, diamine	
Putrescine	$C_4H_{12}N_2$	butane-1,4-diamine	Aliphatic amine, diamine It is a tetramethylenediamine organic chemical compound $NH_2(CH_2)_4NH_2$ (1,4- diaminobutane or butanediamine) that is related to cadaverine	
Cadavarine	$C_4H_{12}N_2$	pentane-1,5-diamine	Aliphatic amine, diamine It is a toxic diamine with the formula $NH_2(CH_2)_5NH_2$, which is similar to putrescine	
β-phenylethylamine (β-PEA)	$C_8H_{11}N$	2-phenylethylamine	Cyclic aromatic amine, monoamine	
Tryptamine	$C_{10}H_{12}N_2$	2-(1*H*-Indol-3-yl) ethanamine	Heterocyclic amine, Monoamine It contains an indole ring structure, and is structurally similar to the amino acid tryptophan	

[1]IUPAC = International Union of Pure and Applied Chemistry.
Data compiled from EFSA (2011), data from Chapter 4, and (http://en.wikipedia.org/wiki/) [Accessed 6th April 2015].

cannot be made if the specification for *Staph. aureus* is set at impractically and unnecessarily low levels. The management of food safety in dairy products manufacture must be based on good and informed judgement. Reliable and informative sources able to impart the knowledge and understanding required to inform judgement are, therefore, important to milk processors and dairy products manufacturers. It is hoped that this chapter, and the book of which it is part, will serve those who work in the dairy industry, as well as those who have academic or scholarly interests in the topic, enabling them to make appropriate decisions about matters of microbial food safety and dairy products, and particularly those concerning microbial toxins.

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