

## Microbial toxins in fermented foods: health implications and analytical techniques for detection

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### Abstract

Recently, demand for fermented foods has increased due to their improved nutritional value, taste, and health-promoting properties. Worldwide consumption of these products is increasing. Fermented foods are generally safe for human consumption. However, some toxins, primarily biogenic amines (putrescine, phenylethylamine, histamine, tyramine, and cadaverine), mycotoxins (fumonisins, aflatoxins, ochratoxin A, zearalenone, and trichothecenes), and bacterial toxins (endotoxins, enterotoxins, and emetic toxins) can be produced as a result of using an inappropriate starter culture, processing conditions, and improper storage. These toxins can cause a multitude of foodborne illnesses and can lead to cardiovascular aberration and adverse gastrointestinal symptoms. Analytical techniques are in use for the detection of toxins in fermented foods for monitoring and control purposes. These include culture, chromatographic, immunoassays, and nano sensor-based techniques. These detection techniques can be used during the production process and along the food chain. On an industrial scale, HPLC is widely used for sensitive quantification of toxins in fermented foods. Recently, biosensor and nano sensor-based techniques have gained popularity due to accuracy, time efficiency, and simultaneous detection of multiple toxins. Other strategic methods being investigated for the removal of toxins from fermented foods include the use of specific starter cultures for bio-preservation, aflatoxin-binding, and biogenic amine-degradation agents that may help to appropriately manage the food safety concerns associated with fermented foods.

**Keywords:** Fermented food, HPLC, Nanosensors, PCR, Toxin

### 1. Introduction of fermented foods

Food fermentation is a food processing technology that uses the growth and metabolic activity of beneficial microorganisms naturally present or added to raw food materials for stabilization and transformation. Secondary metabolites formed as a result of fermentation contribute to the organoleptic properties of flavor and texture, functional properties, and the nutritive value of foods [1]. Based on the substrate used, the fermentation process is

classified into the nine major groups of fermented cereals, fermented vegetables, fermented legumes, fermented roots/tubers, fermented milk products, fermented and preserved meat products, fermented, dried, and smoked fish products, miscellaneous fermented products, and alcoholic beverages [2]. Fermenting microorganisms can be incorporated into foods in two ways. First, wild or spontaneous fermentation is a natural phenomenon where naturally occurring microorganisms in food or present under processing conditions conduct the

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fermentation [3]. Examples include fermented soy foods, sauerkraut, and kimchi. A culture-dependent fermentation or “controlled fermentation” is a second process in which a starter culture is inoculated into a food product. Kefir and kombucha are examples of food products produced through starter culture inoculation [3].

Fermented foods provide beneficial effects to humans by two main mechanisms; modulation of gut microbiota, and/or formation of different bioactive compounds, such as exopolysaccharides, oligosaccharides, peptides, GABA-gamma aminobutyric acid, conjugated linoleic acids, and vitamins [3]. Fermentation of some foods also yields angiotensin-converting enzyme (ACE) inhibitory peptides which have an anti-hypertensive effect. Kefir, a yogurt-based drink, has been reported to improve protein digestibility [4]. Soy-based fermented foods including soy paste and soy sauce have shown anti-inflammatory effects both in vitro and in vivo [5].

The lactic acid bacteria (LAB) *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Weissella* are the most common fermenting bacteria. Other bacterial consortia which can be isolated from fermented foods or are detected during fermentation include *Bacillus* in legume-based products, *Bifidobacterium*, *Brachy bacterium*, *Brevibacterium*, and *Propionibacterium* in cheese, and *Arthrobacter* and *Hafnia* in meat products [1]. Several yeast taxa have also been isolated from fermented foods, including *Candida*, *Debaryomyces*, *Geotrichum*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, *Wickerhamomyces*, and *Zygosaccharomyces* [1].

Owing to the benefits of fermented foods, there has been a remarkable increase in the worldwide consumption of fermented foods. A wide variety of indigenous fermented foods are consumed around the world. According to an estimate, there are approximately 5000 varieties of fermented foods and beverages globally [1]. The global market share of fermented foods was valued at approximately 149.5 billion US dollars in 2016 [6]. This value was based on industrial production only. Daily indigenous household preparation of fermented foods was excluded. Also, the availability of ready-to-eat fermented foods and beverages in the market enhances consumption due to accessibility for consumers. An increase in demand for fermented foods is expected at a compound annual growth rate of 4.3% during 2019–2024 and is expected to reach 205.5 billion US dollars by 2023 [6,7]. Of the different fermented foods available, fermented dairy products have the greatest demand worldwide. Among these, Kefir and

drinking yogurts experienced significantly greater sales in recent years [7]. Out of the 172.2 million tons of milk produced by the European Union in 2018, 64.9 million tons (37.7%) were used for fermented cheese products, and 7.4 million tons (4.3%) were used for fermentation of other acidified milk products [3]. Although the dairy fermentation industry has had the greatest market value in the recent years, there are regional differences in the types of fermented foods consumed. Cereal-based fermented foods are widely consumed in the United States and Europe, while fermented dairy foods, fermented soybean, and fermented fish products are more commonly consumed in Asia [7,8].

Fermented foods consumption has recently increased due to their health benefits. However, fermented foods may pose a potential food safety risk due to various microbial toxins, including biogenic amines, mycotoxins, and bacterial toxins. These toxins may be produced during the fermentation process or may be added during the food processing stages.

## 2. Toxins in fermented foods- emerging challenges & public health concerns

Fermented foods are generally safe for human consumption but are not entirely devoid of potential health risks associated with toxins. Multiple factors account for the presence of toxins in fermented foods, including poor hygiene, an unfit starter culture, unsafe storage conditions, and the use of contaminated raw materials. Table 1 presents an overview of toxins that can be present in fermented foods. These toxins can be classified into three categories: biogenic amines, mycotoxins, and bacterial toxins [3,9–11].

### 2.1. Biogenic amines

In fermented foods, LAB used in the fermentation process are the main reasons for the production of biogenic amines. LAB produce amino-acid decarboxylase enzymes which convert amino acids to biogenic amines. The presence of biogenic amines in foods can cause intoxication symptoms such as heart palpitations, redness, headaches, vertigo, and altered blood pressure and can be fatal at high concentrations [12] (Fig. 1).

Biogenic amines are most commonly reported in wine, beer, fermented soybeans, meat, dairy, and vegetable products. Soy-based foods, such as soy sauce, miso, natto, stinky tofu, and tempeh, have been reported to cause biogenic amine toxicity (Table 1). The dietary groups with the maximum

Table 1. Toxins in different fermented food products.

Fermented food products	Toxins	Microbes	Harmful effects	Reference
Alcoholic beverages (South Africa-based)	Aflatoxins <sup>a</sup> , Zearalenone <sup>a</sup> , Ochratoxin <sup>a</sup> , Penicillic acid <sup>a</sup> , Shiga toxin <sup>b</sup>	<i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Rhizopus</i> spp., <i>E-coli</i> (STEC)	Neurotoxicity, nephrotoxicity, hemolytic uremic syndrome	[11,28]
Meju (fermented soybeans)	Aflatoxin B1 <sup>a</sup> , Ochratoxin A <sup>a</sup>	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Cytotoxicity, carcinogenic, immunosuppression	[37]
Iru and ogiri (Nigerian fermented foods)	Aflatoxins <sup>a</sup> , Endotoxins <sup>b</sup>	<i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Rhizopus</i> spp., <i>Enterobacteriaceae</i>	Neurotoxicity, carcinogenic, immunosuppression, vomiting	[28]
Fish sausage (fermented)	Cadaverine <sup>c</sup> , putrescine <sup>c</sup> , histamine <sup>c</sup> , tyramine <sup>c</sup>	<i>Enterobacteriaceae</i>	Acute and chronic toxicities, such as, vomiting	[72]
Sausages	Shiga toxin <sup>b</sup>	<i>E. coli</i>	Hemolytic uremic syndrome	[73]
Fermented sausages (dry)	Verotoxigenic (Shiga toxin) <sup>b</sup>	<i>E. coli</i>	Hemolytic uremic syndrome	[74]
Fermented milk products	Aflatoxins M1 <sup>a</sup>	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Neurotoxicity, nephrotoxicity, carcinogenic	[75]
Maize meal (fermented)	Fumonisin B1 <sup>a</sup> , Aflatoxin B1 <sup>a</sup> , Zearalenone <sup>a</sup> , Ochratoxin A <sup>a</sup>	<i>Fusarium graminearum</i>	Neurotoxicity, carcinogenic, immunosuppression	[28]
Fermented pasta	Deoxynivalenol <sup>a</sup> and T-2 toxin <sup>a</sup>	<i>Fusarium graminearum</i> , <i>F. asiaticum</i> , <i>F. meridionale</i> , <i>F. boothii</i>	Cytotoxicity, carcinogenic, immunosuppression, neurotoxicity, nephrotoxicity	[76]
Fermented soyabean (Doenjang)	Enterotoxin <sup>b</sup>	<i>Bacillus cereus</i>	Vomiting, diarrhea	[28]
Fermented soya sauce products	Deoxynivalenol <sup>a</sup> , Enterotoxin <sup>b</sup>	<i>Fusarium</i> spp., <i>Bacillus cereus</i>	Immunosuppression, neurotoxicity, nephrotoxicity	[32]
Kimchi	Toxic trace elements (As, Cd, In, Pb, and Tl)	–	Neurotoxicity, neurodegenerative diseases	[77]
Pickled vegetables	Cadaverine <sup>c</sup> , tyramine <sup>c</sup> , Nitrosamines	<i>Firmicutes</i> , <i>Leuconostoc</i> spp.	Cardiac palpitations, headache, flushes, nausea	[78]
Milk tofu	Toxic elements (V, Ba, Sr, Pb, Cd),	–	Neurotoxicity, neurodegenerative diseases	[79]
Fumigated cheese	Citrinin <sup>a</sup> , cyclopiazonic acid <sup>a</sup> , roquefortine C <sup>a</sup>	<i>Aspergillus flavus</i> , <i>Penicillium roqueforti</i> , <i>Fusarium oxysporum</i>	Neurotoxicity, nephrotoxicity, carcinogenic, immunosuppression, cytotoxicity	[80]
Ogi baba	Aflatoxins B1 <sup>a</sup>	<i>Aspergillus flavus</i>	Carcinogenic, neurotoxicity	[28]
Ugba (fermented African oil bean seeds)	Aflatoxins B1 <sup>a</sup> , Fumonisin B1 <sup>a</sup> , Sterigmatocystin <sup>a</sup>	<i>Aspergillus</i> spp., <i>Fusarium</i> spp.	Neurotoxicity, carcinogenic, immunosuppression, cytotoxicity	[28]
Soumbala	Enterotoxins <sup>b</sup>	<i>Bacillus cereus</i>	Vomiting, diarrhea	[28]
Bikalga	Enterotoxins <sup>b</sup>	<i>Bacillus cereus</i>	Vomiting, diarrhea	[28]
Kenkey (fermented maize)	Aflatoxins <sup>b</sup>	<i>Aspergillus</i> spp.	Neurotoxicity, carcinogenic	[81]
Yogurt	Aflatoxins <sup>b</sup>	<i>Aspergillus</i> spp.	Neurotoxicity, carcinogenic	[75]

<sup>a</sup> Classification of mycotoxins.

<sup>b</sup> Classification of bacterial toxins.

<sup>c</sup> classification of endotoxins.

average values for the total concentration of biogenic amines include fish sauce with an average total biogenic amine concentration of 582–588 mg/kg followed by fermented vegetables (375–390 mg/kg), cheese (177–334 mg/kg), and fermented sausages (281–283 mg/kg) [13]. The primary biogenic amines produced during the fermentation of these products are putrescine, phenylethylamine, histamine, tyramine, and cadaverine. Histamine and tyramine are the most common and important concerning food safety [11]. Biogenic amines are thermostable, non-volatile compounds with a low

molecular weight that resist heat treatments applied during food processing.

Histamine, produced from histidine, is a notorious causative agent for food poisoning outbreaks. Cheese (mainly gouda, Swiss, cheddar, gruyere, and Cheshire) and wine are among the reported outbreak sources of histamine poisoning. In these outbreaks, the histamine content of cheeses ranged between 850 and 1870 mg/kg [14]. In wine, a histamine concentration of 2 mg/l was the permissible limit for import and export purposes [15]. In Taiwan, histamine content was reported to be greater than

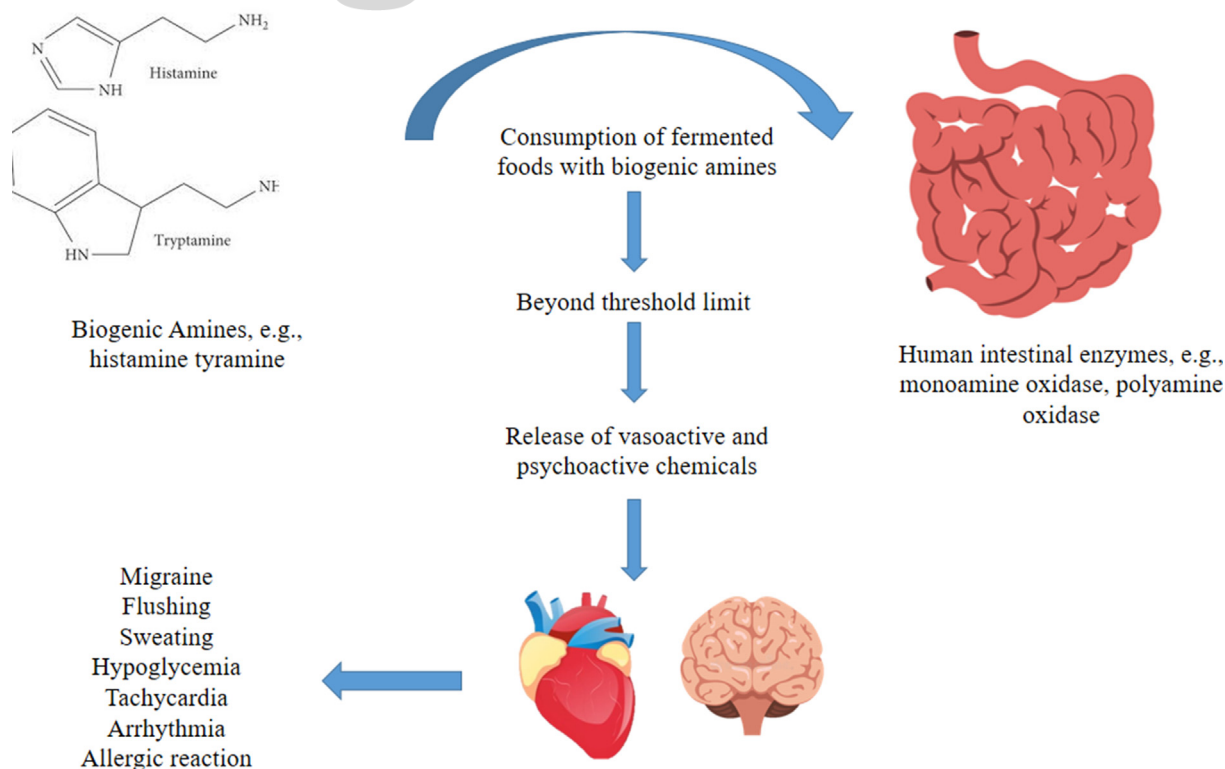


Fig. 1. The harmful health effects of biogenic amines from fermented foods and the detoxification process by the intestinal enzymes in the body.

50 mg/kg in kimchi [16]. The Commission Regulation (EC) 2073/2005 has established the permissible limits for histamine only for fish and fish products in terms of food safety. These permissible limits range between 100 mg/kg and 200 mg/kg. For fish products that are subjected to enzymatic treatment in brine solution have a bit higher permissible histamine between 200 mg/kg and 400 mg/kg [13].

Histamine poisoning can have adverse health effects [17]. Symptoms of histamine poisoning include abdominal pains, arrhythmia, extrasystoles, urticaria, redness, headache, nasal discharge, respiratory failure, asthma, hypotension, hemoconcentration and eyelid swelling [14]. Microbial strains responsible for histamine production in fermented foods are *Oenococcus oeni*, *Pediococcus parvulus*, *Pediococcus pentosaceus*, *Tetragenococcus species*, *Leuconostoc species*, *Ligilactobacillus saerimneri 30a*, *Lentilactobacillus hilgardii*, *Lentilactobacillus buchneri* and *Lactobacillus curvatus* [18].

*Streptococcus thermophilus* is an important thermophilic LAB used as a starter culture in cheese making. In order to make cheese from pasteurized milk, it is combined with thermophilic Lactobacilli. It is also commonly used in many natural whey cultures for making classic raw milk cheeses. Research has shown that *S. thermophilus* contains histidine decarboxylase (hdcA) gene, which has

novel properties and may considerably increase the histamine accumulation in cheese [19].

Tyramine is another potential biogenic amine that can be found in cheese, fermented vegetables, alcoholic beverages, and fermented sausages at toxicologically relevant levels. Tyramine indirectly increases the blood noradrenaline concentration and acts as a vasoconstrictor leading to hypertension, migraine, brain hemorrhage, and heart failure [17,20]. Concentrations higher than 100 mg/kg of food weight are considered harmful to humans [21].

A study was conducted for the quantitative determination of various biogenic amines in twenty-two different food products from the Belgian market. The most significant biogenic amines present in blue cheeses were tyramine and cadaverine, with tyramine contents up to 1306 mg/kg [22].

In another study conducted in Switzerland, tyramine was detected in 46 fermented sausage samples out of 62 samples with a maximum concentration of 785.2 mg/kg [14]. Similarly, tyramine accumulation has been reported in the Thai fermented shrimp Kung-som because fermented shrimp is rich in tyrosine (10.78 mg/g dry mass), which is a precursor for tyramine formation [23]. Korean fermented foods, such as doenjang (fermented soybean paste), have also been reported for tyramine toxicity with concentrations up to 1430.7 mg/kg [24]. The Gram-



positive bacteria *Enterococcus* (*E. faecalis* and *E. faecium*) and *Lactobacillus* species (*Latilactobacillus curvatus* and *Levilactobacillus brevis*) are the major groups responsible for tyramine formation and accumulation in fermented foods [12]. *Carnobacterium*, *Leuconostoc*, *Staphylococcus*, and *Lactococcus* can also contribute to tyramine toxicity in fermented foods. *L. brevis*, *L. hilgardii*, *L. plantarum*, and *Leuconostoc* species can cause tyramine production in fermented beverages [25].

## 2.2. Mycotoxins

Mycotoxins are also a potential food safety concern in fermented foods [26]. Mycotoxins are secondary toxic metabolites produced by fungi that primarily contaminate agricultural products, such as cereals, grains, wheat, maize, rye, oats, and legumes including peanuts, beans, peas, lentils, and soybean. Therefore, cereal-based fermented foods are vulnerable to mycotoxin contamination [9]. Mycotoxins are thermostable molecules, which increase the difficulty of elimination once produced in or after entry into any food.

Beer is vulnerable to contamination by mycotoxins during all stages of brewing [27]. Aflatoxins, fusarium toxins, and zearalenone may enter the malt from cereal grains due to thermostability, or directly contaminate produced beer due to water solubility [27]. A study conducted in Nigeria showed that 82% of fermented food samples (maize gruel Ogi, sorghum gruel Ogi-baba, melon seed Ogiri, locust bean Iru) contained mycotoxins, mainly aflatoxin B1, fumonisin B1, and sterigmatocystin [28]. *Aspergillus*, *Fusarium*, *Penicillium alternaria*, and *Cladosporium* were prominent contaminant microorganisms. These fungal taxa produce several toxins, including fumonisins, aflatoxins, ochratoxin A, zearalenone, and trichothecenes, which can be nephrotoxic, carcinogenic, and immunotoxic [28]. The blue mold cheese fermenting fungi *Penicillium roqueforti* produces mycotoxins, cyclopiazonic acid, and rugulovasine A and B [29]. *Aspergillus flavus* and species of *Fusarium* are responsible for aflatoxin B1 and trichothecene T-2 toxins in the fish sauce during fermentation [30]. Deoxynivalenol, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol are harmful for both human and animals and present in grains and wheat products. Based on research on animals and clinical findings involving humans, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) decided that the provisional highest tolerable intake of 1 g/kg body weight/day for the total amount of deoxynivalenol and its acetyl derivatives [31]. However, China has set a limit of 1000 µg/kg for deoxynivalenol in cereal-based products, both 3-

acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol legal upper limits have not yet been established [32].

Different mycotoxins have specific detrimental effects on the health of an individual. The major mycotoxins include aflatoxins, ochratoxin A, patulin and zearalenone. Even small amounts of mycotoxins in foods can pose a considerable health risk. Aflatoxins have carcinogenic potentials and mainly affect the liver, causing hepatotoxicity. Ochratoxin A is nephrotoxic and accelerates the formation of free radicals, which are harmful to the human body. Patulin toxicity can lead to adverse gastrointestinal conditions and neurotoxicity. Zearalenone has carcinogenic and teratogenic potentials [33]. Regulatory authorities in many countries have defined the permissible limits of mycotoxins in different foodstuffs. For example, the Commission of the European Communities (EC regulation No. 1881/2006) has set an upper limit for mycotoxins in some fermented foods at 50 µg/kg of patulin in apple cider and other apple-based fermented drinks, 0.050 µg/kg of aflatoxin M<sub>1</sub> in raw milk and milk-based products, and 2.0 µg/kg of ochratoxin A in wine [27]. The Codex Committee on Food Additives and Contaminants has specified 0.5 mg/kg for aflatoxin M<sub>1</sub> in milk, whereas the US Food and Drug Administration (FDA) has defined 20 ppb as the permissible limit for aflatoxin M<sub>1</sub> in milk [34].

## 2.3. Bacterial toxins

Another potential food safety risk in fermented foods is the presence of bacterial toxins. Some bacteria and their toxins become incorporated into fermented products via substrate contamination. Studies have specifically highlighted the presence of bacterial toxins in both meat and vegetable-based fermented foods [28]. A review of studies showed that several vegetable-based fermented foods, such as cucumbers, mustard greens, young melons, cabbage, Chinese cabbage, papayas, and bamboo shoots are at high risk of microbial contamination due to indirect contamination via a raw substrate [9]. *Nem Chua* (fermented pork sausage) consumed in Vietnam has a high risk of causing food intoxication due to processing without exposure to heat or any cooking. Thus, *Nem Chua* is vulnerable to contamination and growth of toxin-producing bacteria, such as *Staphylococcus aureus* [9]. New Zealand mussel (*Perna canaliculus*) is a traditional fermented food in New Zealand that is commonly contaminated with *Clostridium botulinum*. *Clostridium* spores survive during fermentation due to tolerance of anaerobic conditions and germination and growth of vegetative cells, leading to the production of heat-resistant

toxins that cause food intoxication. Traditionally consumed fermented foods in the Northeast regions of India have also been investigated for potential foodborne toxin contamination. A significant microbial load of the toxin-producing bacteria *Bacillus cereus*, *P. mirabilis*, and *C. botulinum* was found in soybean, fish, and pork-based fermented foods [35].

Cell membranes of Gram-negative bacteria contain lipopolysaccharide complexes, known as endotoxins that are heat stable and, thus, can survive processing conditions and pose a significant risk of food-borne illnesses. The presence of bacterial endotoxins has been reported in fermented ogiri, ugba, iru, ogi, and ogi baba in Africa [28]. Among the fermented foods tested, iru, a condiment derived from alkaline fermentation of locust beans, had the highest endotoxin concentration at  $5.5 \times 10^4$  endotoxin units/g. *Sphingomonas*, *paucimobilis*, and *Escherichia coli* were the dominant Gram-negative taxa responsible for endotoxin production. A significant correlation between microbial load and toxin concentration was also revealed [28].

Sorghum is an important staple crop in Africa made edible through fermentation. Several studies have reported the occurrence of opportunistic bacteria and their toxins in sorghum-based fermented foods [26]. *B. cereus*, *Clostridium perfringens*, *E. coli*, *Listeria monocytogenes*, and *Enterococci* contaminate fermented weaning cereals. *Gowé*, a traditional cereal-based fermented beverage in Benin, has food safety concerns due to contamination with *E. coli* and Enterobacteriaceae [10].

*Bacillus* species are predominant in oil-bean seeds. These taxa produce less worrisome, heat-labile enterotoxins in ugba and more hazardous, emetic, heat-stable toxins in baobab seeds [36]. *B. cereus sensu lato* is a foodborne pathogen in some Korean fermented soybean products, such as doenjang, gochujang, ssamjang, and chokochujang [37]. Shiga toxin-producing *E. coli* (STEC) is another causative agent of foodborne illnesses worldwide. In a study conducted in Nigeria, STEC was detected in 2% of raw milk samples, 6% of fresh local cheese, and 9.6% of fresh local cheese. All these bacterial toxins can lead to food poisoning, have deleterious effects on health, and cause adverse gastrointestinal symptoms [38].

### 3. Analytical techniques

#### 3.1. Techniques for biogenic amines detection

Detection of biogenic amines in fermented foods is crucial. Early detection of biogenic amines in foods allows timely preventive measures to be

implemented for avoiding food intoxication. In addition to toxin detection, it is imperative to detect bacteria containing decarboxylase enzymes to facilitate a preliminary risk estimation of biogenic amine formation in fermented foods and to take measures to counter accumulation.

Traditionally, culture-dependent techniques have been used to detect the presence of biogenic amine-forming bacteria in fermented foods (Table 2). These techniques are based on pH changes in differential growth media. Another culture-based method relies on detecting carbon dioxide produced by the enzymatic action of bacterial decarboxylases [17]. Other culture-based techniques involve growing LAB on a culture media followed by DNA extraction and sequencing to evaluate the genetic potential of these bacteria for the production of biogenic amines [39]. Culture-based processes are labor-intensive, time-consuming, and sometimes yield false-positive due to the development of alkali-based compounds or false-negative results due to the acid produced during fermentation along with biogenic amines, causing interference with the accuracy of toxin detection [40].

Compared to conventional culture-dependent methods, modern molecular methods (Table 2) for detecting bacteria containing decarboxylases are more rapid and reliable [40]. These methods enhance the early detection of biogenic amine-producing bacteria. Molecular techniques include DNA hybridization and polymerase chain reaction (PCR) [41]. DNA hybridization involves aligning nucleotide sequences to identify similar lines. Primers are then designed using these similar sequences to amplify a DNA fragment that is then used as a probe in a whole-cell dot-blot hybridization assay. However, this method also yields false results [12].

PCR is another molecular method that is faster and more sensitive than other methods. PCR facilitates specific detection of amino acid decarboxylase genes, which are amplified from biogenic amine-producing bacterial strains. Thus, PCR enables prior risk assessment of biogenic amine formation by designing oligonucleotide primers comparable to the nucleotide sequences in decarboxylase genes [12]. This method has been used to detect histidine decarboxylases (pyruvic-dependent and pyridoxal phosphate-dependent) and tyrosine decarboxylases. These molecular techniques have been modified to fit conditions for fermented food products, including cheese and sausages [41].

Recent advancements have enabled simultaneous detection of histamine, tyramine, and putrescine-producing LAB using multiplex PCR (mPCR) [17]. Successful implementation of real-time PCR (q-

Table 2. Analytical techniques with merits and demerits used for detection in fermented foods.

Analytical Technique	Fermented Food	Toxin/ Bacteria Detected	Merits	Demerits	Reference
Culture-dependent method	Stinky Tofu, Chinese traditional fermented food (grasshopper sub shrimp paste), Japanese sake, Chinese Luzhou-flavor liquor, Grape wine, Vietnamese alcoholic beverage	Biogenic amines	Investigation of microbial diversity at the species level	Laborious, Time-consuming, Culture media may not be suitable for every bacterium, False-positive results	[17,40]
HPLC	Cheese, Wine, Cider, Fermented fish sauce, Thai fermented pork	Biogenic amines	Reliable, sensitive quantification of toxins, commercially available	Tedious, Time-consuming	[25,48]
	Red, rose, and white wine, Beer, Cheese, Red yeast rice, Cereal-based ferments, Maize gruel (ogi), Sorghum gruel (ogi-baba), Melon seed (ogiri), Locust bean (iru), African oil bean seed (ugba)	Mycotoxins			[33,34,53,54,60]
TLC	Fermented seed condiments, Wine, Cider	<i>Bacillus</i> sp., Biogenic amines, Mycotoxins	Effective separation of toxins	Time-consuming	[82] [25,83,84] [53]
PCR	Wine, Chinese traditional fermented food grasshopper sub shrimp paste, Fermented locust bean, Fermented melon	Biogenic amine-producing bacteria, Mycotoxins	Convenient, Sensitive, High differentiation	Less sensitive compared to biosensors, False positive results	[40,85] [41,60]
	Alkaline fermented seed condiments (tayohounta, soumbala, Ogiri, Ugba), Doenjang, a Korean fermented soybean paste, Fermented fish (Pla Som and Pla Ra), Indian fermented pork and fish, Iru, ogi and ogi baba, Fermented beverages (mahewu and umqombothi)	<i>Bacillus</i> sp., <i>Enterobacter</i> sp., Endotoxins			[28,82]
Biosensors/ Nano sensors	Wine, Yogurt, Cheese, Roquefort, Monascus fermented food, soybean	Biogenic amines, Mycotoxins, Enterotoxins, Diarrheal toxins ( <i>Nhe</i> and <i>Hb1</i> ), <i>P. mirabilis</i> , <i>B. cereus</i>	Rapid, Latest technique, Highly sensitive and allows simultaneous detection of toxins	Not common at industrial scale	[33,35,36,46,53,60,64]

PCR) for quantitative estimation of histamine-producing LAB [42] and tyramine-producing strains [43] in cheese has also been documented. Putrescine is another common biogenic amine found in dairy products. It is synthesized when LAB deaminates agmatine, which is made from arginine in milk. A culture-independent multiplex qPCR technique based on the specific amplification of the particular domain of the agmatine deaminase gene cluster (AGDIc) has been developed for the detection, quantification, and identification of LAB capable of producing putrescine from agmatine [44].

The cider samples were examined using qPCR methods designed for the quantification of biogenic amine producers from cheese and wine. A strong correlation was found between the amount of biogenic amine and the presence of microorganisms that produce biogenic amine [45]. However, PCR may also yield false-positive results if the selected genes have been taken from the database in which the function is assigned by homology but not by function. False-positive results may also occur if any biogenic amine-producing microorganism has not yet identified and described. Other detection methods include enzymatic assays that are exclusively histamine-specific. The most commonly used assay uses the formation of hydrogen peroxide in response to the enzymatic activity of oxidase on histamine. However, this method also yields false positives because other cultures also produce hydrogen peroxide exclusive to histamine. Another form of the enzymatic assay using chromogen is leucocrystal violet. This method effectively screens out histamine-producing bacterial strains but does not provide the concentration of histamine produced because of culture media interference. This drawback has been rectified by filtering the culture medium and replacing leucocrystal violet with chromogen 3,3-Diaminobenzidine, which paved the way for quantifying histamine produced by bacterial strains [17].

Novel methods of detecting biogenic amines in fermented foods use biosensors and nano-sensors [46] (Fig. 2). Enzyme sensors are also used to detect biogenic amines in fermented foods. Sensors have been used to detect the biogenic amines histamine, tyramine, and putrescine in food products [46]. Recently, enzyme-based sensors have been investigated for sensitivity to the detection of biogenic amines in foods. These enzyme-based biosensors work by detecting signals generated due to changes in physicochemical properties after the interaction of specific amine oxidases with biogenic amines. Changes in pH, heat generation, and gas production are detected by the biosensors. Another type of

biosensor is based on the detection of light emitted due to interaction between the enzyme and an analyte. The major biosensors for biogenic amine detection are enzyme-based electrochemical biosensors. These enzyme sensors act swiftly and can detect biogenic amines in food within 20 min [46]. A novel fluorescence sensing method for tyramine detection has also been developed that integrates molecular imprinting and the Quantum Dot-Graphene technique for tyramine detection. This method has been used for cost-effective and rapid tyramine detection in rice wine with a high sensitivity for tyramine [47].

Chromatographic techniques are also used to detect biogenic amines. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are the most commonly used [17]. TLC works by separating biogenic amines from samples in the form of spots on layers of silica gel/cellulose attached to glass plates or aluminum sheets. The highly polar nature of amines can alter the chromatographic resolution by forming streaks. The amines are, therefore, first converted into derivatives using dansyl chloride. The relatively stable sulphonamide derivatives improve fluorescence and provide satisfactory chromatographic results [17]. The biogenic contents of the Japanese soy-based fermented food natto and the Italian blue cheese gorgonzola were successfully detected using HPLC with fluorescence detection [48].

The most popular chromatographic technique for detecting biogenic amines in food is HPLC (Fig. 3). HPLC was used to measure the variety and amount of biogenic amines in fermented milk from cows and goats [49]. The ultra-high performance liquid chromatography (UHPLC) technique had also been used to determine biogenic amines in a variety of cheese samples. Particularly, high-resolution mass spectrometry and UHPLC using columns packed with sub-2  $\mu$ m tiny particles have provided significant opportunities to improve accuracy, sensitivity, and quickness of biogenic amine measurement in food samples [50]. This method uses pre- and post-column derivatization and then uses fluorescence or UV (ultraviolet) radiation for detection. Different derivatization reagents are used for the pre and post-columns, including dansyl chloride, benzoyl chloride, fluoresceine, 9-fluorenylmethyl chloroformate, ophthalaldehyde, naphthalene-2,3-carboxaldehyde, n-acetylcysteine, and 2-mercaptoethanol [17]. Reversed-phase-HPLC, multichannel UV detection, post-column derivatization, and fluorescence detection have been used to identify biogenic amines in fermented sausages and meat [17]. Douchi, sufu, fermented sausage,



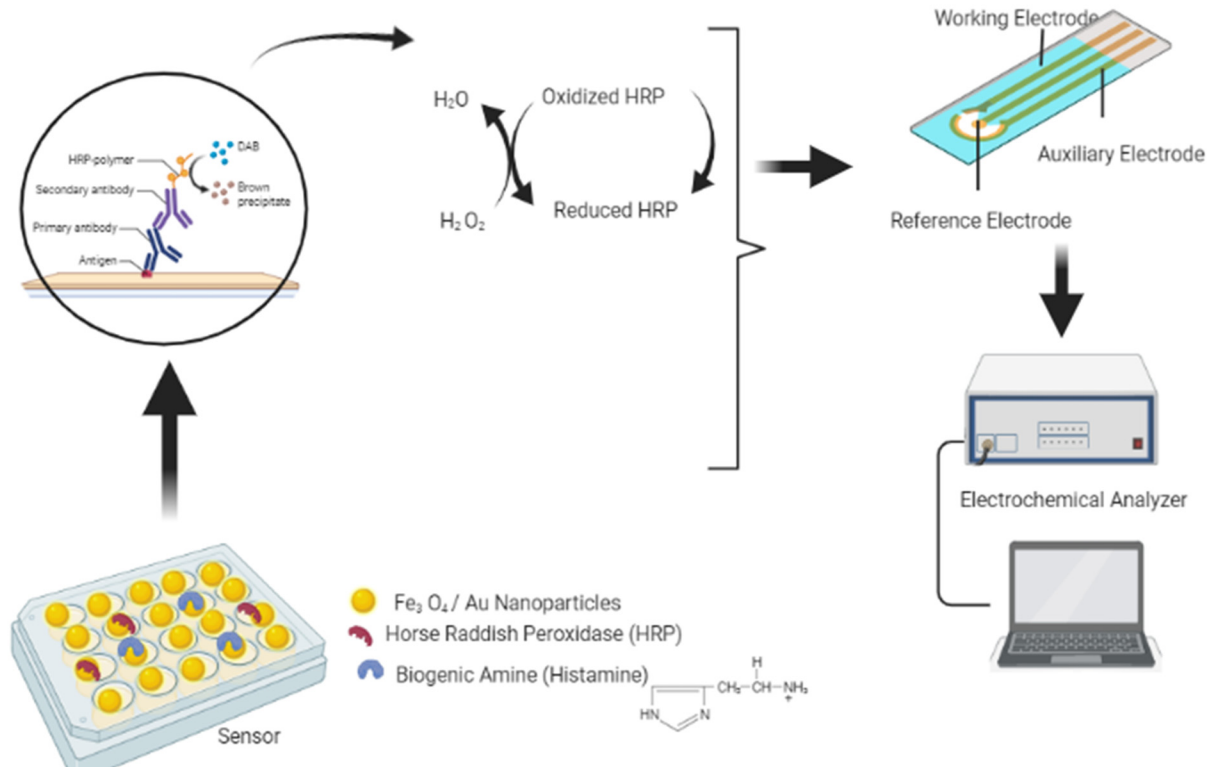


Fig. 2. Mechanism of electrochemical detection of biogenic enzymes using nanosensors: the shift in the redox potential of the HRP enzyme after reaction with biogenic amines is detected by an electrode attached to the electrochemical analyzer, which displays the electrical signals indicating the presence of biogenic amines in fermented foods.

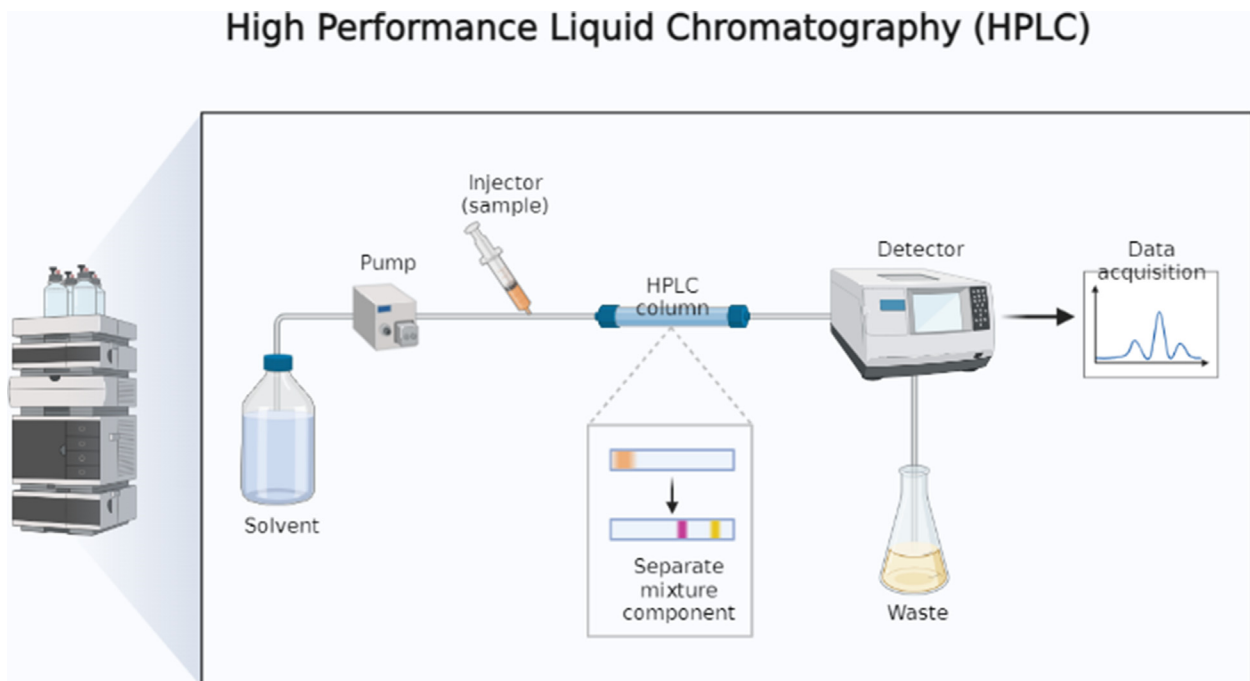


Fig. 3. Mechanism of high-performance liquid chromatography (HPLC).

yulu, and shrimp paste are few examples of traditional Chinese fermented dishes. The biogenic amine content of these food had also been determined using reversed-phase HPLC-DAD using an Inertsil ODS-SP column after pre-column derivatization with dansyl chloride [51]. HPLC is the official reference method for the detection of histamine (Regulation EC No 2073/2005, microbiological criteria for foodstuffs) [17]. There is potential with available analytical methods for biogenic amine detection in fermented foods. Each method has merits and demerits. However, the latest molecular techniques using nanosensors have shown better reliability and less time [46].

### 3.2. Techniques for mycotoxins detection

Different methods of mycotoxin detection have been investigated for efficacy and accuracy (Table 2). These include the chromatographic techniques of gas chromatography (GC), HPLC, TLC, and immunological assays coupled with immunosensors [52].

One method for mycotoxin detection in fermented foods is HPLC. It is often the standard method for mycotoxin detection at the industrial level, and numerous protocols are in place for the detection of mycotoxins, particularly for the estimation of ochratoxin A amounts [53]. HPLC is often coupled with UV and fluorescence detectors (FD) for the detection of mycotoxins, particularly aflatoxins. Also, the use of 2.6  $\mu\text{m}$  core-shell particles in the chromatography column has improved the analytical performance of HPLC. The use of HPLC coupled with tandem mass spectrometry (LC-MS/MS) has been used for a more sensitive and specific screening of mycotoxins in food samples [54]. Utilizing liquid chromatography-tandem mass spectrometry, twenty-three mycotoxins, including aflatoxin B1 (AFB1), fumonisin B1 (FB1), and sterigmatocystin (STE) have been estimated in fermented food samples from Southwest Nigeria, including maize gruel (ogi), sorghum gruel (ogibaba), melon seed (ogiri), locust bean (iru) [55].

UHPLC (ultrahigh-performance liquid chromatography) is another technique for mycotoxin detection. It is a solid-phase extraction procedure used for the separation of particles. Mycotoxins are then detected using multiple reaction modeling (MRM) in positive electrospray ionization mode. Multiple mycotoxins can be detected using this method, including aflatoxins B1, B2, G1, G2, ochratoxin A, fumonisins B1, and B2, zearalenone, deoxynivalenol, T-2 toxin, and HT-2 toxin. This method has been used in the brewing industry for mycotoxin analysis of wine and beer [54]. *Fusarium*

mycotoxins in beers and spices have also been quantified using this technique in Nigeria [56].

TLC is also a common technique for detecting mycotoxins, especially aflatoxins. Modifications in TLC have improved its efficacy, compared to HPLC with 5000 theoretical plates for a 5 cm migration in high-performance TLC (HPTLC) compared with 5000 for a 20 cm long HPLC column. The method uses formation of plates with an adsorption material, such as silica gel, followed by a volatile solvent for spotting purposes. Mycotoxins are then quantified using fluorescence, color, or UV adsorption. The TLC method can be efficiently used for screening purposes by ruling out negative samples. Multi-toxin detection is also possible by applying extracts to different TLC plates and grouping the mycotoxins according to chromatographic properties. This method can be applied in the detection of aflatoxins, ochratoxin A, citrinin, sterigmatocystin, zearalenone, trichothecenes, patulin, and penicillic acid [52]. Mycotoxigenicity of Spanish fermented meat sausage has been investigated by TLC [57].

GC can also be used for mycotoxin detection in food products when the mycotoxins are converted to volatile compounds. The GC system is linked to an MS (mass spectrophotometry) flame ionization detector (FID) or Fourier transform infrared spectroscopy (FTIR) to detect volatile mycotoxins [53]. GC-MS is a suitable method for detection of mycotoxins in aromatic fermented foods, such as Chinese horse bean chilli paste [58].

An enzyme-linked immunosorbent assay (ELISA) is another method for mycotoxin detection. The citrinin level in *monascus* (red yeast rice) has been detected using ELISA [59]. In contrast to conventional ELISA, the latest immunoassays use biosensors or immunosensors. These are broadly classified into labeled and label-free. Labeled immune sensors working on the principle of competitive immunoassay involve a sample analyte competing with a conjugated or labeled analyte for attachment to antibodies. On the contrary, in labeled non-competitive (sandwich) immunoassay, secondary antibodies generate a signal when an antigen is captured. The sandwich-type labeled assay is commonly used for aflatoxin B1 estimation. Alternatively, in the label-free immunosensor, non-competitive method, the transducer reads the interaction between the analyte and immobilized antibodies [60]. However, this method gives false-positive results due to nonspecific binding. Instead of immune sensors, mycotoxins can also be detected using aptasensors that involve the use of aptamers, which are synthetic oligonucleotide ligands (either single-stranded DNA or RNA) containing 10–50

variable bases. These aptamers have high specificity, stability, and cost-effectiveness and can, thus, be valuable replacements for antibodies [60]. Ochratoxin A was detected in wine using enzyme-linked aptamer assays (ELAAS) based on a competition format [61]. All of these methods can be used for mycotoxin detection in fermented foods. However, the TLC, HPLC, and immunosensor methods offer significant reliability for industrial applications.

### 3.3. Techniques for bacterial toxins detection

Considering the risk of foodborne illnesses associated with bacterial contamination, use of highly sensitive analytical techniques for detection is mandatory. Culture-based techniques facilitate detection of bacterial strains, while some methods are explicitly focused on detection of bacterial toxins. Detection of endotoxins of Gram-negative bacteria by chromogenic limulus amoebocyte lysate (LAL) assay has been performed [28]. This technique uses the LAL test kit that contains a standard endotoxin, the chromogenic substrate LAL, and endotoxin-free water used as a blank. A microplate reader (wavelength set at 405 nm) is used to measure the absorbance of the sample mixture. The endotoxin concentration is determined using a standard curve for a known concentration of endotoxin standards in endotoxin-free water [28]. Microbiological quality of fermented foods (ogiri, ugba, iru, ogi and ogi baba) and beverages (mahewu and umqombothi) from selected Nigerian and South African markets has been evaluated using LAL kits [62].

For phenotypic detection of toxic shock syndrome toxin (TSST-1) produced by coagulase-negative *Staphylococci*, a reversed passive latex agglutination (RPLA) test kit has been used [63]. This technique has also been used to detect hemolysin BL (*Hbl*) enterotoxin produced by *B. cereus* [36]. Using this method, a positive result is indicated by the formation of a lattice structure (agglutination) after adding a test sample to the latex suspension of each super-antigenic toxin control [63]. A *Bacillus* diarrheal enterotoxin visual immunoassay (BDEVIA) toxin detection kit has also been used specifically for the detection of the non-hemolytic (*Nhe*) enterotoxin complex [36]. *B. cereus* enterotoxins from doenjang, a Korean fermented soybean paste was analyzed using BDEVIA and RPLA kits [64].

Novel approaches for bacterial toxin detection also aim at the identification of genes responsible for toxin production. Such techniques help in the preliminary detection of the toxigenic potential of

bacterial strains. Toxin-producing genes are identified using mPCR [65]. This method has been successfully used for the detection of the four toxin-producing genes *hbl*, *nhe*, *cytK2*, and *cesB* in *B. cereus* [66]. The genes responsible for TSST-1 (*sea*, *seb*, and *sec*) production have also been detected using this method [63]. The mPCR technique requires DNA extraction, followed by a comparison with specific primers for gene detection. Also, modification of mPCR has allowed simultaneous amplification of all the toxin genes. The nucleotide sequences are then compared with the GenBank database to identify the specific toxin genes [63].

The PCR technique has been used to detect bacterial strains in fermented foods. *B. cereus*, *P. mirabilis*, and *C. botulinum* were isolated from indigenous fermented soybean, fish, and pork foods in India using qPCR (quantitative PCR) (Table 2). The diarrheal toxins (*Nhe* and *Hbl*) and enteric toxins (cereulide) were also detected in soybean samples through qPCR and immunoassay [35].

In contrast to conventional labor-intensive culture-based methods, modern molecular methods using PCR offer rapid, convenient, and early estimation of the food safety risks associated with foodborne pathogenic bacteria. Also, PCR has higher sensitivity and specificity, thus, producing reliable and accurate results [63].

## 4. Recent strategies for minimizing toxins in fermented foods

Apart from early detection of toxins during the production stage and application of good manufacturing practices (GMPs), other methods have been implemented to reduce amount of toxins in fermented foods. One recent approach is bio-preservation, which includes the use of an effective starter culture to improve microbial control of toxins in fermented foods [9]. For instance, heat-treated *Bacillus subtilis* (a GRAS bacterium), isolated from kimchi has been investigated as a biocontrol agent to reduce ochratoxin A levels in fermented foods [67]. This bacterium disrupted the hyphae of the toxin-producing species *A. ochraceus* and *A. carbonarius*. *B. subtilis* also produced lipopeptides (surfactins, iturins, and fengycins) that causes the degradation of ochratoxin A [67]. Miso prepared using *L. plantarum* as a starter culture had a reduced biogenic amine content and produced KL-1, a potent bacteriocin that inhibits the growth of the amine-producing microorganisms *Latilactobacillus sakei*, *Leuconostoc mesenteroides*, and *E. faecalis* [68].

Amine-negative *Pediococcus pentosaceus* strains isolated from cheonggukjang (Korean soybean

fermented food) and fermented fish sausage can be used as a starter culture because these have been effective in reducing histamine and tyramine contents in soy-based ferments by 14.7–23.7% and 15.7–25.9%, respectively. These strains prevent the development of biogenic amine-producing bacteria and, thus, inhibit formation of biogenic amines in fermented sausages and soybean [68]. *B. subtilis* and *Bacillus amyloliquefaciens* isolated from the Korean soybean fermented foods doenjang, cheonggukjang, and gochujang also showed biogenic amine degradation potentials as these taxa contain the enzyme amino-oxidase that catalyzes deamination biogenic amines [15]. Nine amine oxidases have been identified in *L. plantarum*, CAU 3823 strain, which can degrade 40% of the biogenic amines in Chinese rice wine at the end of fermentation process [69]. Some specific *L. paracasei* strains can also lower the levels of histamine and tyramine in cheese [70].

*Saccharomyces cerevisiae* was reported to be an effective aflatoxin binder in dairy-based fermented products, such as yogurt. The aflatoxin adheres to the mannan bacterial cell wall component, which is a bioactive glycoprotein that binds aflatoxin [71].

Specific starter cultures are preferred for indigenous preparation of fermented foods that are at high risk for toxin contamination. These starter cultures exhibit a broad range of function as bio-preservatives, aflatoxin-binders, and degraders of biogenic amines. Further research is required regarding the choice of starter cultures to eliminate the risk of toxin formation in fermented foods. Also, the latest technologies are now targeting preparation of genetically designed starter cultures [68].

## 5. Conclusion and future perspectives

Biogenic amines, mycotoxins, and bacterial toxins are a significant hazard in fermented foods. Food-borne illnesses due to toxins have been documented in fermented foods and beverages, including biogenic amines (particularly, histamine and tyramine) in cheeses, soy foods, and wine, mycotoxins in cereal-based fermented foods and alcoholic beverages, and bacterial toxins in sausages and sorghum-based fermented foods. To combat the risk of toxins associated with fermented foods, it is important to apply the latest detection techniques at the industrial level. The conventional, labor-intensive, and time-consuming culture-based techniques that have been used for the estimation of toxins in fermented foods sometimes yield false-positive results. Modern analytical techniques have been investigated for sensitivity and specificity in detection of toxins in fermented foods, including

chromatography, PCR, and immunoassays linked with biosensors and nano-sensors. The chromatographic techniques of HPLC and TLC are mostly used at industrial scales. A safer and more desirable fermentation technique uses specially designed starter cultures at an industrial scale to produce amine degrading enzymes, aflatoxin binding proteins, and bacteriocins for bio-preservation. Owing to the growing demand for fermented foods, it is imperative that the latest techniques for toxin detection are industrialized, GMPs are implemented, proper storage conditions are maintained, and the choice of a starter culture is made wisely.

## Conflict of interest

The authors declare no conflict of interest.

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