chapter two

Natural toxins

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2.1 Introduction

Man's diet contains many thousands of substances, of which many are unknown. Relatively few are of nutritional significance. The majority contribute to the sensoric quality of food.

A number of toxic components of natural origin have been identified, and the mechanisms underlying their toxicities elucidated. For example, the potato contains more than 250 substances including solanine, which is known to cause neurotoxic effects in animals and man.

Usually, natural toxins are not acutely toxic, except in a few cases in animals. An example is tetrodotoxin, a neurotoxin first identified in puffer fish, a Japanese delicacy. Expert cleaning of the fish prevents transmission of the toxin to the edible parts of the fish. Yet, accidents happen each year. Most of the natural toxins, particularly those occurring in plant-derived foods, induce adverse effects only after chronic ingestion or by allergic reactions.

So far, many minor plant food components have not been chemically identified yet and, consequently, have not been evaluated for any toxic properties. Indications of their presence have been obtained from chromatographic and spectroscopic studies. It may even be expected that with the further development of analytical techniques still more components will be found, and of these more may appear to be toxic. This chapter deals with endogenous toxins of plant origin (Section 2.2) and contaminants of natural origin (Section 2.3), including toxins of microbial origin (Section 2.3.3).

2.2 Endogenous toxins of plant origin

There is no simple way of classifying toxic food components of plant origin, since this category comprises many different types of substances. These are classified on the basis of common functional groups (Sections 2.2.1 to 2.2.3), physiological action (Section 2.2.4) and type of effect (Sections 2.2.5 and 2.2.6). Several important representatives of the various types will be highlighted in this section.

2.2.1 Toxic phenolic substances

More than 800 phenolic substances have been detected in plants. Many of them contribute to the (bitter) taste and flavor of foods, and some also contribute to color.

These substances can be divided into two major groups on the basis of frequency of occurrence, structural relationship, and relative toxicity:

1. widespread phenolic plant substances, often used in the production of foods and beverages. About 25 have been identified and only a few are present in relatively high concentrations in certain plant foodstuffs; the majority are only present in trace amounts. This group includes phenolic acids such as caffeic acid, ferulic acid, gallic acid, flavonoids, lignin, hydrolyzable and condensed tannins, and derivatives. At the levels present in food, these substances are devoid of acute toxicity. Presumably, evolutionary adaptation gave animals and man the ability to detoxicate them;

2. a more heterogeneous group of highly toxic phenolic substances. Examples are coumarin, safrole, myristicin, and phenolic amines also known as catecholamines, and gossypol (see Chapter 3).

A number of phenolic substances will be discussed in more detail in the following subsections.

2.2.1.1 Flavonoids

A class of plant pigments that are widely present in human food, are the flavonoids. These pigments are polyhydroxy-2-phenylbenzo-γ-pyrone derivatives, occurring as aglycones, glycosides and methyl ethers. They are divided into six main subgroups (see Figure 2.1). Most flavonoids are present as β-glucosides. The enzymes catalyzing the formation of the active agents have not yet been found.

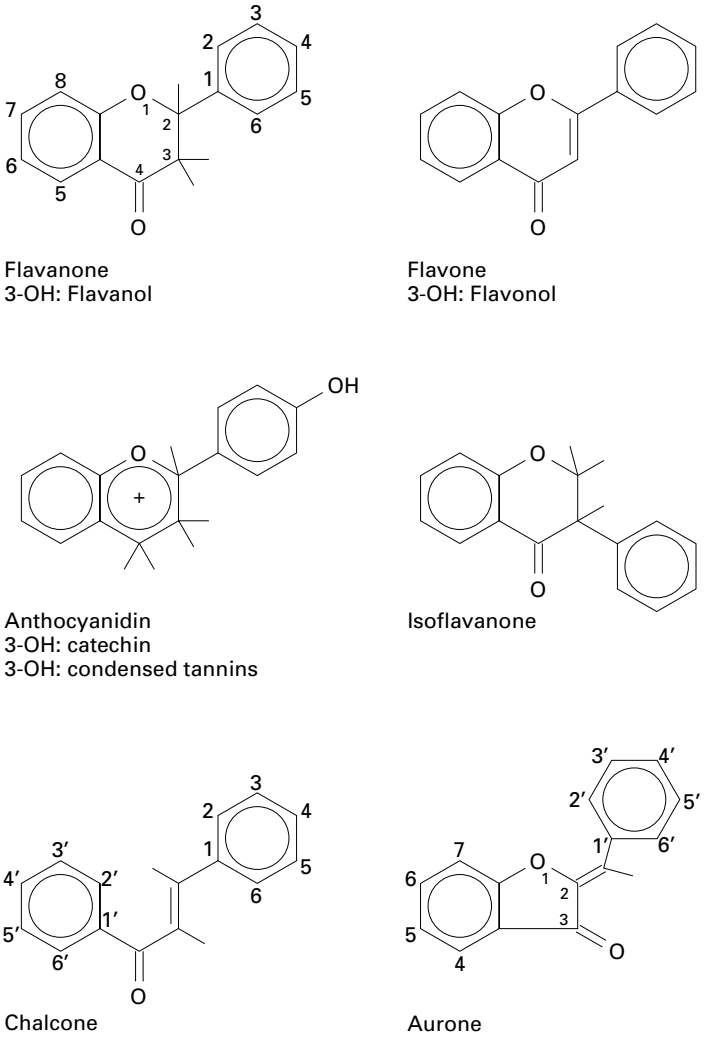
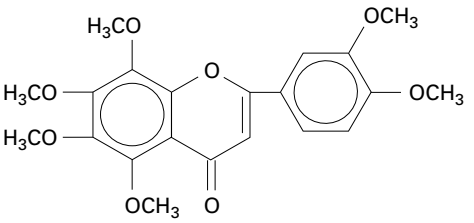


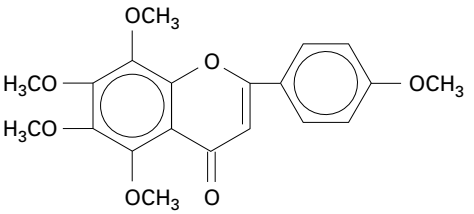
Figure 2.1 Flavonoid classification.

Because of their wide distribution, the effects of the flavonoids on human well-being are of considerable interest. More than 1 g of various flavonoids are ingested daily in the diet of the Western world.

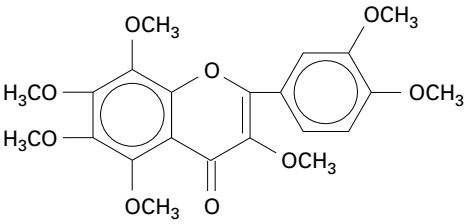
A group of yellow pigments that occurs abundantly is the flavones. Examples are nobiletin, tangeretin and 3, 3′, 4′, 5 ,6 ,7, 8-heptamethoxyflavone. The former two are found in citrus fruits such as tangerines, mandarines and oranges, the latter in grapefruit.



Nobiletin



Tangeretin



3,3′,4′,5,6,7,8 - Heptamethoxyflavone

The flavones are generally located in the oil vesicles of the fruit peel. Flavones are apolar, and therefore readily soluble in the oil. They can be found in the juice after pressing the peel. The oily material from orange peel can contain about 2 mg nobiletin per 100 ml oil and 0.3 mg tangeretin per 100 ml oil.

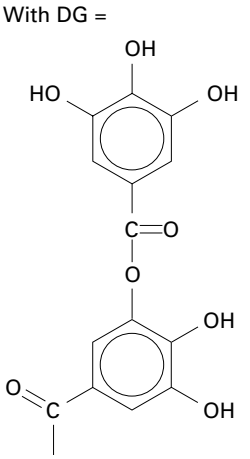
The flavones group has been extensively investigated for mutagenicity. A well-known mutagenic representative is quercetin, occurring, for example, in cereal crops. Quercetin is the only flavonoid shown to be carcinogenic in mammals after oral administration. Its structure can be found in Chapter 3.

2.2.1.2 Tannins

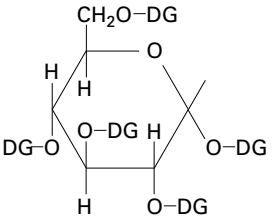
Tannins are a heterogeneous group of broadly distributed substances of plant origin. They are considered to include all polyhydric phenols of plant origin with a molecular mass higher than 500. Two types of tannins can be distinguished on the basis of degradation behavior and botanical distribution, namely hydrolyzable tannins and condensed tannins.

The *hydrolyzable* tannins are gallic, digallic, and ellagic acid esters of glucose or quinic acid. An example of this group is tannic acid, also known as gallotannic acid, gallotannin,

or simply tannin. Tannic acid has been reported to cause acute liver injury, i.e., liver necrosis and fatty liver.

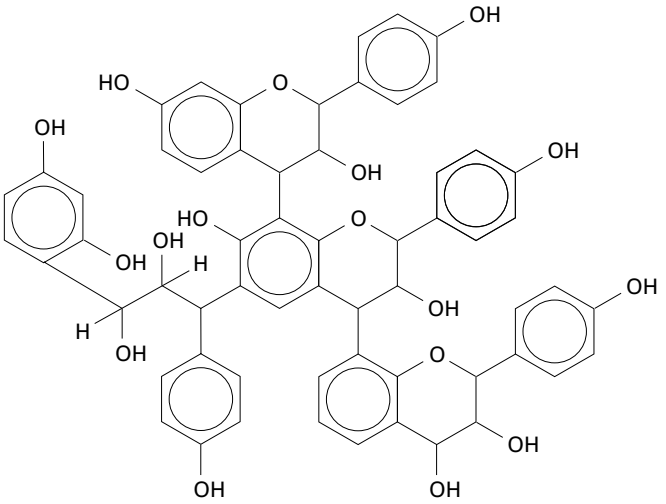


digallic



Tannic Acid

The *condensed* tannins are flavonoids. They are polymers of leukoanthocyanidins (Figure 2.1). The monomers are linked by C–C bonds between positions 4 and 6, or positions 4 and 8.



Proposed structure of
a condensed tannin

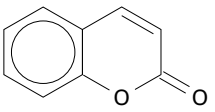
Tannins occur in many tropical fruits, including mango, dates, and persimmons. The contribution of the tannins in tea, coffee, and cocoa to the total tannin intake by humans is of particular importance. A cup of regular ground coffee was found to contain 72 to 104 mg of tannins, a cup of instant coffee 111 to 128 mg, and a cup of decaffeinated instant coffee 134 to 187 mg. One particular brand of cocoa was found to contain 215 mg tannins per cup. Tea has the highest tannin content. It has been shown that on maximum extrac-

tion, black tea leads to 431 to 450 mg tannin per cup. Green tea may yield more soluble tannins, while black tea contains tannins with a higher molecular mass, as a result of oxidation of phenolic precursors during fermentation. From these data it can be estimated that a person may easily ingest 1 g or more tannins per day. Other important sources of tannins are grapes, grape juice, and wines. The tannins in grapes are mostly of the condensed type. The highest levels are found in the skin of the fruit. On average, grapes contain 500 mg per kg, and red wines 1 to 4 g per l of wine. Tannins are also found in large amounts in ferns.

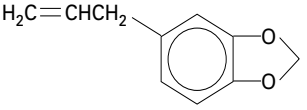
2.2.1.3 Coumarin, safrole, and myristicin

Natural toxins can also be found among the flavorings. Three examples will be discussed in this section: coumarin, a chroman derivative, and safrole and myristicin, both methylenedioxyphenyl substances.

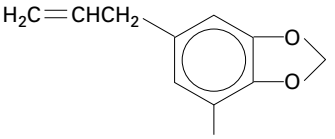
Only a few flavor components of natural origin have been toxicologically evaluated. One reason for this may be that isolation of sufficient quantities for testing is often difficult. More is known about those flavor components that are also synthetically made. This concerns coumarin and safrole.



Coumarin



Safrole



Myristicin

Coumarin widely occurs in a number of natural flavorings, including cassis, lavender, and lovage. These flavorings are extensively used in sweets and liquors. Traces of coumarin occur in citrus oils and some edible fruits.

Safrole has been shown to cause liver tumors in rats. It is found in the oil of sassafras and in black peppers. Both coumarin and safrole are still allowed for food use in the European Community. They are prohibited in the US though, as they have been found to cause liver damage in rats.

One of the most common other methylenedioxyphenyl substances is *myristicin*. It is found in spices and herbs such as nutmeg, mace, black pepper, carrot, parsley, celery, and dill. It has been suggested that myristicin contributes to the toxicity of nutmeg. After nutmeg abuse, tachycardia, failing salivation, and excitation of the central nervous system have been reported. Nutmeg has been abused as a narcotic.

2.2.2 Cyanogenic glycosides

Cyanogenic glycosides are glycosides from which cyanide is formed by the activity of hydrolytic enzymes. They are widely spread in higher plants. More than 1000 plant species have been reported to be cyanophoric, mostly in edible plants (see Table 2.1).

Cyanide doses that are lethal to humans can easily be reached or even exceeded after the intake of a variety of cyanogenic foodstuffs. Lethal intakes by humans range from 0.5 to 3.5 mg per kg body weight. The quantities of cyanide produced by Asiatic varieties of lima beans range from 200 to 300 mg per 100 g (see Table 2.2). American varieties of lima beans produce less than 20 mg HCN per 100 g. Selected breeding of low-cyanide varieties has been started.

Fresh cassava cortex produces cyanide in quantities ranging from 1.0 to more than 60.0 mg per 100 g, depending on several conditions, including variety, source, time of harvest and field conditions. Damaged roots can contain even more cyanide, i.e., 245 g per 100 g.

Table 2.1 Cyanogenic glycosides in edible plants

Glycosides	Aglycone	Sugar	Food found
Amygdalin	D-mandelonitrile	Gentiobiose	Almonds, apple, apricot, cherry, peach, pear, plum, quince
Dhurrin	L-*p*-hydroxymandelonitrile	D-glucose	Sorghums, kaffir corns
Linamarin	α-hydroxyisobutyronitrile	D-glucose	Lima beans, flax seed, cassava or manioc
Lotoaustralin	α-hydroxy-α-methylbutyronitrile	D-glucose	Same as linamarin: cassava
Prunasin	D-mandelonitrile	D-glucose	Same as amygdalin
Sambunigrin	L-mandelonitrile	D-glucose	Legumes, elderberry
Vicianin	D-mandelonitrile	Vicianose	Common vetch, and other vicias

Table 2.2 Hydrogen cyanide contents of some foodstuffs

Food	HCN (mg/100 g)
Lima beans	210–310
Almonds	250
Sorghum sp.	250
Cassava	110
Peas	2.3
Beans	2.0
Chick peas	0.8

Cyanogenic glycosides consist of a saccharide moiety and an aglycone, a β-hydroxynitrile. The saccharide group can be a monosaccharide, e.g., glucose, or a disaccharide, e.g., gentiobiose and vicianose. Glycoside linkages can be hydrolyzed by glycosidases. The nitrile can undergo further degradation by a lyase to hydrogen cyanide and an aldehyde, a ketone or in some cases an acid.

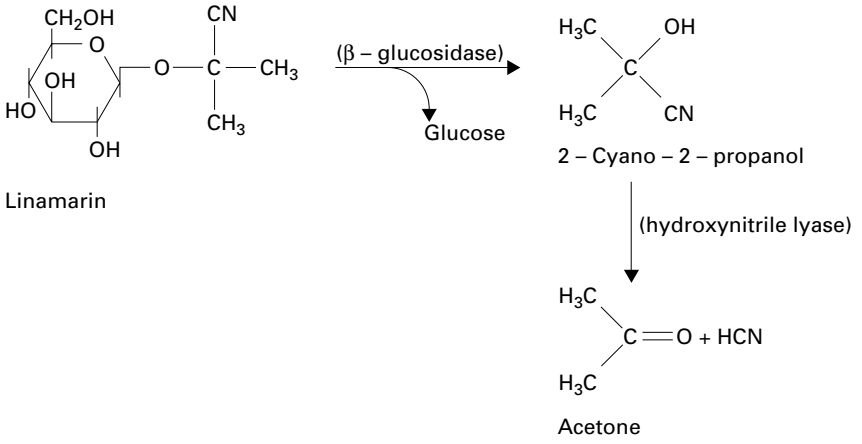


Figure 2.2 Degradation of the cyanogenic glucoside linamarin.

Glycosidases and hydroxynitrile lyase are present in plant cells. They become available when plant tissue is damaged. This inevitably occurs when food is prepared for consumption. As mentioned above, the damaged parts of cassava roots contain high concentrations of cyanide. Nevertheless, cassava, being rich in starch, remains an important food source in Africa, parts of Asia and Latin America, because preparation methods have been developed by which the cyanogenic glycosides are removed or hydrolyzed, and β-glucosidase is destroyed. The cassava is grated, soaked in water, and fermented for several days. The soaked plant tissue is then dried and pounded to flour. Such processes greatly reduce the cyanogen content of food to safe levels. For example, "gari," a fermented cassava preparation, contains an average of 1.0 mg HCN per 100 g. Consumption of cassava may lead to goiter, as the cyanide formed can be metabolized to thiocyanate by the enzyme rhodanase. High consumption of dry, unfermented cassava, containing high levels of cyanogen, accounts for the widespread incidence of goiter in parts of Africa.

Sorghum can be consumed safely, as it is free from or very poor in cyanogen. On germination the sorghum seedling may reach a concentration of 0.3 to 0.5% HCN (dry weight). The young green leaves, however, are rich in cyanogen. This is why cattle are not allowed to graze on young sorghum plants. If sorghum is packed in a silo, cellular degradation and fermentation may lead to the release and elimination of cyanide.

2.2.3 *Glucosinolates*

Glucosinolates are a particular group of substances, occurring in cruciferous plants, such as cabbage and turnips. They can be considered as natural toxins, but also as antinutritives. This Section is limited to the glucosinolates' pathway from raw material to consumer only. Glucosinolates as antinutritives are dealt with in Chapter 3. Concerning toxicity and antinutritive activity, the hydrolysis products are the active agents, not the glucosinolates themselves. Hydrolysis of glucosinolates results in the formation of isothiocyanates and nitriles. The enzyme becomes available for catalysis when cells are damaged on cutting or chewing.

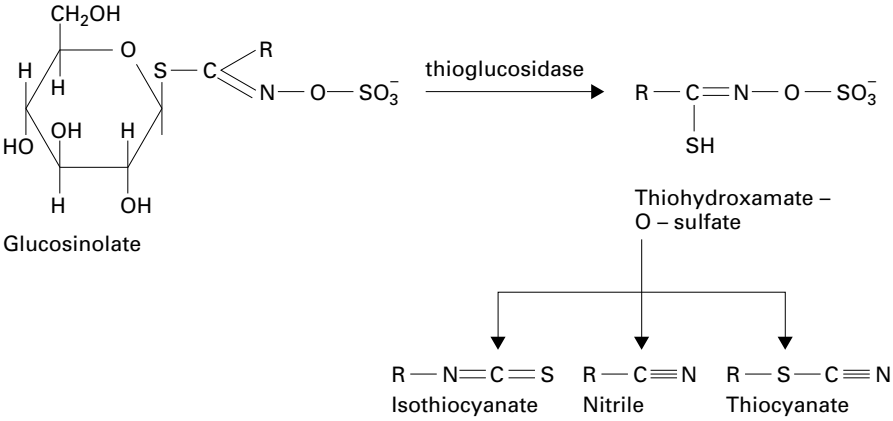
Several isothiocyanates have been shown to be embryotoxic in rats, while *in vitro* studies have proved a number of them to be cytotoxic and mutagenic. Further, several nitriles have been identified as precursors of N-nitroso compounds. These will be discussed in Chapter 5.

2.2.4 *Acetylcholinesterase inhibitors*

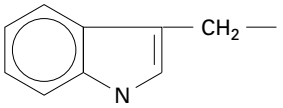
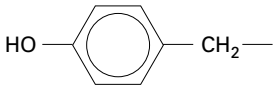
Acetylcholinesterase inhibitors have been detected in several edible fruits and vegetables. Their active components are alkaloids. In many foodstuffs, however, they have not yet been identified. These include broccoli, Valencia oranges, sugar beet, cabbage, pepper, carrot, strawberry, apple, lima bean and radish. In potato, eggplant and tomato — members of the Solanaceae family — the principal alkaloids have been identified. The most potent inhibitors are found in potatoes, and of these the most active component is the glycoalkaloid solanine.

The toxicity of solanine has been the subject of extensive study. Oral administration results primarily in gastrointestinal and neurological symptoms.

The solanine concentration of potato tubers varies with the degree of maturity at harvest, rate of nitrogen fertilization, storage conditions, variety, and greening by exposure to light. Commercial potatoes contain 2 to 15 mg of solanine per 100 g fresh weight. Greening of potatoes may increase the solanine content to 80 to 100 mg per 100 g. Most of the alkaloid is concentrated in the skin. Sprouts may contain lethal amounts of solanine.



The identities of R in the predominant glucosinolates of a number of vegetables:

Cabbage		Indolylmethyl
Other brassicas, horseradish, black mustard	$CH_2{=}CH{-}CH_2{-}$	Allyl
White mustard		*p* - Hydroxybenzyl
Rape	$CH_2{=}CH{-}CHOH{-}CH_2{-}$	2 - Hydroxybut - 3 - enyl

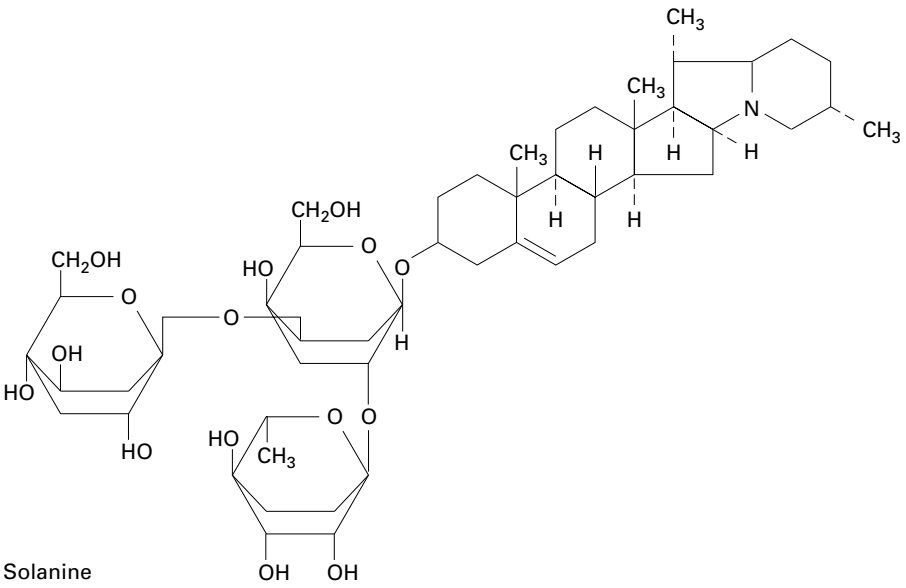
General structures of glucosinolates and their hydrolysis products.

Since potatoes also contain other glycoalkaloids, namely chaconine and tomatine, with biological properties similar to solanine, the symptoms seen in potato poisoning may be due to combined actions of the alkaloids. All existing and newly developed varieties of potatoes are now monitored for alkaloid content. Solanine is heat stable and insoluble in water. Hence, toxic potatoes cannot be rendered harmless by cooking. It is generally accepted that 20 mg solanine per 100 g fresh weight is the upper safety limit.

2.2.5 *Biogenic amines*

Natural toxins also include certain amines which can be of plant as well as of microbial origin. The latter source is dealt with in Section 2.3.3. The most important biogenic amines found in plants are listed in Table 2.3.

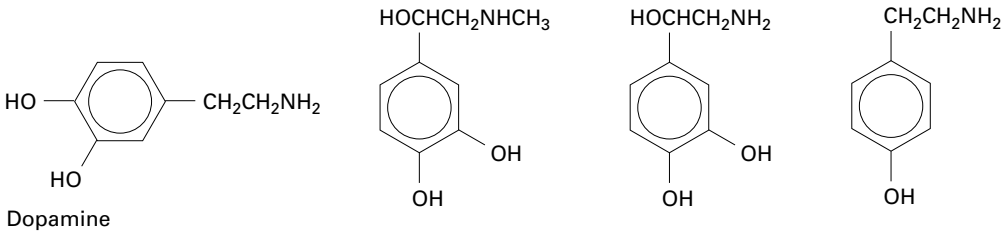
The dietary intake of biogenic amines may pose risks. A well-known harmful effect of all three of the phenethylamines, dopamine, norepinephrine, and tyramine is hypertension. The risk is greater when combinations of biogenic amines and monoamine oxidase (MAO) inhibitors are ingested. Monoamine oxidases mediate the oxidative deamination of



Solanine

the three phenethylamines. Monoamine oxidase inhibitors are a heterogeneous group of drugs. Clinically-used MAO inhibitors include hydrazine derivatives such as the antidepressent iproniazid. Several phenalkylamines are found in citrus fruits.

Amines may be formed by the metabolic transformation of precursors endogenously present in food of plant origin. Fava beans *(Vicia faba)* contain dihydroxyphenylalanine (DOPA), which may be decarboxylated to dopamine.



Dopamine Epinephrine Norepinephrine Tyramine

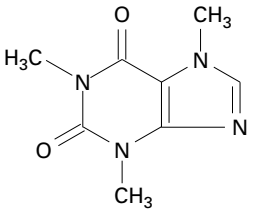
Table 2.3 Biogenic amine content of some fruits and vegetables (mg per 100 g fresh weight)

Amines	Avocado	Banana pulp	Eggplant	Orange	Red plum	Tomato (ripe)	Potato
Dopamine	0.4–0.5	66–70		0.1			
Epinephrine		<.25					
Norepinephrine		10.8					0.01–0.02
Serotonin	1.0	2.5–8.0	0.2		1.0	1.2	
Tyramine	2.3	6.5–9.4	0.3	1.0	0.6	0.4	0.1

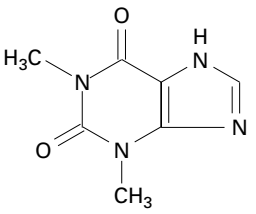
				Pineapple			Plantain		
	Date	Fig	Pawpaw	Green	Ripe	Juice	Green	Ripe	Cooked
Dopamine	<0.08	<0.02	0.1–0.2						
Epinephrine	<0.08	<0.02							
Norepinephrine	<0.08	<0.02					0.2	0.25	
Serotonin	0.9	1.3	0.1–0.2	5.0–6.0	2.0	2.5–3.5	2.0–6.0	4.0–10	4.7

2.2.6 *Central stimulants*

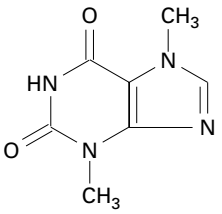
For most people the everyday diet contains a considerable amount of stimulants. These substances increase the state of activity of the nervous system. A particular class of stimulants is the methylxanthines. They include caffeine, theophylline, and theobromine.



Caffeine



Theophylline



Theobromine

Caffeine is found in coffee beans, tea leaves, cocoa beans, and colanuts. In our diet the primary source of caffeine, however, is coffee: one cup of coffee is estimated to contain 100 to 150 mg of caffeine. The caffeine content of cola drinks ranges from 0.1 to 0.15 mg/ml.

In general, methylxanthines cause effects on the peripheral nervous system, but they also induce significant stimulation of the central nervous system. Caffeine is a little more potent than theophylline, and theobromine is relatively inactive.

Further, caffeine has been reported to cause premature aging, a lower growth rate and a lower body weight in experimental animals. In rodents, the oral LD_{50} (see Part 2, Chapter 8, Section 8.9.1) ranges from 127 to 355 mg/kg. Adverse effects of caffeine on cardiac function are questioned, since no relationship has been found between drinking tea and heart disease; the caffeine content of tea is similar to that of coffee. *Theophylline* is present in small amounts in tea. *Theobromine* is the principal alkaloid of the cocoa bean. It is also found in tea leaves and cola nuts.

2.3 *Natural contaminants*

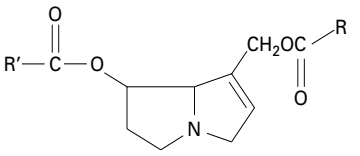
Natural contaminants can also originate from biological systems different from those in which they occur. There are three important sources. First, raw materials of plant origin can become contaminated if they are mixed with toxic non-nutritive plant species. Secondly, raw materials of animal origin, mainly fish and milk, can also become contaminated if the animal has ingested toxic substances of natural origin. Thirdly, contaminants of natural origin can be the products of microorganisms. This section deals with a number of important examples of contamination with natural toxins.

2.3.1 *Mixing of edible plants with toxic plants*

Several intoxications have been reported following the consumption of contaminated cereals. The causative agents are pyrrolizidine alkaloids, produced by the genera *Senecio*, *Crotalaria* and *Heliotropium*.

Pyrrolizidine alkaloids can be the cause of acute liver damage and vein lesions. These substances may also contribute to the liver cancer incidence in humans.

Epidemics of pyrrolizidine intoxication have been reported in India and Afghanistan in 1973 and 1976.



Pyrrolizidine alkaloids

In India, millet, the principal cereal in the diet, appeared to be heavily contaminated with *Crotalaria* seeds. The alkaloid content of the seeds was estimated at 5.3 mg/g, while the precentage of *Crotalaria* seeds in millet varied from 0.0 to 0.34% in unaffected households and 0.0 to 1.9% in affected households.

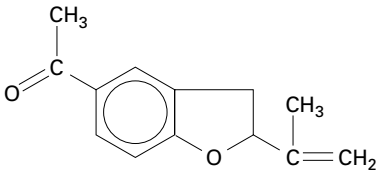
In Afghanistan, the consumption of wheat bread heavily contaminated with *Heliotropium* seeds was found to be the cause of the intoxication. In this epidemic, the minimum daily consumption per person during 2 years was estimated at 2 mg. The disease had been observed in preceding years, but worsened after the occurrence of a severe drought which caused the wheat fields to become heavily infested with *Heliotropium*.

2.3.2 *Contamination resulting from the intake of toxic substances by animals*

Contamination of meat with toxic substances of plant origin rarely occurs. Only in a few cases the intoxication appeared to be related to the consumption of wild animals which had ingested highly toxic plant material shortly before they were consumed. Toxic contaminants in milk and aquatic organisms can originate from feed.

2.3.2.1 *Contamination of milk with plant toxins*

Many foreign substances have been detected in milk. Milk is readily contaminated when lactating animals or women ingest toxins. Contamination of milk with plant toxins has been observed in the US in rural areas, where the inhabitants depend on the local milk supply. The toxin originated from either white snakeroot *(Polygonum)*, or the rayless goldenrod *(Solidago)*. Especially during periods of drought, when feed plants are scarce and the weeds are in flower, the milk may contain sufficient toxin to give rise to outbreaks of "milk sickness." In this case, the major toxic component appeared to be tremetone.

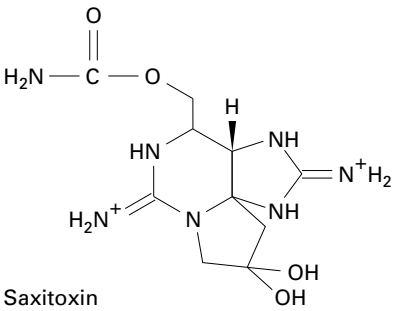


Tremetone

The symptoms were weakness, followed by anorexia, abdominal pain, vomiting, muscle tremor, delirium and coma, and eventually death. A characteristic accompanying phenomenon is the expiration of acetone. The mortality rate was between 10 and 25%.

2.3.2.2 *Natural toxins in aquatic organisms*

Paralytic shellfish poisoning is attributed to the consumption of shellfish that have become contaminated with a toxin or group of toxins from the ingestion of toxic plankton, in particular toxic dinoflagellates. The shellfish involved are pelecypods, a family of mollusks, including mussels and clams. The dinoflagellates produce a complex mixture of toxins. One of the components has been identified as saxitoxin.



Saxitoxin

Shellfish poisoning symptoms include tingling and burning in face, lips, tongue, and ultimately the whole body, and parathesia followed by numbness, general motor incoordination, confusion, and headache. These symptoms develop within 30 minutes after ingestion. Death, preceded by respiratory paralysis, occurs within 12 hours. The chance of contamination and poisoning is highest during a so-called red tide. In many parts of the world, the sea sometimes suddenly becomes colored, as a result of dinoflagellate bloom. The phenomenon is referred to as red tide, although the bloom may also be yellowish, brownish, greenish, and bluish in color. The red color is probably due to the xanthophyll peridinin.

In spite of the frequent occurrence of red tide and the high toxicity of the paralytic shellfish poisons, intoxication rarely occurs. This is largely due to strict regulations set by many countries and the awareness in coastal areas of the risks associated with eating shellfish during red tides. Although ordinary cooking destroys up to 70% of the toxin(s) and pan-frying destroys even more, there may be sufficient toxin left in the mollusks to cause serious poisoning.

2.3.3 *Microbial toxins*

2.3.3.1 *Introduction*

Section 2.3.3 deals with the way in which toxic substances produced in food and feed by microorganisms enter the pathway from raw material to consumer.

Microorganisms are ubiquitous. Any environment supporting higher organisms contains microorganisms too, while the converse is not true. Absence of microorganisms in an environment indicates that special or unusual conditions have occurred, such as heating and filtration for sterilization or preservation.

During food production, raw food materials of plant or animal origin are exposed to soil, water, air, machinery parts, packaging materials, human hands, etc. As these invariably carry microorganisms, all raw food materials have in principle been inoculated with a variety of microbes. The opportunity for these microorganisms to grow is determined by the food environment. Major environmental factors include availability of water (referred to as water activity or a_w) and nutrients, temperature, pH, and presence or absence of atmospheric oxygen. Growth also depends very heavily on how long suitable environmental conditions prevail. The majority of naturally occurring microbial contaminators are unable to multiply, or succumb to other microbes in a food environment. However, even if an infective microorganism remains alive without multiplying, the food may serve as a vehicle to transfer it to the human body and cause illness. Microorganisms which multiply usually degrade the food components enzymatically and excrete their metabolites. In many cases, the resulting loss of structure, or formation of off-smells is regarded as spoilage. However, a wide variety of fermented foods are manufactured of which the desirable taste, flavor, and other properties are especially due to microorganisms and their metabolic activity.

Table 2.4 Food hazards: perception of the consumer versus epidemiological data

Cause	Perception[1]	Relative importance[2]
Microbial contamination	22	49.9
Nutritional imbalance		49.9
Environmental contaminants	31	0.05
Natural toxins	10	0.05
Food additives	30	0.0005
Others, e.g., packaging materials	7	
	100%	100%

[1] Survey held in the Netherlands, 1990.

[2] Ranking based on objective scientific criteria including the severity, incidence, and onset of biological symptoms.

Table 2.5 Food-borne bacterial pathogens and associated diseases

Organism	Pathogenicity	Incubation time (hours)	Duration of disease (days)
Salmonella	infection	6–36	1–7
Shigella	infection	6–12	2–3
Escherichia coli	infection	12–72	1–7
Yersinia enterocolitica	infection	24–36	3–5
Campylobacter jejuni	infection	3–5 (days)	5–7
Listeria monocytogenes	infection	variable	—[a]
Vibrio parahemolyticus	infection	2–48	2–5
Aeromonas hydrophila	infection	2–48	2–7
Staphylococcus aureus	toxin in food	2–6	≤1
Clostridium botulinum	toxin in food	12–96	1–8[b]
Clostridium perfringens	toxin in intestine	8–22	1–2
Bacillus cereus[c]	toxin in food	1–5	≤1
Bacillus cereus[d]	toxin in intestine	8–16	>1

[a] Affects people with a predisposing factor; high mortality rate.

[b] High mortality rate; complete convalescence takes 6–8 months.

[c] Emetic type.

[d] Diarrheal type.

Section 2.3.3 deals with some harmful aspects of microbial food contamination, namely the production of toxic substances causing food-borne disease.

2.3.3.2 Food-borne diseases

Epidemiological evidence has shown that microbial contamination is a major risk factor associated with food consumption. However, the average consumer does not always realize this, and is, for example, more concerned about environmental contaminants in food. This discrepancy between the incidence of food-borne diseases and the perception of the consumer is illustrated in Table 2.4.

Food-borne diseases can be either food-borne infections or food-borne intoxications, depending on whether the pathogen itself or its toxic product (a microbial toxin or toxic metabolite, produced in the food) is the causal agent. Table 2.5 lists the most important bacterial food-borne pathogens.

Of all reported food-borne diseases with microbiological etiology which occurred in Canada in 1984, infections with *Salmonella* and *Campylobacter* spp. constituted 67% and 8%,

and intoxications originating from *Clostridium perfringens*, *Staphylococcus aureus* and *Bacillus cereus* 16%, 7% and 1%, respectively.

Other microbial agents causing food-borne intoxications include toxins produced by fungi (mycotoxins) and by algae, and toxic metabolites such as biogenic amines and ethyl carbamate produced by bacteria and yeasts. The various causative factors of food-borne diseases are summarized in Figure 2.3. In Section 2.3.3.3 the major food-borne toxins will be discussed. Although intoxications by biogenic amines and ethyl carbamate are of microbial origin, they can also be regarded as chemical poisonings.

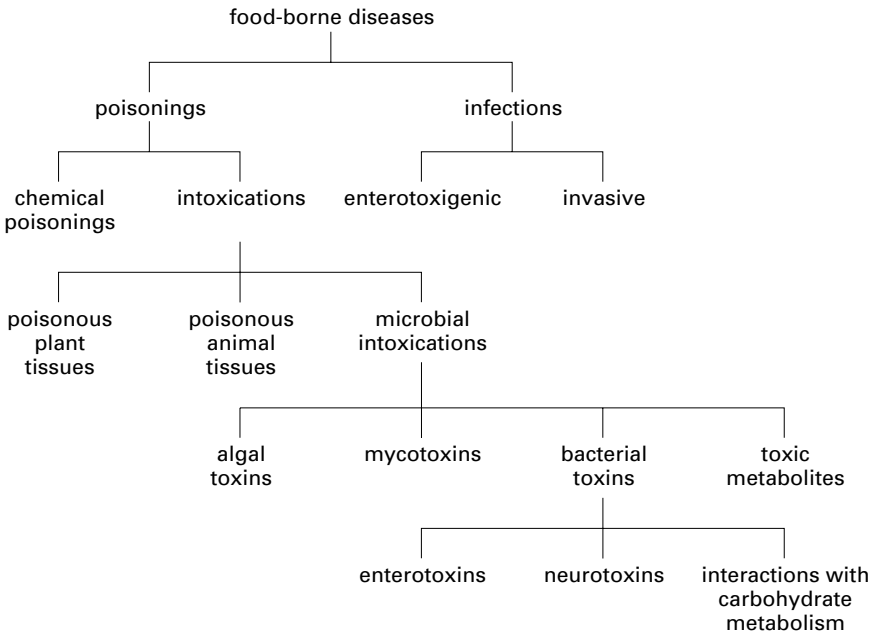


Figure 2.3 Classification of food-borne diseases.

2.3.3.3 *Bacterial toxins*

According to the mechanisms underlying the effects of bacterial toxins, they can be classified as follows:

- sub-unit toxins (e.g., *Clostridium botulinum* toxins, see Subsection 2.3.3.3.1)
- membrane-affecting toxins (e.g., *Staphylococcus aureus* toxins, see Subsection 2.3.3.3.2)
- lesion-causing toxins (e.g., *Clostridium perfringens* and *Bacillus cereus* toxins, see Subsection 2.3.3.3.3)
- immuno-active endotoxins (e.g., Gram-negative bacteria toxins, see Subsection 2.3.3.3.4).

These toxins will be discussed in relation to the properties of the causative bacteria, the conditions favoring toxin production, as well as the structure and stability of the toxin. Their toxicity will be discussed in detail in Part 2 of this book.

2.3.3.3.1 Sub-unit bacterial toxins. To this group belong the toxins produced by *Clostridium botulinum*. *C. botulinum* are motile, Gram-positive rod-shaped spore-forming anaerobic bacteria. *C. botulinum* is not one species, but a group of bacteria which are all

capable of producing neurotoxins. Biochemically, *C. botulinum* is very similar to *Clostridium sporogenes* and *Cl. novii*. However, the latter do not produce toxins and are therefore not relevant here. According to the toxin they produce, there are eight types of *C. botulinum*: A, B, C1, C2, D, E, F, and G.

Toxicity and symptoms. Botulism, caused by the ingestion of food containing the neurotoxin, is the most severe bacterial food-borne intoxication known. The type A toxin is the most lethal. Types A, B, E, and F are toxic to humans; types B, C, and D to cattle; and types C and E to birds.

After an incubation period of 12 to 72 hours, symptoms may start with nausea and vomiting, followed by tiredness, headache, muscular paralysis, double vision, and respiratory problems, often with fatal results. The duration of botulism is 1 to 10 days, mortality is relatively high (30 to 65%). In most foods, botulinum spores are of no consequence unless they are able to germinate and produce the toxin. The exception is infant foods in which botulinum spores are potentially infective and may give rise to toxicogenesis in the infant intestine. A good example of this is infant botulism caused by contaminated honey.

Recent outbreaks involved yogurt with hazelnut (UK 1989: 27 cases, 1 death; type B), fermented seal oil (Canada 1989: 4 cases, 2 deaths; type E), white fish (1989: 8 cases, 1 death; type E), traditional Eskimo fish product (1984, 1989: type E), and infant botulism (1987–1989: 68 cases).

Chemical properties (structure and stability) of botulinum toxin. C. botulinum produces an intracellular protoxin consisting of a non-toxic progenitor toxin (a hemagglutinin with molecular mass approximately 500,000) and a highly toxic neurotoxin (molecular mass approximately 150,000). The protoxin is released upon lysis of the vegetative bacterial cell. The neurotoxin is formed by proteolytic degradation of the protoxin. This proteolysis is caused by *C. botulinum* (type A, and some B and F) proteolytic enzymes, or by exogenous proteases e.g., trypsin when non-proteolytic *C. botulinum* (type C, D, E, and some B and F) are involved.

Botulinum toxin is heat-sensitive (inactivated at 80°C for 10 minutes or 100°C for a few minutes). It is acid-resistant and survives the gastric passage. Botulinum toxin is an exotoxin: it is excreted by the cell, but most of it is released upon lysis of the cell after sporulation.

Environmental conditions. C. botulinum grows best at pH >4.6 at temperatures of approximately 37°C (type E at 30°C). The minimum temperature for growth is 12.5°C (type E at 3.5°C).

Type of food involved; prevention. At particularly risk is food of low to neutral pH (>4.5) which has undergone inadequate heating. Examples include home-preserved vegetables which carry soil-borne *C. botulinum*, but also meat and fish which are contaminated during slaughtering with *C. botulinum* originating from the intestines. Of increasing importance are chilled vacuum-packed foods which usually have had minimal heat treatment, and contain no preservatives other than any naturally occurring antimicrobial substances, and are not reheated or only mildly heated prior to consumption.

Preventive measures include adequate heat processing to reduce the number of *C. botulinum* spores with a factor 10^{12} (the "botulinum cook" or "12-D concept", with D being the time required for a tenfold reduction in the population density at a given temperature). The heat-resistance of the spores varies: D-values of 1 minute at 80°C (type E), 100°C (type C), or 113°C (type A and D). Germination of spores surviving the heat treatment can be prevented by the addition of nitrite, lowering the pH or the a_w, addition of salt, thorough heating of food prior to consumption (the toxin is heat-labile) and refrigerated storage (less adequate for type E).

2.3.3.3.2 Membrane-affecting bacterial toxins. A well-known example of a bacterium-producing membrane-affecting toxins is *Staphylococcus aureus*. *S. aureus* are non-motile,

non-sporeforming, Gram-positive bacteria which can excrete enterotoxins in food. These enterotoxins show clear antigenic activity, and based on their antigenic properties, they are differentiated in A, B, C1, C2, C3, D and E. Most enterotoxicoses are caused by toxins A, or A and D. A characteristic of *S. aureus* is the formation of the enzyme coagulase which can cause the clumping (coagulation) of blood serum. However, coagulase-negative staphylococci have also been incriminated in food-borne gastroenteritis.

Toxicity and symptoms. A quantity of 1 to 25 μg of the enterotoxin is required to cause sickness in adult humans. After a very short incubation period ($^1/_2$ to 6 hours), symptoms of staphylo-enterotoxicose include violent vomiting and diarrhea, sometimes followed by shock but no fever. Serious dehydration may result from the diarrhea. The duration of the illness is 24 to 72 hours, and the mortality is very low. Everyone who consumes the poisoned food, becomes ill ("maladie du banquet": buffet disease).

Chemical properties (structure and stability) of the enterotoxin. The extracellular enterotoxins are a heterogenous group of globular proteins consisting of linear peptide chains. The molecular mass ranges from approximately 30,000 to 235,000. The enterotoxin is heat-resistant (it withstands boiling at 100°C for more than 1 hour).

Environmental conditions. Growth is possible at temperatures between 7 and 46°C (optimum is 37°C), pH 4 to 9 (optimum is pH 7), $a_w \geq 0.86$, and salt (NaCl) concentrations up to 15%. *S. aureus* is a facultative anaerobe, but grows better under aerobic conditions. It is a poor competitor: it hardly grows in the presence of competitive microflora. About 70% of *S. aureus* of human origin are able to produce enterotoxins. The environmental conditions required for toxin production include: temperature ≥12°C, $a_w \geq 0.90$, pH ≥4.6, aerobic conditions, and little microbial competition.

Type of food involved; prevention. S. aureus is a very common microorganism. About 30 to 50% of humans carry the organism in the mucous membrane of the nose and throat, or on the skin. Animals also carry *S. aureus*. Particularly with this microorganism, the human factor plays a very important role in the transfer to food. For instance, sneezing behind the hand increased the *S. aureus* load of a test surface from about 100 to ≥5000 per 25 cm^2. The types of food favoring enterotoxin production include dairy cream, ice cream, cured meats (ham, sausages, meat pies), and opened canned foods (in which fast growth is possible without competition). See also Section 2.4.3 for this aspect. *S. aureus* growth and toxin production can be prevented by proper storage (refrigerated, or too hot for growth), heating (this is not of help if the toxin has already been produced), adequate personal hygiene, cleanliness, and good disinfection practice.

2.3.3.3.3 Lesion-causing bacterial toxins. Two examples of this type of toxin-producing bacteria will be discussed: *Clostridium perfringens* and *Bacillus cereus*.

Clostridium perfringens. Clostridium perfringens are Gram-positive, anaerobic (aerotolerant) spore-forming rod-shaped bacteria. Several serotypes are distinguished (A, B, C, D, E, F) which produce different enterotoxins. Particularly, serotype A is associated with food-borne intoxications.

Toxicity and symptoms. Although enterotoxin formation in food (i.e., meat and poultry) may occur, still the incidence of *C. perfringens* food poisoning due to preformed enterotoxin in the food is rare. (Therefore, in Table 2.5, *C. perfringens* itself is listed as the causal agent.) A large number ($>10^8$) of vegetative *C. perfringens* cells need to be consumed to release sufficient enterotoxin. After an incubation period of 8 to 24 hours, abdominal cramps (much gas produced) and diarrhea with nausea but rarely vomiting can last for 24 hours. A number of enterotoxins have been found to damage the intestinal wall; the glucose resorption is inhibited and the bowel movement is stimulated. The mortality of serotype A poisoning (mainly in the US) is 3 to 4%; serotype C (Europe) is rarely fatal.

Chemical properties (structure and stability) of the enterotoxin. The toxins are protein-type enterotoxins (molecular mass approximately 34,000 dalton). Release of the enterotoxins in

the intestine occurs during sporulation and lysis of the *C. perfringens* cells. The proteinous nature of the enterotoxin makes it rather heat-sensitive.

Environmental conditions. Growth can take place at 15 to 50°C (optimum 40°C), pH 5 to 8 and a_w ≥0.93.

Type of food involved; prevention. C. perfringens causes mostly problems in meats. In the live animal, the microorganism can penetrate into the body through the intestinal wall. The thermal resistance of the spores varies from heat-labile (D-value 0.3 minutes at 100°C) to relatively heat-resistant (D-value 17.6 minutes at 100°C). The heat resistance also depends on the composition of the food. When contaminated meat receives inadequate heating (e.g., in the center of large pieces of roasted meat) or when the cooked meat is not sufficiently cooled prior to storage, germination of surviving *C. perfringens* spores may occur. Prevention measures include good hygiene, adequate meat heating (≥65°C at the center) followed by refrigerated storage (≤7°C).

Bacillus cereus. Bacillus cereus are Gram-positive spore-forming aerobic rod-shaped bacteria. They produce enterotoxins as well as several enzymes of pathogenic relevance, including lecithinase and hemolysin. Two different enterotoxins are known: type I and type II.

Toxicity and symptoms. Type I diarrheagenic enterotoxin occurs most frequently and is mildly toxic. After an incubation period of 8 to 16 hours, 50 to 80% of the consumers develop abdominal cramps and diarrhea which may last for 24 hours. Type II emetic enterotoxin is less common. After a short incubation period of 1 to 6 hours, violent vomiting occurs. Symptoms may last for 8 to 10 hours.

Chemical properties (structure and stability) of the enterotoxin. Type I is a proteinous enterotoxin (with molecular mass approximately 50,000). It is formed in the intestine (relatively long incubation period; large number of cells ≈10^6 required). This enterotoxin is heat-sensitive and, being a protein, undergoes degradation by trypsin. Type II is a toxin with molecular mass ≤5000. It is formed in the food during the logarithmic phase of bacterial growth. Type II is stable at pH 10 and is heat-resistant.

Environmental conditions. Growth can take place at 10 to 50°C (optimum 37°C), pH 5 to 9.

Type of food involved; prevention. Particularly cereal products contain *B. cereus* spores. There is no evidence that human factors are involved in the contamination. Cooking with cereal containing dishes followed by inadequate cooling enables germination of the spores that survived the heating. Type I toxin is associated with sauces, pastries, etc.; type II toxin is associated with cooked or fried rice. The main prevention measure is adequate and immediate cooling after cooking. This should be carried out in shallow layers enabling fast heat transfer; storage should be at ≤10°C.

2.3.3.3.4 Immuno-active bacterial endotoxins. Endotoxins are found in the cell wall of Gram-negative bacteria. Examples of bacteria with active endotoxins are *Salmonella abortus equi* and *Escherichia coli*. The endotoxins can be released upon lysis of the vegetative cells.

Toxicity and symptoms. Endotoxins are capable of stimulating the immune system in a non-specific way, and causing inflammations. Symptoms of intoxication include fever, shivering, painful joints, and influenza-like complaints, lasting for approximately 24 hours.

Chemical properties (structure and stability) of the endotoxin. Immuno-active endotoxins consist of lipopolysaccharides (LPS) bound covalently to protein and lipid fractions (Figure 2.4). The polysaccharide part consists of a lipid A fraction and a long polysaccharide chain. The lipid A fraction is identical in almost all bacteria. In the polysaccharide chain, a central part and an O-chain are distinguished. The central part has a similar structure in many bacteria, but the O-chain is rather characteristic.

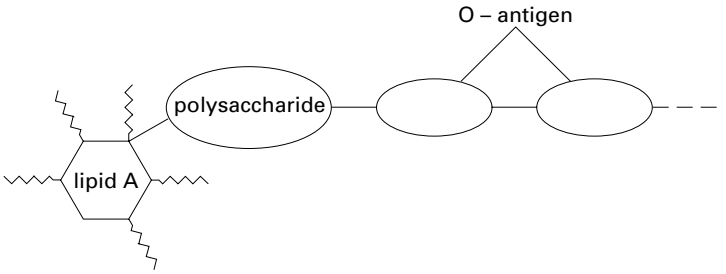


Figure 2.4 General structure of endotoxins.

Little is known about the covalently-bound protein. It is assumed that it is bound to the lipid A and is thus referred to as lipid A associated protein (LAP) or endotoxin protein (EP). The biological activity of the endotoxin is associated with its LPS part. LAP or EP appear to play a minor role. This might explain the different activities of endotoxins of various bacteria.

Environmental conditions. Endotoxins are released at the end of the growth curve, i.e., after death of the bacteria. Favorable conditions for growth of Gram-negative bacteria include pH 4.5, a_w >0.99, and temperatures ranging from 15 to 40°C.

Type of food involved; prevention. In principle, any type of food can serve as a vehicle. The release of endotoxins may take place in the intestine as a result of a food infection. Preventive measures against food infections include avoidance of cross-contamination of cooked food with raw foods, adequate heating, refrigerated storage, and adequate personal hygiene.

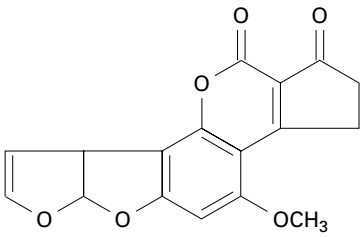
2.3.3.4 Mycotoxins

2.3.3.4.1 General. Mycotoxins are secondary metabolites of fungi which can induce acute as well as chronic toxic effects (i.e., carcinogenicity, mutagenicity, teratogenicity, and estrogenic effects) in animals and man. Currently, a few hundred mycotoxins are known, often produced by the genera *Aspergillus*, *Penicillium*, and *Fusarium*.

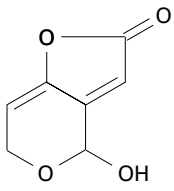
Toxicity and symptoms. Toxic syndromes resulting from the intake of mycotoxins by man and animals are known as mycotoxicoses. Although mycotoxicoses have been known for a long time ("Holy Fire" in the Middle Ages in Europe caused by the mold *Claviceps purpurea*; "Alimentary Toxic Aleukia" in the Soviet Union in 1940 caused by *Fusarium* spp.; "Yellowed Rice Disease" in Japan caused by *Penicillium* spp.), the mycotoxin-induced disorders remained the neglected diseases until the early 1960s, when the aflatoxins were discovered. This discovery was followed by much scientific research on mycotoxins.

Chemical properties (structure and stability). The chemical structures of some important mycotoxins are shown in Figure 2.5. The mycotoxins that will be discussed below are chemically stable and resistant to cooking. Several other mycotoxins have been shown to be unstable in foods. As this strongly reduces their toxicity, these will not be discussed here.

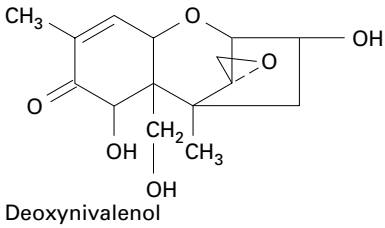
Environmental conditions. Mycotoxin contamination of food and feed highly depends on the environmental conditions that lead to mold growth and toxin production. The detectable presence of live molds in food, therefore, does not automatically indicate that mycotoxins have been formed. On the other hand, the absence of viable molds in foods does not necessarily mean there are no mycotoxins. The latter could have been formed at an earlier stage prior to food processing. Because of their chemical stability, several mycotoxins persist during food processing, while the molds are killed.



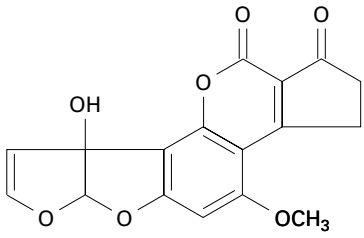
Aflatoxin B1



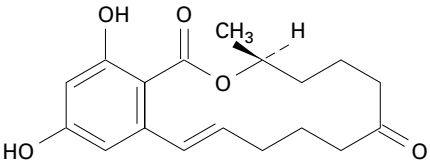
Patulin



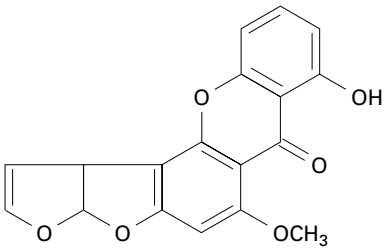
Deoxynivalenol



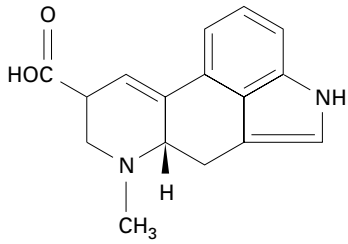
Aflatoxin M 1



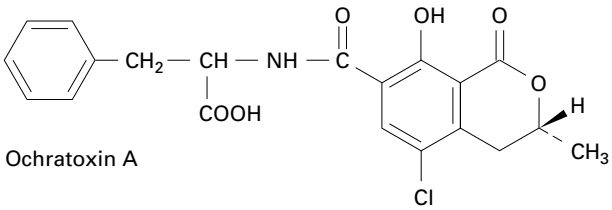
Zearalenone



Sterigmatocystin



Lysergic acid



Ochratoxin A

Figure 2.5 Some important mycotoxins.

Type of food involved; prevention. Many foodstuffs and ingredients may become contaminated with mycotoxins. The occurrence of various mycotoxins in foods and feeds has often been reported. Since the discovery of the aflatoxins, probably no commodity can be regarded as absolutely free from mycotoxins. Also, mycotoxin production can occur in the field, during harvest, processing, storage, and shipment of a given commodity.

2.3.3.4.2 Aflatoxins. The aflatoxins are the most important mycotoxins. They are produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*.

Toxicity and symptoms. Aflatoxins are potent toxins. They are well-known for their carcinogenicity. In view of occurrence and toxicity, aflatoxin B1 is the most important of them, followed by G1 > B2 > G2. Aflatoxin B1 is a very potent hepatocarcinogen in various experimental animal species including rodents, birds, fish, and monkeys. It appears that the aflatoxins themselves are not carcinogenic but rather some of their metabolites. Primary liver cancer is one of the most prevalent human cancers in the developing countries. Epidemiological studies carried out in the 1970s provide statistical support for the association of food consumption, contamination with aflatoxins, and incidence of hepatocellular carcinoma. It is now believed that there are combined actions of aflatoxins and hepatitis B virus infection leading to primary liver cancer. Due to worldwide commercial activities, the threat of aflatoxins to human health is not limited to those countries where the mycotoxins are produced. Moreover, the international trade in animal feed ingredients has contributed to the potential hazard for public health, because milk and dairy products may become contaminated with aflatoxin M1 (see Figure 2.5), the 4-hydroxy derivative of aflatoxin B1 formed in cows after ingestion of aflatoxin B1 with their feed. Aflatoxin M1 is also a suspect carcinogen, although its carcinogenic potency is probably less than that of aflatoxin B1.

Chemical properties (structure and stability). Aflatoxins are derivatives of coumarin. (The structure of coumarin can be found in Section 2.2.1.3.)

The most important types of aflatoxins are B1, B2, G1, and G2 (Figure 2.6). Aflatoxins are heat-stable and are hard to transform to non-toxic products. However, two methods of detoxication should be mentioned. First, the fate of aflatoxin B1 during food fermentation has been investigated in a variety of products. It appeared that fungi involved in food fermentation, for instance *Rhizopus oryzae* and *R. oligosporus*, are capable of reducing the cyclopentanone moiety, resulting in the formation of aflatoxicol A (Figure 2.7). This reaction is reversible. Under suitable environmental conditions (e.g., presence of organic acids), aflatoxicol A is irreversibly converted to its stereoisomer aflatoxicol B. Aflatoxicol A is approximately 18 times less toxic than aflatoxin B1.

In lactic fermentations at pH ≤4.0, aflatoxin B1 is readily converted to aflatoxin B2a (Figure 2.7) which is also less toxic. Both transformations thus reduce the toxicity, but the detoxication is not complete unless the lactone ring of the aflatoxin molecule is opened (Figure 2.8).

This would correspond to the loss of fluorescence at 366 nm. It has been found that loss of fluorescence correlates with reduced mutagenicity. Screening fungi for their ability to reduce the fluorescence of aflatoxin B1 solutions revealed that certain *Rhizopus* spp. were able to transform 87% of aflatoxin B1 into non-fluorescent substances of as yet unknown nature. A similar detoxication by opening of the lactone ring is achieved by treatment with ammonia (NH_4OH) at elevated temperature and pressure, which is applied at industrial scale to detoxicate animal feed ingredients, e.g., groundnut press-cake. At high pH the lactone ring of the aflatoxin molecule is hydrolyzed.

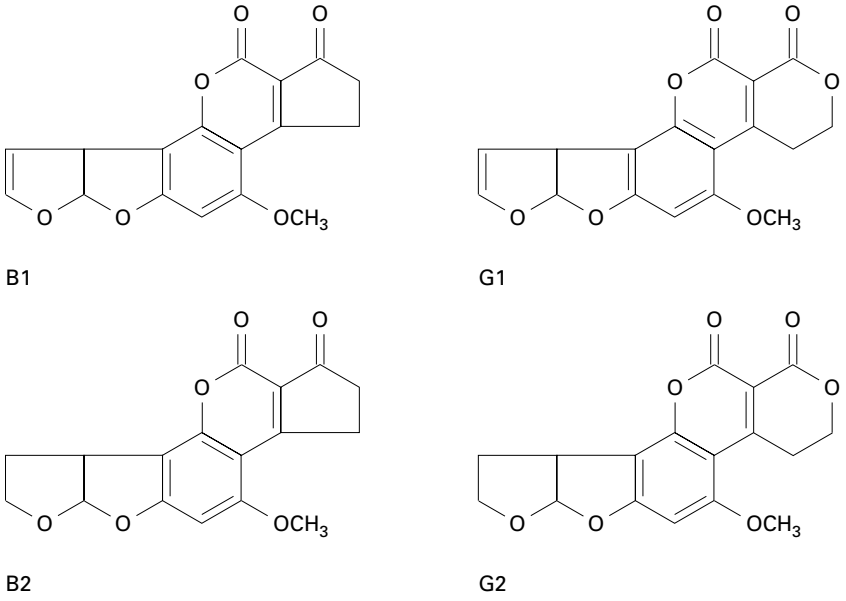


Figure 2.6 Structures of the major aflatoxins.

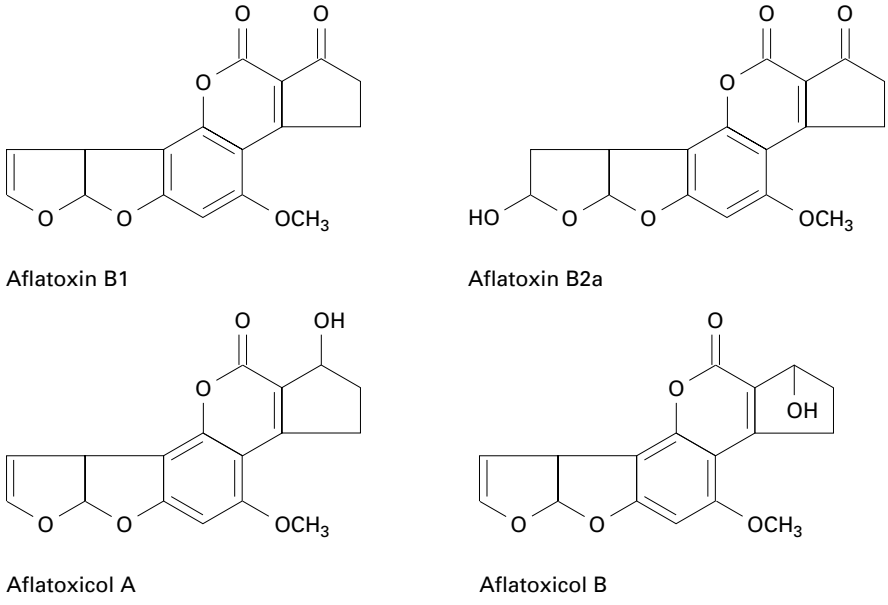


Figure 2.7 Detoxication of aflatoxin B1.

Environmental conditions. The fungi grow best at approximately 25°C at high relative air humidity (≥80%). Aflatoxins are produced both pre- and post-harvest, at relatively high moisture contents and relatively high temperatures.

Type of food involved; prevention. Aflatoxins can occur on various products, such as oilseeds (groundnuts), grains (maize) and figs. Problems with aflatoxin contamination

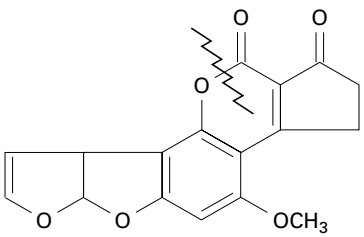


Figure 2.8 Detoxication of aflatoxin B1 by opening the lactone ring.

occur in industrialized countries (US) as well as in the developing countries in Latin America, Asia, and Africa. Aflatoxin M1 can be detected in low concentrations in milk samples from around the world, because of the high sensitivity of the current analytical methods. Prevention of aflatoxin contamination is achieved by discouraging fungal growth. Particularly, adequate post-harvest crop-drying is essential to reduce the chance of fungal growth.

2.3.3.4.3 Deoxynivalenol. Deoxynivalenol (DON) is a mycotoxin belonging to the group of trichothecenes (see Figure 2.5). It is produced by *Fusarium graminearum*.

Toxicity and symptoms. The trichothecenes, including T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol, fusarenon-X, nivalenol, and DON, induce a wide variety of toxic effects in experimental animals: diarrhea, severe hemorrhages, and immunotoxic effects. DON occurs worldwide. The toxin is of particular interest in the zootechnic sector, because feeding pigs with DON may lead to economic loss due to refusal of the feed and vomiting.

Chemical properties (structure and stability). DON is quite resistant to conventional food processing conditions.

Environmental conditions. The *Fusarium* producer strains prefer high relative air humidity at moderate temperatures (10 to 30°C).

Type of food involved; prevention. Fusarium species particularly occur on grains, e.g., maize, wheat and rye, in the moderate climate zones.

2.3.3.4.4 Ergot alkaloids. Ergot alkaloids are produced by *Claviceps purpurea*, which grows in the ears of grasses and cereals. The fungus forms sclerotia (2 to 4 cm large ergot kernels), which are the hibernation stage. During the harvest the sclerotia may end up between the cereal grains.

Toxicity and symptoms. Ergot alkaloids act particularly on the smooth muscles. Severe poisoning leads to constriction of the peripheral arteries, followed by dry gangrene of tissue and loss of extremities. Neurological disorders (itching, severe muscle cramps, spasms and convulsions, and psychological disorders) may also occur.

Chemical properties (structure and stability). The sclerotia contain derivatives of lysergic acid (Figure 2.5), the ergot alkaloids. Figure 2.9 illustrates the basic structure of these substances, taking ergotamine as an example. Ergometrine, ergotamine, and ergocristine are among the most important.

Environmental conditions. Ergot formation is favored especially in pre-harvest rye by high relative air humidity and temperatures of 10 to 30°C.

Type of food involved; prevention. Claviceps pupurea is common in pre-harvest grains. Consequently, a strict quality control of grain before milling is required. Taking into account the present-day grain quality assurance systems and its relatively high no-effect level, ergot is not considered a serious threat to human or animal health.

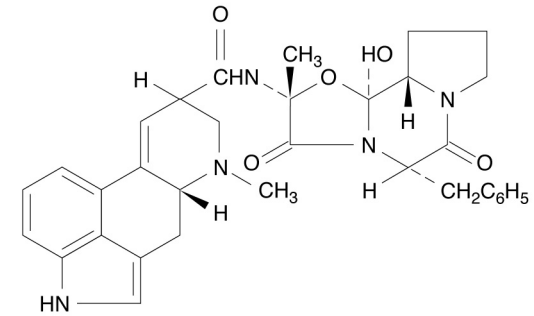


Figure 2.9 Structure of ergotamine.

2.3.3.4.5 Patulin. Patulin is mainly produced by *Penicillium expansum, Penicillium patulinum* and *Byssochlamys nivea*.

Toxicity and symptoms. Patulin causes hemorrhages, formation of edema, and dilatation of the intestinal tract in experimental animals. In subchronic studies, hyperemia of the epithelium of the duodenum and kidney function impairment were observed as main effects.

Chemical properties (structure and stability). The structure of patulin is shown in Figure 2.5. It is stable under conditions required for fruit juice production and preservation (see below).

Environmental conditions. Moderate temperatures, high moisture content, and relatively low pH (3 to 5) favor the growth of the fungi involved and patulin formation.

Type of food involved; prevention. The toxin occurs in vegetables and fruits (apples). Patulin is an indicator of bad manufacturing practice (use of moldy raw material) rather than a serious threat to human and animal health, as recent subacute and chronic toxicity studies have revealed. Thus, regulatory action based on safety evaluation would not be necessary.

2.3.3.4.6 Sterigmatocystin. Sterigmatocystin is produced by *Aspergillus versicolor* and *Aspergillus nidulans*.

Toxicity and symptoms. Sterigmatocystin is considered to be a carcinogen. Experiments with animals have shown that it causes liver and lung tumors in rats and mice. In comparison to the doses that induce tumors in rats, sterigmatocystin appeared to be a less potent carcinogen than the very potent aflatoxin B1.

Chemical properties (structure and stability). Sterigmatocystin is structurally related to the aflatoxins (Figure 2.5) and is equally stable.

Environmental conditions. Among the factors stimulating fungal growth and toxin production on cheese are lactose, fat, and some fat hydrolysis products.

Type of food involved; prevention. The natural occurrence of sterigmatocystin in food is probably limited. However, investigations on the occurrence of sterigmatocystin in food are, as yet, also limited. Sterigmatocystin occurs occasionally in grains and the outer layer of hard cheeses, when these have been colonized by *Aspergillus versicolor*. The concentration of sterigmatocystin in the outer layer of contaminated cheeses decreases rapidly from outside to inside. Insufficient data are available on the occurrence of sterigmatocystin, for example, in grated cheese to allow an evaluation of the health hazard caused by this product.

2.3.3.4.7 Zearalenone. Zearalenone is produced by some *Fusarium* species, i.e., *Fusarium roseum* and *Fusarium graminearum*.

Toxicity and symptoms. Zearalenone has estrogenic and anabolic properties. Pigs are among the most sensitive animals. The International Agency for Research on Cancer has placed zearalenone in the category "limited evidence of carcinogenicity."

Chemical properties (structure and stability). Zearalenone (Figure 2.5) is structurally related to the anabolic zeranol. Few data are available on its stability.

Environmental conditions. The conditions favoring zearalenone production are similar to those favoring DON formation, i.e., high relative air humidity at moderate temperatures.

Type of food involved; prevention. Zearalenone often co-occurs with DON in various grains, in particular maize and wheat. A risk assessment study on zearalenone carried out in Canada revealed that currently no adverse health effects are anticipated from zearalenone due to the intake of maize products. Other food sources such as wheat, flour, or milk may also contribute to the exposure to zearalenone. For the time being, no regulatory action has been recommended.

2.3.3.4.8 Ochratoxin A. Ochratoxin A can be produced by both *Aspergillus ochraceus* and *Penicillium viridicatum*.

Toxicity and symptoms. Ochratoxin A is a potent nephrotoxin in birds, fish, and mammals. Ochratoxin A is also teratogenic in mice, rats, hamsters, and chickens. The primary target organ is the developing central nervous system. There is a hypothesis that a renal disease observed in some areas of the Balkan countries is associated with exposure to ochratoxin A.

Chemical properties (structure and stability). The structure of ochratoxin A is shown in Figure 2.5. It is a fairly stable substance which is not easily metabolized.

Environmental conditions. Ochratoxin A production in cereals is favored under humid conditions at moderate temperatures.

Type of food involved; prevention. Ochratoxin A occurs in grains and, following transfer, in the organs and blood of a number of animals, especially pigs. Recently, a risk assessment study on ochratoxin A has been published. (Limited) Canadian data on estimated human intakes indicate that the tolerable daily intakes, estimated from carcinogenicity data of ochratoxin A, have been exceeded occasionally. More data are required to estimate the dietary exposure to ochratoxin A and to assess the need for regulatory controls or other control mechanisms. The current concern about ochratoxin A has led the International Union of Pure and Applied Chemistry (IUPAC) to the recent launching of a project in which the worldwide occurrence of ochratoxin A in food and animal feed will be mapped.

2.3.3.5 Toxic microbial metabolites

2.3.3.5.1 Biogenic amines. The main producers of biogenic amines in foods are Enterobacteriaceae and Enterococci. Most lactic acid bacteria which are used to produce fermented foods do not produce significant levels of biogenic amines.

Toxicity and symptoms. Biogenic amines have a stimulatory or toxic effect on the consumer. The symptoms of intoxication, persisting for several hours, include burning throat, flushing, headache, nausea, hypertension, numbness and tingling of the lips, rapid pulse, and vomiting. Especially, histamine has been indicated as the causative agent in several outbreaks of food intoxication. A level of approximately 1000 ppm of total biogenic amines in food is supposed to elicit toxicity, but from a Good Manufacturing Practice (GMP) point of view, levels in food of 50 to 100 ppm, 100 to 200 ppm and 30 ppm for histamine, tyramine, and phenylethylamine, respectively, or a total of 100 to 200 ppm are acceptable. The toxicity of histamine appears to be enhanced by the presence of other

biogenic amines found in foods that can inhibit histamine-metabolizing enzymes in the small intestine. Estimating the frequency of histamine poisoning is difficult because most countries have no regulations for histamine levels in foods, nor do they request notification of histamine poisoning. Also, because histamine poisoning closely resembles food allergy, it may often be misdiagnosed.

Chemical properties (structure and stability). Biogenic amines are a group of moderately toxic substances which can be formed in fermented foods, mainly by decarboxylation of amino acids (Figure 2.10).

Environmental conditions. The levels of biogenic amines increase with the presence of free amino acids (precursors), low pH of the product, high NaCl concentrations, and microbial decarboxylase activity.

Type of food involved; prevention. Biogenic amines are especially associated with lactic fermented products, particularly wine, cheese, fish, and meat. Also, very low levels occur in fermented vegetables (Figure 2.11).

Biogenic amines also occur naturally in fruits, vegetables, and fish; they may be produced by microbial decarboxylase activity. For instance, fresh fish (mackerel, tuna, skipjack) contain high levels of histidine which is readily decarboxylated to histamine by Gram-negative bacteria, e.g., *Proteus morganii*.

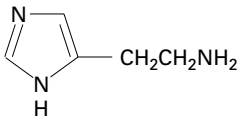
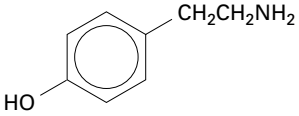
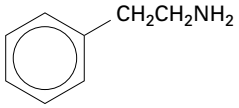
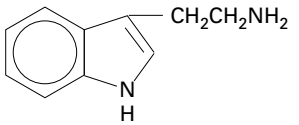
Amine	*Formula*	*Precursor*
Ethylamine C_2H_7N	$CH_3CH_2NH_2$	Alanine
Putrescine $C_4H_{12}N_2$	$H_2N(CH_2)_4NH_2$	Ornithine
Histamine $C_5H_9N_3$		Histidine
Cadaverine $C_5H_{14}N_2$	$H_2N(CH_2)_5NH_2$	Lysine
Tyramine $C_8H_{11}NO$		Tyrosine
Phenylethylamine $C_8H_{11}N$		Phenylalanine
Tryptamine $C_{10}H_{12}N_2$		Tryptophan

Figure 2.10 Major biogenic amines.

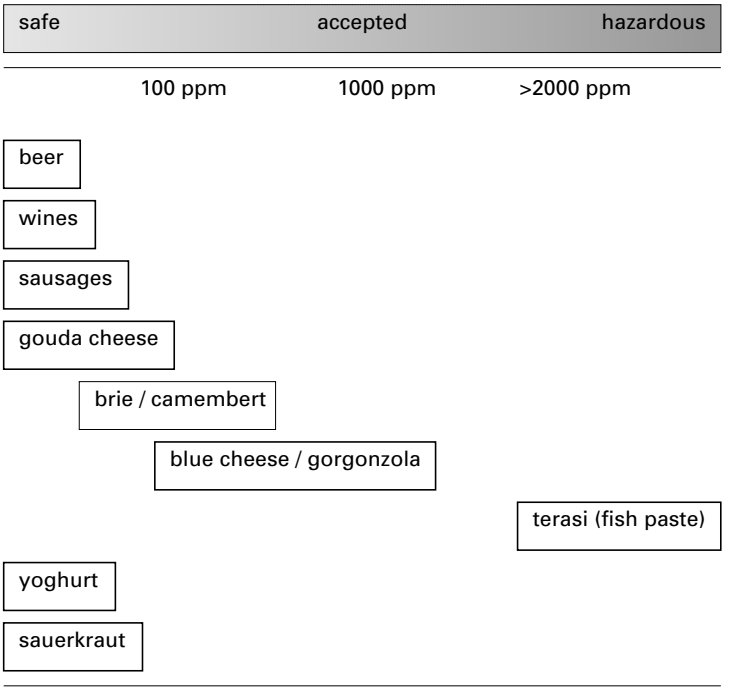


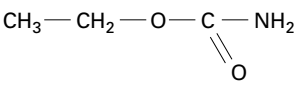
Figure 2.11 Presence of biogenic amines in fermented foods in relation to health hazards.

In meat products, species of Enterobacteriaceae have been found to be associated with cadaverine formation, and lactobacilli with tyramine formation. Also, sauerkraut may contain varying levels of biogenic amines, due to the large variations in the naturally selected microflora. In cheese, Enterobacteriaceae, heterofermentative lactobacilli, and *Enterococcus faecalis* were shown to be associated with the production of up to 600 ppm of biogenic amines including phenylethylamines. *Lactobacillus buchneri* has been shown to be involved in cheese-related outbreaks of histamine poisoning. Pasteurization of cheese milk, good hygienic practice, and selection of starters with low decarboxylase activity are measures to prevent the accumulation of these undesirable products.

2.3.3.5.2 Ethyl carbamate. Ethyl carbamate (urethane) is associated with yeast fermented foods and beverages.

Toxicity and symptoms. Ethyl carbamate is a mutagen as well as a carcinogen.

Chemical properties. The structure of the carbamic acid moiety of ethyl carbamate may originate from several substances including naturally occurring citrulline, and urea and carbamyl phosphate resulting from the metabolism of L-arginine and L-asparagine by yeast. In addition, vicinal diketones and HCN released from cyanogenic glycosides can act as precursors. Ethanol (the other precursor) is formed as a result of alcoholic fermentation by yeasts, or as one of the products of heterofermentative lactic acid fermentation.



Ethyl carbamate (Urethane)

Table 2.6 Occurrence of ethyl carbamate in fermented foods and beverages[1]

Product	Number of samples	Average level (ppb)	Range (ppb)
Yogurt	12	0.4	ND–4
Cider	8	0.6	ND–4
Bread	30	1.7	ND–8
Malt beverages	69	1.8	ND–13
Bread, toasted	9	5.2	2–14
Soya sauce	12	18	ND–84
Wine	6	18	7–40
Sake	11	52	3–116

Note: ND = not detectable.

[1] Data found in literature.

Environmental conditions. Heat and light enhance the formation of ethyl carbamate.

Type of food involved; prevention. Ethyl carbamate occurs in a variety of fermented foods and beverages (Table 2.6).

In most countries there is no legislative limit value, but the Food and Agriculture Organization World Health Organization (FAO/WHO) suggest a level of 10 ppb for softdrinks, and the Canadian Government recommends 30 to 400 ppb for various alcoholic beverages. Research on wine and stone fruit (cherry, plum) fermentations indicate that reduction of the levels of the precursors by enzymatic treatment, selection of yeast strains, control of fermentation conditions, and treatment of the pH-adjusted fermented pulp with $CuSO_4$ may be useful in keeping the ethyl carbamate levels at a minimum.

2.4 *Recent developments in food safety assurance*

2.4.1 *Good manufacturing practice*

In principle, prevention of food-associated intoxications starts at the level of primary, i.e., pre-harvest plant and animal production. However, this is very difficult to achieve on a large scale and can only be considered as a long-term objective.

Consequently, it is important to prevent bacteria and mold spores from starting to grow during food and feed processing and storage. The establishment of, and strict adherence to hygiene guidelines and rules for Good Manufacturing Practice contribute to the systematic microbiological control of industrial processes. The main techniques to achieve growth prevention include: drying (reduction of water activity), control of keeping and storage temperatures (the lower the better) and modified atmosphere storage (CO_2 levels in the gas phase exceeding 35% v/v inhibit microbial growth). Other techniques include the application of gamma irradiation, or fungicides to kill fungal spores. However, some of the methods may have the disadvantage of not being fully effective and of leaving chemical residues in the food product.

2.4.2 *Consumer education*

It is also important that the consumer should protect him/herself by safe handling of food. Surveys have shown that most food intoxications originate from inadequately refrigerated storage, or use of left-overs which were not or inadequately re-heated. Also, foods of animal origin should not be consumed raw. Cross-contamination of cooked food with raw food must be avoided by keeping raw and cooked foods separated.

***Table* 2.7** Critical control points in the manufacturing process of sweetened concentrated milk with regard to growth of and enterotoxin production by *Staphylococcus aureus*

Processing stage	Can contamination with *S.aureus* occur?	Can *S.aureus* grow or produce enterotoxins?	Can *S.aureus* survive?
Raw milk	yes	no, if temperature <10°C	yes
Pasteurization	no, if overpressure is maintained		no, if adequate time/temperature combination is used
Concentration	no	no, if temperature >45°C	yes
Seeding with lactose crystals	yes, if not done aseptically		yes
Bottling or can filling	yes, if not done aseptically		yes
Storage	no	growth if $a_w > 0.86$; no toxin formed	yes
Home use	yes, after opening	yes, after dilution if temperature > 15°C	yes

2.4.3 *Hazard analysis at critical control points*

The introduction in the 1970s of the Hazard Analysis at Critical Control Points (HACCP) (see Part 3, Chapter 21, Section 21.3) concept has marked a change in the philosophy with regard to the microbiological quality assurance of food. This concept provides a means for identifying the microbiologically important stages in food processing and the means to control them. Introduction of this system starts with a detailed analysis of the hazards associated with the manufacture, distribution, and use of the food, and leads to the identification of the critical control points. Systematic and frequent monitoring and control are carried out at these points. In applying these principles, greater assurance of product safety is achieved than would be possible with the traditional procedures.

The above is illustrated by the production of sweetened concentrated milk. In this product, *Staphylococcus aureus* can grow and produce enterotoxins. The stages in the manufacturing process which are of importance from a microbiological point of view are summarized in Table 2.7.

At each stage, the chance of contamination with *S. aureus* is assessed, and the conditions determining growth and toxin production are given. As can be seen, the safety of the process can be monitored comfortably and quickly by regular measurements of temperature and pressure. In addition, maintenance of asepsis during the process and adequate instructions for use and storage at the consumer level are key factors to reduce the risk of intoxication.

2.5 *Summary*

Some of the many thousands of natural substances present in food have been found to induce toxic effects. Usually, natural toxins are not acutely toxic, except in a few cases in animals. Particularly, those natural toxins occurring in plant-derived foods may induce adverse effects only after chronic ingestion or by allergic reactions.

In this chapter, the natural toxins are divided into endogenous toxins of plant origin and contaminants of natural origin. Endogenous toxins of plant origin comprise many different types of substances. There is no simple way of classifying this group of toxic food components. The way they are dealt with here is based on a classification according to common functional groups (toxic phenolic substances, cyanogenic glycosides, and glucosinolates), the physiological action (acetylcholinesterase inhibitors), and the type of toxic effect induced (biogenic amines and central stimulants). Toxins in food can also be contaminants of natural origin. There are three important sources of this group of natural toxins. First, raw materials of plant origin may be mixed with toxic non-nutritive plant species, e.g., cereals have been reported to be contaminated by pyrrolizidine alkaloids. Secondly, raw materials of animal origin, mainly fish and milk, can also be contaminated if the animal has ingested toxic substances of natural origin. A well-known case is that of paralytic shellfish poisoning. This is attributed to the consumption of shellfish that have become contaminated with a toxin (saxitoxin) on ingestion of toxic plankton. Thirdly, contaminants of natural origin can be products of microorganisms. Several microorganisms, including bacteria and fungi, can cause food-borne diseases in this way. The most important bacterial toxins, their chemical properties, environmental conditions required for their formation, type of food involved, and prevention measures are discussed. In particular, the bacteria *Clostridium botulinum* (botulinum toxin), *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, and endotoxin-forming Gram-negative bacteria are of importance as far as food safety is concerned. Toxins of fungal origin, the so-called mycotoxins, are produced by the genera *Aspergillus*, *Penicillium*, and *Fusarium*. The most important mycotoxins are the aflatoxins *(Aspergillus flavus)*. Another major fungal toxin is ochratoxin A (*Aspergillus ochraceus* and *Penicillium vindicatum*). A special group of microbial toxins are metabolites of microorganisms. Important examples are the biogenic amines (formed by decarboxylation of free amino acids during spoilage and in some fermentations) and ethyl carbamate (occurring in yeast-fermented foods and beverages).

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