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Mycotoxins in soybean-based foods fermented with filamentous fungi: Occurrence and preventive strategies

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Abstract

Fermented soybean products are widely consumed worldwide, and their popularity is increasing. Filamentous fungi, such as *Actinomucor*, *Aspergillus*, *Monascus*, *Mucor*, *Penicillium*, *Rhizopus*, and *Zymomonas*, play critical roles in the fermentation processes of many soybean foods. However, besides producing essential enzymes for food fermentation, filamentous fungi can release undesirable or even toxic metabolites into the food. Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi and may be detected during the food production process. Without effective prevention strategies, mycotoxin contamination in fermented soybean products poses a risk to human health. This review focused on the changes in mycotoxigenic fungal abundance and mycotoxin contamination at different stages during the production of soybean-based fermented foods, as well as effective strategies for preventing mycotoxin contamination in such products. Data from relevant studies demonstrated a tendency of change in the genera of mycotoxigenic fungi and types of mycotoxins (aflatoxins, alternariol, alternariol monomethyl ether, deoxynivalenol, fumonisins, ochratoxin A, rhizoxins, T-2 toxin, and zearalenone) present in the raw materials and the middle and final products. The applicability of traditional chemical and physical mitigation strategies and novel eco-friendly biocontrol approaches to prevent mycotoxin contamination in soybean-based fermented foods were discussed. The present review highlights the risks of mycotoxin contamination during the production of fermented soybean products and recommends promising strategies for eliminating mycotoxin contamination risk in soybean-based fermented foods.

KEYWORDS
fermented food, filamentous fungi, mycotoxin, prevention strategy, soybean

1 | INTRODUCTION

Fermented foods are produced through desired microbial growth and enzymatic conversions of food components and have remained a staple of human food for centuries (Marco et al., 2021). In the last two decades, interest in the beneficial effects of fermented foods on human health has increased and made fermented foods an increasingly popular food category. Reportedly, over 5000 varieties of fermented foods are currently produced and consumed worldwide (Tamang et al., 2016).

Fermented soybean products have been an important part of the human diet in Asian countries since ancient times and are recently gaining popularity in Western countries (Qin et al., 2022). Soybean-based fermented foods are consumed by hundreds of millions of people every day. For example, soy sauce is one of the most important condiments in Asian countries, where it is consumed in most families regularly. The annual production of soy sauce in the world was reported at 10 million metric tons (Hoang et al., 2016). In China, more than 50% of the households in the major cities, such as Shanghai, Guangzhou, and Wuhan, normally possess more than two bottles of soy sauce, and the daily soy sauce intake among Chinese adults was reported at 8.2 g/day (Yu et al., 2018). The soy sauce market has been prosperous with a market size of approximately 150 billion dollars in 2019, and in recent years, the soy sauce consumption in the global market has been increasing (Devanthi & Gkatzionis, 2019; Jin et al., 2022). In Korea, soy sauce (ganjang) and soybean paste (doenjang) are consumed nationwide as essential seasoning materials to flavor and characterize traditional Korean cuisine (Kim et al., 2020). The annual production of soy sauce and soybean paste in Korea is about 304,511 tons and 89,822 tons, respectively (The Ministry of Agriculture, Food and Rural Affairs and the Korea Agro-Fisheries & Food Trade Corporation, 2021a, 2021b), and the daily intake of soy sauce and soybean paste by Koreans is 6.1 g/day and 4.2 g/day, respectively (Korea Centers for Disease Control & Prevention, 2020). On the other hand, the production volume of soybean paste (miso) and soy sauce (shoyu) in Japan amounted to around 482,000 metric tons and 744,000 metric tons in 2019, respectively (Allwood et al., 2021). Over the last two decades, fermented soybean products have attracted worldwide attention for their beneficial health effects and related bioactive contents in soy isoflavones, including daidzin, genistin, and glycitin, including their aglycone forms (Gupta et al., 2015).

Through fermentation, the functional properties of the raw materials are improved, and soybeans are transformed into tasty, nourishing, and less toxic food products. Fermentation could also improve food tolerability and reduce the glycemic index by reducing certain sugars in the raw materials (Nyyssölä et al., 2020). Enzymatic transformations occurring during the fermentation process might also detoxify toxic components and remove antinutritive factors in soybeans, such as trypsin inhibitors (Aviles-Gaxiola et al., 2018). Beyond these benefits, fermented soybean products are also helpful in building resistance to diseases, such as cardiovascular ailments, cancers, and osteoporosis (Martinez-Gonzalez et al., 2019).

Similar to other fermented foods, the flavor, taste, texture, and functional properties of fermented soybean products are defined by the fermentation strain (monoculture fermentation) or the interplay of microbial communities (multicultural fermentation) and their complex metabolites (Singh et al., 2017). Bacteria, filamentous fungi, and yeasts are the major groups of microorganisms associated with soybean fermentation. In Asian countries, filamentous fungi, such as *Actinomucor*, *Aspergillus*, *Monascus*, *Mucor*, *Penicillium*, *Rhizopus*, and *Zymomonas*, are the predominant organisms used in fermentation processes (Table 1).

During the fermentation process, filamentous fungi produce enzymes such as α-amylase, amyloglucosidase, cellulase, β-galactosidase, hemicellulase, invertase, lipase, maltase, pectinase, acid and alkaline proteases, and degradative antinutritive factors, thus, improving the bioavailability of nutrients and minerals (Tamang & Kailasapathy, 2010). Soybeans fermented using filamentous fungi also exhibit increased phenolic content and enhanced radical scavenging activity (Ghanem et al., 2020). However, some undesirable or even toxic metabolites of filamentous fungi might be released into the food during fermentation.

Mycotoxins are toxic secondary metabolites produced by filamentous fungi from various genera such as *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium*, which can contaminate foods and cause serious and acute toxic effects to human health (Lee & Ryu, 2017; Marroquín-Cardona et al., 2014). Mycotoxins have variable molecular structures. It is estimated that about 300 to 20,000 different kinds of mycotoxins exist in nature, and among them, aflatoxins (AFs), deoxynivalenol (DON), fumonisins (FBs), ochratoxins, and zearalenone (ZEA) are the most commonly found mycotoxins that contaminate foods throughout the world (Lee & Ryu, 2017). Consuming foods that are contaminated with mycotoxins above the safety levels can have severe acute and chronic toxic effects on human body such as carcinogenicity, genotoxicity, mutagenicity, organ toxicity, and teratogenicity (Yang et al., 2020b). Hence, public health and governmental authorities around the world, such as the European Food Safety Authority (EFSA), Food and Agriculture Organization (FAO), US Food

TABLE 1 Soybean foods fermented using filamentous fungi

Fermented soybean products	Functional filamentous fungal species	Reported health benefits	Country/region	Reference
Fermented whole soybean				
Douchi	*Aspergillus* spp., *Mucor* spp., *Rhizopus* spp.	Antidiabetic, antihypertensive, and antioxidative	China	Chen et al. (2015)
Tempeh	*Aspergillus* spp., *Mucor* spp., *Rhizopus* spp.	Anticancer, antidiabetes, antimicrobial, antiobesity, antioxidant, and improved gut health	Indonesia	Ahnan-Winarno et al. (2021)
Fermented soy curd				
Furu (or sufu)	*Actinomucor* spp., *Mucor* spp., *Rhizopus* spp.	Antioxidative, hypocholesterolemic, antihypertensive, and antimutagenic	China, Southeast Asia	Han et al. (2001)
Tofuyo	*Aspergillus oryzae* or *Monascus purpureus*	Antihypertensive, antioxidative, and hypocholesterolemic	Japan	Yasuda et al. (2012)
Fermented soy block				
Meju	*Aspergillus* spp., *Botrytis* spp., *Mucor* spp., *Rhizopus* spp.	NA	Korea	Ryu et al. (2021)
Fermented soy paste:				
Doenjang (or toenjang)	*Aspergillus* spp., *Mucor* spp., *Rhizopus* spp.	Anticancer, antidiabetic, anti-inflammatory, antimutagenic, and antiobesity	Korea	Mun et al. (2019)
Doujiang (or chiang)	*Aspergillus* spp., *Mucor* spp., *Rhizopus* spp.	Anticancer, antihypertensive, antioxidative, and cholesterol lowering	China	An et al. (2021)
Miso	*Aspergillus oryzae*	Anticancer and antimutagenic	Japan	Allwood et al. (2021)
Tauco (or taoco)	*Aspergillus oryzae*, *Rhizopus oligosporus*, and *Rhizopus oryzae*	Antioxidative	Indonesia	Nandiyanto et al. (2018)
Fermented soy pulp				
Meitauza	*Actinomucor* spp., *Zymomonas* spp.	Antioxidative	China	Xu et al. (2012)
Okara koji	*Aspergillus oryzae*	Antioxidative	Japan	Matsuo (1997)
Okara tempeh	*Rhizopus oligosporus*	Antioxidative and cholesterol-lowering effect	Indonesia	Matsuo and Hitomi (1993)
Fermented soy sauce				
Chiang-yu	*Aspergillus oryzae*	Antioxidative	China	Gao et al. (2019)
Kanjang (or ganjang)	*Aspergillus* spp., *Penicillium* spp.	Anticancer, antidiabetic, antihypertension, and antimutagenic	Korea	Han et al. (2020)
Shoyu	*Aspergillus oryzae*	Anticancer, antihypertensive, antimicrobial, and enhanced gastric juice secretion	Japan	Kataoka (2005)

and Drug Administration (FDA), and the World Health Organization (WHO), are paying serious attention to mycotoxin contamination, and most countries in the world have established limits on the presence of major mycotoxins in foods (Alshannaq & Yu, 2017; Lee & Ryu, 2017). For example, the European Commission has set the maximum levels of AFs, DON, FBs, ochratoxin A (OTA), and ZEA in major food grains at 4–15, 50–200, 200–1000, 2–10, and 20–100 ppb, respectively. The US FDA set the regulatory limits for AFs, DON, and FBs in major food grains at 20, 1000, and 2000–4000, respectively. In particular, Asian countries have set the maximum levels for major mycotoxins specifically for soybean-based fermented foods. For example, the State Food and Drug Administration of China has set the regulatory limits for both AFs and OTA in fermented soybean products at 5 ppb. The Korea Food and Drug Administration sets the regulatory limits for AFs and OTA in fermented soy block (meju) at 15 and 20 ppb, respectively.

Mycotoxigenic fungi may be present in or on the ingredients, utensils, containers, and manufacturing or storage environments during the production of fermented foods. Although many food fermentation processes have been scaled up to industrial levels, most types of fermented soybean products are still manufactured at a small scale, with relatively simple processing facilities under variable levels of hygiene. Thus, such products can be easily contaminated by mycotoxigenic fungi due to the lack of sterility and the use of spontaneous fermentation or fermentation starters with poor quality. Mycotoxin contamination has been reported in many fermented foods, including fermented soybean products (Sivamaruthi et al., 2019). Considering the popularity of soybean-based fermented products, the regulations available on appropriate mycotoxin contents in fermented foods are currently inadequate. Without proper preventive measures, mycotoxin contamination in fermented soybean products may exceed a safe level and can lead to serious health issues among consumers. To ensure the consumption safety of fermented soybean products, it is vital to identify the causes of mycotoxin contamination along with its prevention strategies, and a comprehensive review of the subject area is necessary.

2 | FUNGAL AND MYCOTOXIN CONTAMINATION RISKS IN TRADITIONAL MANUFACTURING PROCESSES

Fermentation is originally a method for food preservation that transforms food ingredients into microbiologically stable products, which can be safer to eat due to the prevention of contamination by food spoilage and foodborne pathogens as well as mycotoxigenic fungi. When properly controlled, microbes associated with food fermentation tend to outcompete these harmful pathogens (Adams & Mitchell, 2002). However, poorly controlled or spontaneous fermentation using autochthonous microorganisms without careful understanding and control of the processes may result in mycotoxigenic fungal and mycotoxin contamination. In particular, during traditional fermentation processes, unsterilized or minimally pretreated soybean and other adjuncts must be exposed to the environment for a long period, making mycotoxin contamination nearly unavoidable.

Based on the shapes and textures of the final products, traditional fermented soybean foods can be classified into fermented whole soybean (douche and tempeh), fermented soy curd (furu/sufu and tofuyo), fermented soy block (meju), fermented soy paste (doenjang/toenjang, doujiang/chiang, miso, and tauco/taoco), fermented soy pulp (meitauza, okara koji, and okara tempeh), and fermented soy sauce (chiang-yu, kanjang/ganjang, and shoyu) (Table 1). Traditionally, the manufacture of soybean-based fermented foods included the preparation of soybeans, the inoculation of functional strains, and the fermentation (Figures 1 and 2).

During the preparation of soybeans, soaking is an essential step in as it can increase the moisture content in the soybeans. The soaking process can also reportedly remove antimicrobial substances and bitter components naturally occurring in the soybean seeds (Silva et al., 2020). This step enables microbial activity in the subsequent fermentation process and makes soybean susceptible to microbial contamination. Therefore, storing or processing soaked soybeans requires extra caution to minimize potential contamination by undesired microbes. Cooking is another procedure necessary for fermented soy pastes, soy sauces, as well as douchi (fermented whole soybean). In the cooking step, soaked soybeans are boiled or steamed at approximately 100°C for a period varying from 10 to 120 min, depending on the final product desired, or appliance used. The cooking step can inactivate potential microbial contaminants; however, cooking may have a minimal effect on the detoxification of mycotoxins. Most mycotoxins are heat stable, which facilitates their survival through conventional cooking processes, and can adversely affect human health (Wu et al., 2020). In addition, soybeans have been suggested to contain heat-labile antifungal components (Cleveland et al., 2009; Silva et al., 2020). Heating processes could further reduce the defense capacity of soybean against fungal infection. Therefore, after the heating processes, the hot soybeans need to be dried to remove free water and be cooled down as soon as possible to minimize microbial spoilage risk.

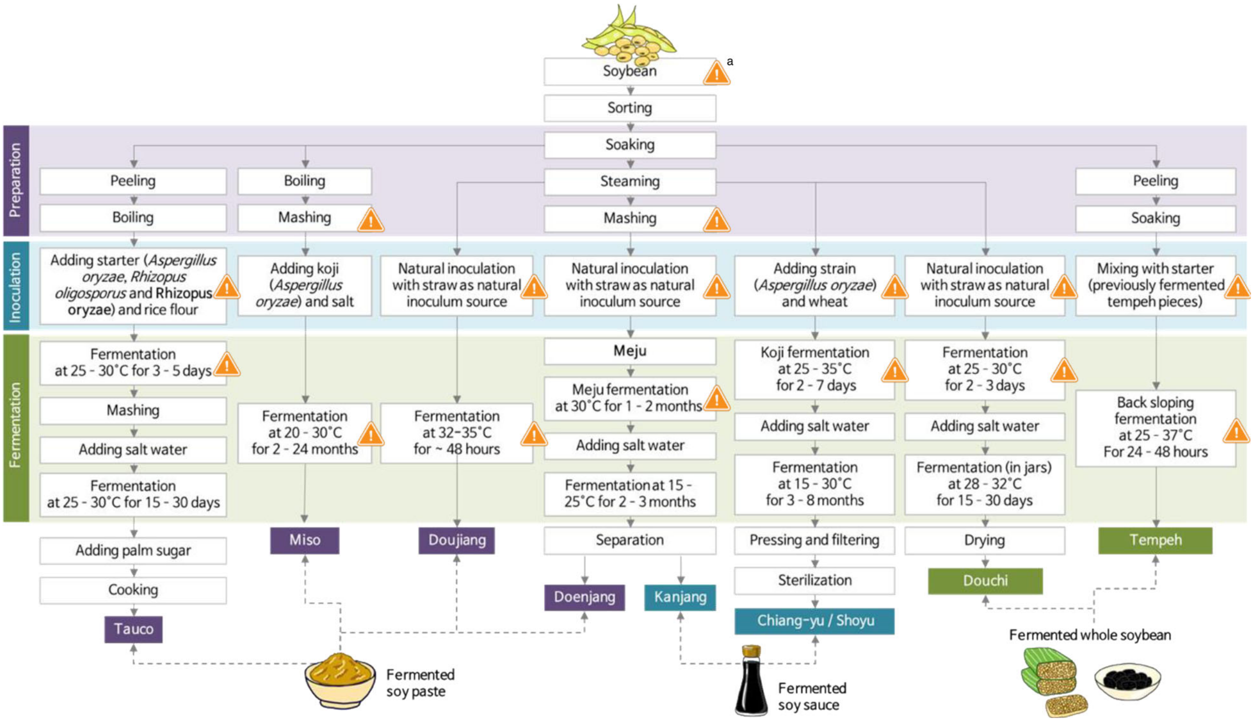

FIGURE 1 Flowchart of traditional fermented soy paste, soy sauce, and whole soybean production, and potential risk points of mycotoxin contamination. [a]Potential risk points of mycotoxin contamination

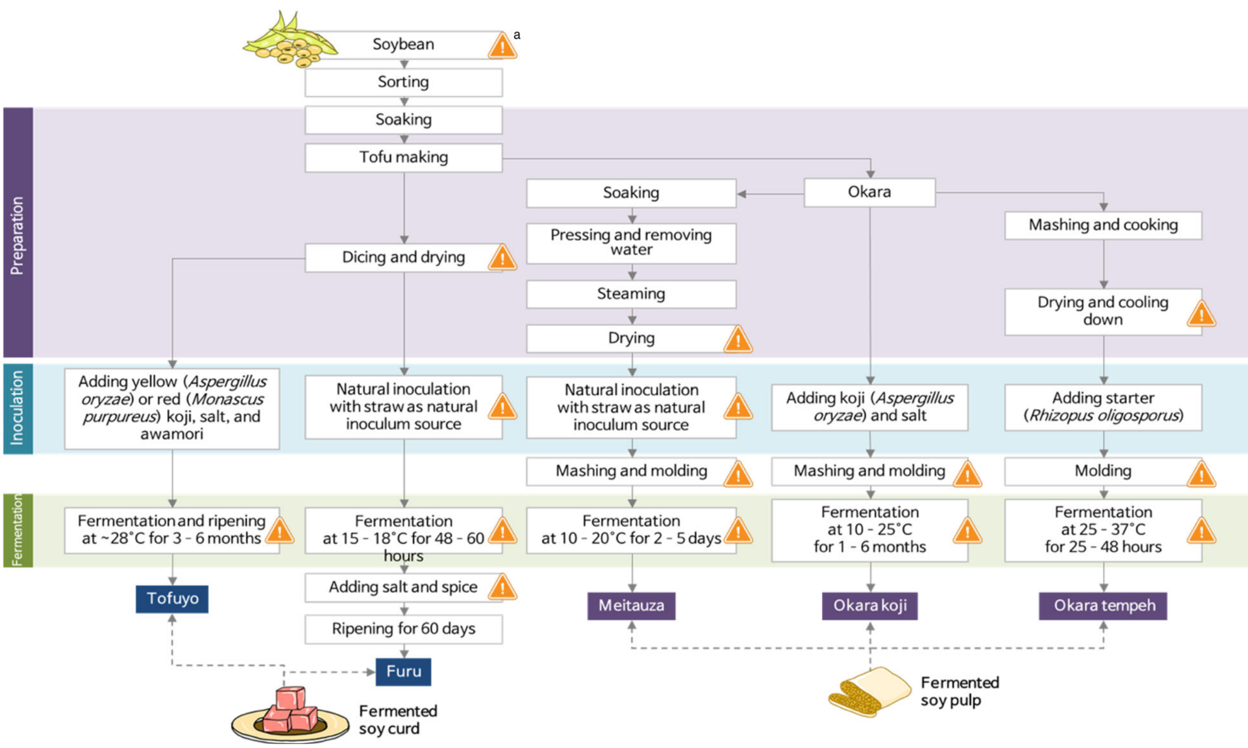

FIGURE 2 Flowchart of traditional fermented soy curd and soy pulp production and potential risk points of mycotoxin contamination. [a]Potential risk points of mycotoxin contamination

The soybean preparation process for fermented soy curds and soy pulps is unique (Figure 2). After soaking, soybeans are blended with water, and the soymilk and okara (soy pulp) are separated through filtration. Okara can be used to produce fermented soy pulp. Tofu (soybean curd) is prepared from soymilk by adding acid or calcium salts to precipitate the soy protein. After pressing, dicing, and drying, tofu blocks are used to produce fermented soy curd. When compared with the cases in other fermented soybean products, mycotoxin contamination in fermented soy curds and soy pulps is extremely rare, with limited reports in the literature. This may be partially attributed to the different soybean preparation processes applied.

Depending on the type of fermenting microorganism adopted, the fermentation can be classified into spontaneous (natural), back-slopping, or controlled fermentation (Mannaa et al., 2021). Spontaneous fermentation primarily uses indigenous natural microorganisms on the food substrate. Covering the prepared soybean substrate with wheat or rice straw is the traditional way of inoculating the substrate with fermentation strains. Back-slopping uses a small portion of a previously successful fermentation product to generate starter cultures for future fermentations. Controlled fermentation with selected starter culture uses defined strains in concentrated form. In food fermentation (cheese, sour cream, beer, sourdough bread, notto, and tempeh), natural fermentation or back-slopping methods have been reported to maintain a healthy gut microbiome due to microbiota diversity (Whittington et al., 2019). However, compared to the controlled fermentation method that uses a selected starter, such methods have issues with quality maintenance and safety since it is difficult to monitor harmful, spoilage-related microorganisms (Mannaa et al., 2021). In particular, there was no study on the effect of the back-slopping method on the growth of mycotoxigenic fungi or mycotoxin content in fermented soybean foods using filamentous fungi.

The fermentation processes of soybean-based foods usually occur at ambient temperatures (10 to 37°C) that favor the growth of fermentation strains and allow contamination by hazardous fungal species from the environment. Fermentation times of soybean-based foods vary from days to months according to the desired product, strains, and protocols used. The early stages of fermentation allow the functional molds to grow and produce their secondary metabolites, enhancing the defense against microbial spoilage during the later stages of the process. Consequently, functional molds are vital for the safety of the final products. During the fermentation process, the populations of filamentous fungi increase during the early stages. The flavor and contents of functional substances increase steadily in the middle stages of fermentation, mostly due to high functional species activity. In the later stages of fermentation, the products become microbiologically stable and can therefore be stored at room temperature for extended periods.

3 | OCCURRENCE OF MYCOTOXINS IN SOYBEAN-BASED FERMENTED FOODS

3.1 | Mycotoxigenic fungal and mycotoxin contamination in soybeans

Raw materials are one of the main sources of mycotoxin contamination in fermented soybean products. Fungal and mycotoxin contaminations are frequently detected in soybeans at both pre- and postharvest stages (Table 2). Fungi from the genera *Alternaria* and *Fusarium* are the most frequently detected contaminants in soybeans (Barros et al., 2011; Park et al., 1999).

Alternaria alternata is the most common *Alternaria* species found on soybean seeds, followed by *A. graminicola*, *Alternaria infectoria*, *Alternaria oregonensis*, and *Alternaria tritimaculans* (Barros et al., 2011). Some *Alternaria* species produce mycotoxins with toxicological significance, including alternariol (AOH), alternariol monomethyl ether (AME), altenuene, altertoxins I, II, and III, and tenuazonic acid, which have been identified as food contaminants with potential toxicological risks to humans (Pfeiffer et al., 2007). In recent years, several studies have investigated the toxicity of AOH and AME and found that both mycotoxins are cytotoxic, genotoxic, mutagenic, and carcinogenic (Brugger et al., 2006; Fehr et al., 2009; Yekeler et al., 2001). Both AOH and AME contaminate soybean seeds during the harvesting stage (Barros et al., 2011).

The *Fusarium* species most frequently detected in soybeans are *Fusarium equiseti*, *Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium semitectum*, and *Fusarium solani* (Park et al., 1999). Many strains of the *Fusarium* genus produce a broad spectrum of mycotoxins, such as trichothecenes, FBs, and ZEA. Among them, trichothecenes, represented by DON, diacetoxyscirpenol, nivalenol, and T-2 toxin (T2), primarily cause toxicity by inhibiting ribosomal protein synthesis and are most strongly associated with chronic and fatal toxicoses among humans (Desjardins & Proctor, 2007). FBs are a group of long-chain amino polyalcohols that inhibit sphingolipid metabolism in animal cells and cause acute renal and liver toxicity, carcinogenicity, and tumor-promoting activity. More than 50 FBs have been reported to date, with FB1, FB2, and FB3 being the most abundant in nature (Mogensen et al., 2009). ZEA is the most prevalent estrogenic mycotoxin. It disrupts the endogenous estrogenic response during the preovulatory stage and blocks the maturation of ovarian

TABLE 2 Mycotoxin levels in contaminated soybeans and fermented soybean products

Soybean products	Mycotoxins	References
Soybeans/soybean meal	Aflatoxins (AFs, 0.1 to 74 ppb) Alternariol (AOH, 25 to 211 ppb) Alternariol monomethyl ether (AME, 62 to 1153 ppb) Deoxynivalenol (DON, 50 to 5500 ppb) Fumonisins (FBs, 25 to 5088 ppb) Ochratoxin A (OTA, 0.2 to 46 ppb) T-2 toxin (T2, 12.2 to 23 ppb) Zearalenone (ZEA, 10 to 807 ppb)	Chilaka et al. (2019); Rodrigues and Naehrer (2012); Oviedo et al. (2012)
Fermented whole soybeans		
Douchi	AFs (0.43 to 31.1 ppb)	Qiu et al. (2019)
Tempeh	AFs (<0.1 ppb) FBs (170 to 2682 ppb) ZEA (8.34 to 24.75 ppb) Rhizoxins	Anggriawan (2017); Borzekowski et al. (2019); Rohm et al. (2010); Yudiono et al. (2021)
Fermented soy curd		
Furu (or sufu)	AFs (0.098 to 0.488 ppb) Rhizoxins	Rohm et al. (2010); Yang et al. (2020a)
Tofuyo	No record	NA
Fermented soy block		
Meju	AFs (0.2 and 48.3 ppb) OTA (0.1 and 193.2 ppb)	Jeong et al. (2019)
Fermented soy paste		
Doenjang (toenjang)	AFs (0.11 and 5.43 ppb) FBs (2.48 and 68.52 ppb) OTA (0.16 and 23.27 ppb) ZEA (4.67—95.08 ppb)	Woo et al. (2019)
Doujiang (or chiang)	AFB1 (<5 ppb)	Zhang et al. (2020)
Miso	No record	Tanaka et al. (2006)
Tauco (or taoco)	No record	NA
Fermented soy pulp		
Meitauza	No record	NA
Okara koji	No record	NA
Okara tempeh	No record	NA
Fermented soy sauce		
Chiang-yu	AFB1 (<1.7 ppb) DON (4.5 to 1245.6 ppb)	Sun et al. (2010); Zhao et al. (2013)
Kanjang (or ganjang)	AFB1 (<1.81 ppb) OTA (<1 ppb)	Lee et al. (2012); Park et al. (2004)
Shoyu	DON (30.5 and 238.3 ppb)	Zhao et al. (2013)

follicles by inhibiting the secretion and release of steroid hormones (Zhang et al., 2018). Moreover, different levels of DON, T2, FB, and ZEA contamination have been observed in soybeans (Barros et al., 2011; Garcia et al., 2016).

Other toxigenic fungal species, such as those from the genera *Aspergillus* and *Penicillium*, have also been isolated from freshly harvested soybeans, but their mycotoxins were often not detected directly (Garrido et al., 2013; Roy et al., 2001).

3.2 | Mycotoxigenic fungal and mycotoxin contamination due to storage and processing environments

Another important source of mycotoxigenic fungal contamination is the storage and processing environments. Mycotoxigenic fungi from contaminated soybeans in the field can be transferred easily to the storage environment through infected seeds and harvest debris. The level of mycotoxin contamination in food crops depends on environmental conditions, such as temperature and humidity. Thus, even though most mycotoxins detected at preharvest and harvest stages of soybeans are usually at safe levels, more serious contamination may occur at the storage stage because of improper storage conditions. Soybeans are resistant to the growth of fungi from the genera *Aspergillus*, especially at the preharvest stage. However, the growth of *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* and the production of AFs have been detected in stored soybeans and soybean meals (Barros et al., 2011; Zhang et al., 2018). AFs (AFB1, AFB2, AFG1, and AFG2) are a group of polyketide-derived secondary metabolites produced by *Aspergillus* spp., including *A. flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Lee et al., 2015). AFB1 is one of the most dangerous mycotoxins threatening food safety and human health. It has potent mutagenicity, teratogenicity, and carcinogenicity and has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC). OTA is another naturally occurring mycotoxin produced in stored soybeans. It is the most toxic member among the isomers of ochratoxins and has been confirmed to cause carcinogenic, nephrotoxic, neurotoxic, and teratogenic effects in humans (Pfohl-Leszkowicz & Manderville, 2007). OTA has been classified as a possible group 2B carcinogen by the IARC. It is produced by different fungal species from the genera *Aspergillus* and *Penicillium*, which have been frequently detected in stored soybeans and soybean meals (Bhattacharya & Raha, 2002; El Khoury & Atoui, 2010). Moreover, *Aspergillus* fungi present in crops after long-term storage and hull-less crops tended to show higher levels of mycotoxin contamination than in hulled crops during storage (Krulj et al., 2019).

3.3 | Mycotoxigenic fungal and mycotoxin contamination in the middle and final products

Most mycotoxins have high stability even at temperatures above 100 C. Conventional cooking, baking, roasting, and pasteurization can lead to no or only limited decomposition of mycotoxins in food grains (Campagnollo et al., 2016; Liu et al., 2020). However, these cooking methods may soften the soybean tissue and destroy the heat-labile antifungal components present in the soybean, which can increase the growth of mycotoxigenic fungi and the corresponding mycotoxin production in the subsequent process (Cleveland et al., 2009; Silva et al., 2020).

Because the detoxification of mycotoxins cannot be achieved by conventional food processing, mycotoxins in raw ingredients might be transferred to the final food products. Mycotoxins, such as AFs, DON, FBs, OTA, and ZEA, are commonly detected in the middle and final products of fermented soybean foods (Table 2). For example, meju is a spontaneously fermented soybean block that is used as a starter culture as well as a nutrient and flavor source for Korean traditional fermented food products, including doenjang, kanjang, and kochujang. Meju is fermented using different fungal species, including those from the genera *Aspergillus*, *Botrytis*, *Mucor*, and *Rhizopus*, and can be contaminated with mycotoxin-producing fungi (Ryu et al., 2021). Meju has reportedly been contaminated with AFs (0.2 to 48.3 ppb) and OTA (0.1 to 193.2 ppb) (Jeong et al., 2019). These mycotoxins might be carried over from the raw material (soybeans) or produced by mycotoxigenic fungi encountered during fermentation. AFs and OTA were also frequently detected in the final products of fermented foods using meju as a starter. For example, Woo et al. (2019) detected the presence of up to 20 mycotoxins in Korean doenjang samples and found that the most frequently contaminating mycotoxins in both commercial and homemade doenjang samples were: AFB1(0.11 to 5.43 ppb); FB1, FB2, and FB3(2.48 to 68.52 ppb); OTA (0.16 to 23.27 ppb); and ZEA (4.67 to 95.08 ppb). Their contamination levels were significantly higher in homemade doenjang than in commercial products. Lee et al. (2022) conducted a 3-year monitoring activity, from 2018 to 2020, on AF contamination of homemade doenjang (n = 1436) in South Korea. The authors reported AF contamination levels ranging from 0.01 to 281.92 ppb in positive samples and an estimated AF exposure level of 0.1012 ng/kg body weight/day based on the average intakes in all age groups. Risk assessment using the Margin of Exposure approach for the carcinogenic and genotoxic potential of aflatoxins revealed values ranging from 3705 to 3954 for the average doenjang intake in all age groups, indicating potential public health risks. AF and OTA contamination in kanjang, another fermented food product prepared using meju, has also been reported at relatively low levels (<10 ppb) (Lee et al., 2012; Park et al., 2004). DON is rarely detected in kanjang, which is the Korean traditional soy sauce, but is frequently detected in soy sauce consumed in China (Chiang-yu) and Germany, with contamination levels ranging from 4.5 to 1245.6 ppb (Schollenberger et al., 2007; Wang et al., 2014; Zhao et al., 2013). Soy sauce produced in Japan (shoyu) is also reportedly contaminated with DON at concentrations ranging from 30.5 to 238.3 ppb (Zhao et al., 2013). In China and Japan, soy sauce is produced using similar procedures by fermenting soybeans with wheat bran or wheat flour (Pacin et al., 1997). At the end of the fermentation, the fermented paste is pressed to yield soy sauce. Therefore, DON contamination in soy sauce produced in China and in Japan could also be due to the high incidence of DON in wheat bran or wheat flour. Furthermore, the addition of wheat bran or wheat flour during chiang-yu and shoyu fermentation could promote contamination by DON-producing strains. In Indonesia, FB1 (170 to 2682 ppb) is frequently detected in tempeh (Anggriawan, 2017). Other *Fusarium* mycotoxins, such as beauvericin and enniatins, with cyclic hexadepsipeptide structures, have also been found in tempeh at very low or unquantifiable amounts (below the limit of quantification).

3.4 | Dynamic changes in mycotoxin contamination levels during the production of fermented soybean products

The fermentation process of soybean products involves complex changes in terms of chemical composition, physical characteristics, and microbial community, which result in dynamic changes in their mycotoxin contamination levels. For example, a study of dynamic changes in AFB1 levels during the fermentation of Chinese soy sauce revealed that approximately 50% of AFB1 was derived from the raw materials (soybeans and wheat), and the remaining 50% was produced at the early stage of fermentation by mycotoxigenic fungi (Xie et al., 2014). Manufacturing processes could remove approximately 48% of AFB1, but approximately 52% was transferred from the middle products (soybean pastes) to the soy sauce. Another study reported a gradual decrease (up to 56%) in the AFB1 levels during a 36-month fermentation of soy sauce (Zhang et al., 2020). Similarly, reductions in the levels of AFs and OTA in meju were observed during the fermentation of doenjang and kanjang (Jeong et al., 2019; Park et al., 2004). Various factors might affect the dynamic changes in mycotoxin levels. The addition of salt during fermentation may create an environment unsuitable for mycotoxigenic fungi and, therefore, reduce mycotoxin production. Meanwhile, high salt content may also favor the growth and proliferation of bacteria involved in the reduction of mycotoxins. A previous study reported that high salt stress on lactic acid bacteria (LAB) strains, such as *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Lacticaseibacillus casei*, promoted their detoxification abilities against AFB1 and T2(Ye et al., 2020). A decrease in pH usually occurs at the later stages of soybean fermentation, which has been suggested to contribute to the reduction of mycotoxins in fermented foods (Adebiyi et al., 2019). The reason for the reduction of mycotoxins caused by a decrease in the pH is still not clear. However, it might be associated with the microorganisms that appear at the later stages of the fermentation process, which may possess the ability to degrade or detoxify mycotoxins (Cho et al., 2016). Furthermore, during fermentation, changes in the contents of bioactive components, such as flavonoids, lignans, and terpenoids, may also affect the dynamic changes in mycotoxin contamination because many of these compounds can regulate mycotoxin biosynthesis (Tian et al., 2021).

4 | PREVENTION OF MYCOTOXIN CONTAMINATION IN FERMENTED SOYBEAN PRODUCTS

Improving the safety of fermented soybean products while retaining good quality and original characteristics is a considerable challenge. Because different mycotoxigenic fungi and mycotoxin contaminations occur in the raw materials and during storage and fermentation stages, different prevention strategies are required (Table 3). In this review, we summarize the strategies that could be applied to prevent mycotoxin contamination in fermented soybean products, and discuss both their advantages and limitations in protecting fermented foods.

4.1 | Preventing mycotoxin contamination in soybeans

4.1.1 | Preharvest prevention

Preventing mycotoxin contamination in raw materials is considered a more effective strategy than subsequent control methods. At the preharvest stage, fungicides are an essential method for controlling diseases and mycotoxin contamination in agriculture. Azoles and strobilurins are currently the most widely used fungicidal chemicals in agriculture (McDonald et al., 2019). Azoles inhibit the growth of the fungi by suppressing the biosynthesis of ergosterol in cytoplasmic membrane, while strobilurins inhibit fungal growth through inhibition of fungal respiration by binding to the complex III (cytochrome bc1) in the mitochondrial respiratory chain. Azole and strobilurin have played prominent role in the fungicide application market for controlling fungal pathogens of soybean for many years (Juliatti et al., 2017). In addition, benzimidazoles (inhibit fungal mitosis and cell division) and phenylamides (inhibit RNA synthesis and protein production) are also important fungicides against fungal pathogens of soybean (Oliver & Hewitt, 2014). These classes of fungicides have a strong activity and a broad antifungal spectrum against a wide range of pathogens, including mycotoxigenic fungi, and can be used in the field or in seed treatment. Some of them have been used to decrease mycotoxin contamination in food crops. For example, triazole fungicides, including metconazole, propiconazole, prothioconazole, and tebuconazole have been used to

TABLE 3 Summary of strategies for preventing mycotoxin contamination during the production of fermented soybean products

Production stages		Strategies		Treatment effects
Soybeans	Preharvest	Chemical	Applying synthetic fungicides (e.g., strobilurin and triazole)	Killing fungal cells or inhibiting fungal growth or spore germination (Oliver & Hewitt, 2014)
		Biological	Applying non-mycotoxigenic biocontrol fungi, applying natural antifungal agents (e.g., essential oils and phytochemicals), and developing resistant plants	Reducing the population of mycotoxigenic strains through growth competition (Abbas et al., 2017), killing fungal cells or inhibiting fungal growth or spore germination (Tian & Chun, 2017), and increasing the contents of antifungal/antimycotoxigenic compounds in the plant (through genetic modification or selective breeding) (Boué et al., 2000)
	Postharvest	Physical	Applying prompt drying, cleaning, and dehulling; avoiding damage to the grains; removing damaged or infected grains; avoiding long-term and mixed grain storage; controlling storage environmental conditions (e.g., low temperature and water activity, low O_2 concentration, and high CO_2 concentration); and applying physical sterilization treatments (e.g., ozone, radiation, ultraviolet light, and ultrasound)	Making grains unsuitable for rapid fungal growth, removing fungal cells from the grains, maintaining the physical defense of the grains to fungal infection, removing grains that are contaminated or easily contaminated with fungi, reducing the probability of contact with fungal cells, creating environmental conditions unsuitable for fungal growth, and killing fungal cells (Liu et al., 2020; Luo et al., 2018)
		Chemical	Applying synthetic fungicides	Killing fungal cells or inhibiting fungal growth or spore germination (Oliver & Hewitt, 2014)
		Biological	Applying natural antifungal agents	Killing fungal cells or inhibiting fungal growth or spore germination (Tian & Chun, 2017)
Fermentation	Preparation	Physical	Sorting, separation, washing, and soaking; cooking, pressure cooking, and autoclaving; and applying mycotoxin-binding materials (e.g., active charcoal and clay minerals) during washing and soaking	Removing damaged or fungal-contaminated grains and certain amounts of mycotoxins (Luo et al., 2018), killing fungal cells, destroying certain amounts of mycotoxins (Cazzaniga et al., 2001), and reducing the mycotoxin content through absorption
		Chemical	Adding organic acids (e.g., citric acid and lactic acid), bases (e.g., ammonium hydrochloride and gaseous ammonia), or oxidants during washing and soaking	Detaching or damaging mycotoxins using acid or base solutions (Lee et al., 2015)

(Continues)

TABLE 3 (Continued)

Production stages		Strategies		Treatment effects
		Biological	Adding inactivated microbial cells during washing and soaking and adding a filtrate of microbial cultures or enzymes during soaking	Reducing the mycotoxin content through absorption (Vila-Donat et al., 2018), reducing the mycotoxin content through biotransformation (Shukla et al., 2018)
	Inoculation	Biological	Using quality fermentation starters, adding biocontrol fungi, mycotoxin biotransformation strains, and mycotoxin-binding strains	Preventing contamination of mycotoxigenic strains in the starter and reducing the population of mycotoxigenic strains through growth competition (Shukla et al., 2020)
	Fermentation	Physical	Adding mycotoxin-binding materials	Reducing the mycotoxin content through absorption Kabak et al., 2006)
		Biological	Using exclusive inhibitors of mycotoxin biosynthesis (e.g., flavonoids and terpenoids) and adding mycotoxin-degrading enzymes	Inhibiting mycotoxin biosynthesis without altering the fermentation microflora (Tian et al., 2021) and reducing the mycotoxin content through enzymic degradation (Loi et al., 2017)
Storage		Physical	Using sealed containers and storing under low temperatures	Creating environmental conditions unsuitable for fungal growth (Luo et al., 2018)
Cooking		Physical	Extrusion cooking and high temperature (e.g., >150°C, affected by the pH and moisture) baking, frying, and roasting	Damaging mycotoxins (Lee et al., 2015)
		Chemical	Alkaline cooking (nixtamalization)	Damaging mycotoxins (Dombrink-Kurtzman et al., 2000)

control DON contamination in wheat (Feksa et al., 2019; Paul et al., 2008). However, most fungicides available nowadays are not completely suitable for controlling mycotoxin production. It has been reported that azoles and strobilurin fungicides reduced fungal growth but induced the production of mycotoxins (Ellner, 2005; Schmidt-Heydt, et al., 2013). Besides, in addition to strict regulations regarding the use of synthetic chemicals for food crops, many filamentous fungi also rapidly evolve resistance to fungicides (Fisher et al., 2018).

A biocontrol strategy using non-mycotoxigenic fungi to compete with the naturally occurring mycotoxigenic strains has been demonstrated to effectively reduce AF, FB, OTA, and patulin levels in food crops (Kagot et al., 2019; Yang et al., 2015). For example, *A. flavus* AF36 and *A. flavus* NRRL 21882 designated Afla-Guard™ are two commercially available biocontrol products registered with the US Environmental Protection Agency (EPA). Both strains, when applied to soil, are able to significantly suppress AF contamination in corn, peanuts (Afla-Guard™), and tree nuts (AF36) in field trials (Abbas et al., 2017). Atoxigenic *F. oxysporum*-based biocontrol agents, FUSAclean and BioFox C, have been used in seed treatment and applied in soil to prevent contamination of vegetables with *F. oxysporum* and Fusarium *verticillioides* (Kaur et al., 2010; Nagraj et al., 2021). However, to date, there is no record of the application of biocontrol agents at the preharvest stage for the prevention of fungal or mycotoxin contamination in soybeans. Moreover, climate change and long-term application requirements pose a challenge to the effectiveness of such strategies (Bandyopadhyay et al., 2016; Gasperini et al., 2019). Furthermore, the large populations of biocontrol strains may increase their sexual reproduction and reassortment of their genes, which may promote competitiveness between these strains and the mycotoxigenic strains, thus further increasing the population of toxigenic strains and compromising the effectiveness of the biocontrol strategy (Mwakinyali et al., 2019).

Another promising biocontrol strategy against mycotoxin contamination involves using natural antifungal and antimycotoxigenic compounds. Many coumarins, flavonoids, terpenoids, and lignins of plant origin, as well as essential oils, exhibit excellent potential as agents to help control mycotoxin contamination in foods (Atanasova-Penichon et al., 2016; Tian & Chun, 2017). Such natural compounds are usually biodegradable and have been reported to have much lower toxicity for humans and animals than synthetic fungicides. Thus, they are desirable in the agriculture and food industry. Antifungal and antimycotoxigenic natural compounds have also been detected in soybeans. For example, an earlier study indicated that phenolic compounds in soybeans protect the crops naturally against fungal contamination (Silva et al., 2020). Glyceollin, which is a soybean phytoalexin produced in response to seed infection by *A. flavus*, has been observed to suppress AFB1 accumulation by up to 95% in an *A. flavus* culture when applied at a concentration of 62.5 ppm (Song & Karr, 1993). Such natural compounds can be used to develop safe and effective antifungal and antimycotoxigenic agents for the control mycotoxin contamination in soybeans.

Another promising use of these compounds is for genetic engineering of host crops to gain resistance to mycotoxin contamination by incorporating the genes for the biosynthesis of natural inhibitors. Developing resistant plants that already possess mycotoxigenic inhibitors is much easier than the production of exogenous inhibitors because the biosynthetic pathways are already present in the host, and an increased inhibitor concentration can be achieved by upregulating the expression of endogenous genes. Glyceollin concentrations in soybean plants can be increased using proper induction, making it a particularly promising candidate for constructing AF-resistant plants (Boué et al., 2000; Daniel et al., 1999). However, most genetic and molecular approaches aimed at preventing mycotoxin contamination in food crops have not yet reached the commercial application stage in the field and require substantial further developments.

4.1.2 | Postharvest and storage prevention

Most of the synthetic and biological antifungal agents applied at the preharvest stage are also promising as protectants during the postharvest stage and storage. In addition, efficient and prompt drying, cleaning, and dehulling of food grains at the postharvest stage have been identified as key critical control points to reduce the risk of mycotoxin contamination (Magan & Aldred, 2007). To prevent mycotoxin contamination, soybeans should be dried as rapidly as possible during or soon after harvest. The critical moisture content for the safe storage of soybeans is approximately 11% (Robertson et al., 1973). Moreover, maintaining a critical moisture content is important to avoid damaging the grains before and during harvest and drying because damaged grains are more prone to fungal invasion and, therefore, mycotoxin contamination. Insect activity is one of the major sources of damage to grains. Thus, the insect population should be kept to a minimum in the field and storage. Furthermore, dehulling soybeans have been found to play an important role in preventing mycotoxin contamination because fungi and mycotoxins are usually present in the outer part of the seed. Chilaka et al. (2019) showed that dehulling of soybeans significantly reduced the concentrations of FBs, ZEN, and T2 in spiked and naturally contaminated soybean samples. However, the efficiency of reduction appears to be dependent on the concentration and extent of penetration of mycotoxins into the grains.

Appropriate storage practice is key to preventing fungal and mycotoxin contamination. Storage environmental conditions, especially temperature, water activity, and atmosphere, have been confirmed in many studies to strongly affect the growth of fungi and the production of mycotoxins (Liu et al., 2020; Luo et al., 2018). Lowering the temperature of the storage environment and the moisture level of the agricultural products can create unsuitable conditions for fungal growth and metabolism during storage (Chulze, 2010). For example, a temperature below 15°C or water activity below 0.87 is unsuitable for AF production in soybeans (Ferna & Vaamonde, 1991). The growth of *A. alternata* and production of AOH and AME in soybeans can be completely inhibited using the combination of a temperature of 15°C and a water activity level of 0.90 (Oviedo et al., 2011). It is also possible to prevent fungal and mycotoxin contamination in stored grains by controlling the storage atmosphere. Relatively low O_2 concentration and high CO_2 concentration contribute to the inhibition of fungal development and mycotoxin biosynthesis (Luo et al., 2018; Magan & Aldred, 2007). For example, *A. flavus* and *Penicillium roqueforti* could not grow in an environment containing 60% and 40% CO_2 and less than 0.5% O_2 balanced with N_2 (Taniwaki et al., 2009). However, controlling the storage atmosphere at a large scale can be very expensive. Furthermore, long-term mixed storage of grains may increase the risk of fungal and mycotoxin contamination; therefore, it should be avoided.

4.2 | Preventing mycotoxin contamination before fermentation

In the manufacturing of most fermented soybean products, the soybeans are processed via a series of physical

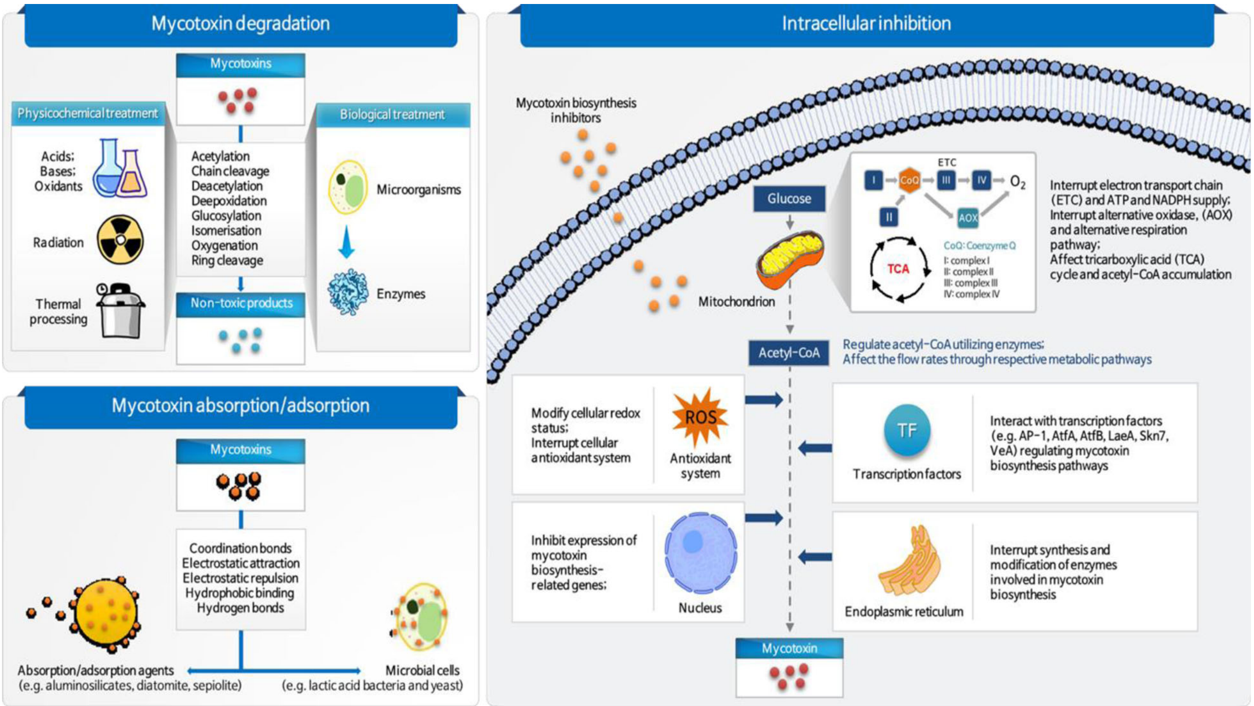

FIGURE 3 Schematic diagram of mechanisms of mycotoxin contamination prevention

pretreatments before the fermentation, which usually includes sorting, separation, washing, soaking, and cooking (Figures 1 and 2). These traditional pretreatments have been proven to help prevent mycotoxin contamination during fermentation and final products (Sivamaruthi et al., 2019). Sorting and removing decayed and poor-quality grains and a simple washing procedure can generally reduce fungal and mycotoxin contamination (Fandohan et al., 2005; Luo et al., 2018). Fungal-infected or insect-damaged grains usually have lower densities than healthy ones and tend to float when soaked in water. Simply discarding the floating fractions can generally eliminate considerable amounts of mycotoxins in the grains (Luo et al., 2018). In addition, adding chemicals, such as organic acids, bases, or oxidants, during the soaking process can help promote the reduction of mycotoxin levels in soybeans (Figure 3). For example, adding 1.0 N citric acid, lactic acid, succinic acid, and tartaric acid during the soaking process (18 h) for AF-contaminated soybeans can decrease the AF levels by 94.1%, 92.7%, 62.0%, and 95.1%, respectively (Lee et al., 2015). Despite the high cost, adding adsorbents or absorbents during the washing and soaking processes is expected to also facilitate the removal of mycotoxins in soybeans (Kabak et al., 2006). Moreover, natural organic binders, such as LAB and yeast cells, have also been proven to be effective in adsorbing or absorbing mycotoxins (Vila-Donat et al., 2018). The conventional cooking process (usually boiling at 100°C) following the soaking can also decrease the level of mycotoxins in grains (Cazzaniga et al., 2001). Pressure cooking or autoclaving of soybeans at different pH levels reportedly promotes the detoxification of AFs (Lee et al., 2015).

4.3 | Preventing mycotoxin contamination during fermentation

The food fermentation process is often sensitive to changes in conditions, which may result in unacceptable final product quality. Most of the physicochemical approaches for preventing mycotoxin production can result in significant alteration of the characteristics of food materials as well as the fermentation conditions. Therefore, they are not suitable to be applied during fermentation. When controlled properly, the fermentation system itself can prevent or suppress the growth of mycotoxigenic fungi and thus mycotoxin contamination. Furthermore, some biological approaches involving selected starter strains or exclusive inhibitors of mycotoxin biosynthesis could overcome the limitations and offer protection during fermentation.

4.3.1 | Improving the fermentation procedures

Adjustment of fermentation conditions, including temperature, pH, salt concentration, and water activity, could

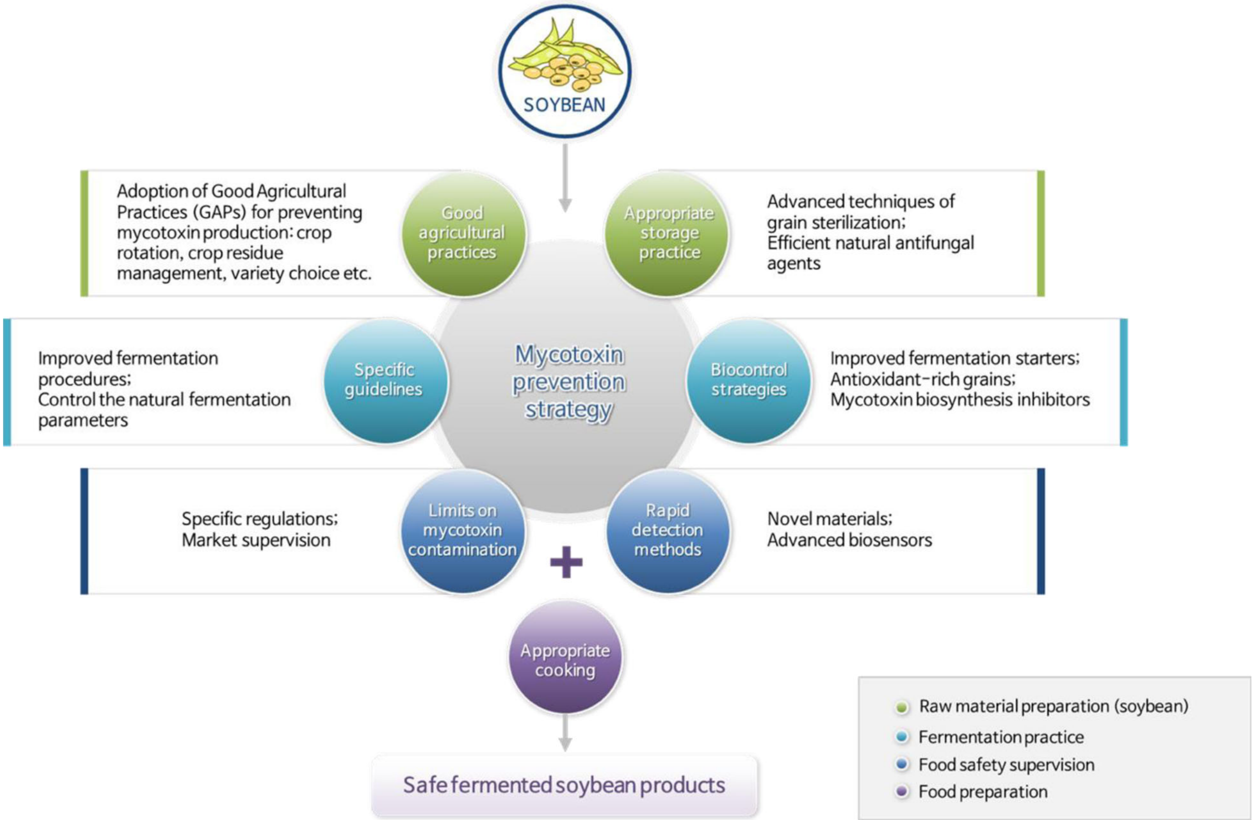

FIGURE 4 Future perspectives of mycotoxin contamination prevention in fermented soybean products

directly affect the survivability of mycotoxigenic fungi and mycotoxin production. At the early stages of fermentation, adjusting the fermentation temperature and water activity of the raw materials to levels that favor the growth and proliferation of functional microbial species can enable them to form the predominant population and therefore prevent contamination by undesired microbial species. Most soybean products require the addition of salt at the early or middle stages of fermentation. The increase in salt content tends to modify the microbial community structure and change their metabolism. Increased salt stress has been reported to promote the mycotoxin detoxification ability of LAB strains (Ye et al., 2020). Therefore, the amount and timing of salt addition could possibly contribute to the prevention of mycotoxin contamination. Similarly, adjusting the pH of the fermentation system during different stages may also result in similar outcomes (Adebiyi et al., 2019; Cho et al., 2016). An in-depth investigation of the dynamic changes of the fermentation microbial community and their metabolism in response to temperature, pH, salt concentration, and water activity would be of great significance in developing improved procedures for food fermentation to prevent mycotoxin contamination.

4.3.2 | Using quality fermentation starters composed of selected microbial strains

Quality fermentation starters composed of selected microbial strains may promote the reduction of undesired microbial species and mycotoxin production in the fermentation process. The use of fermentation starters containing strains that can multiply rapidly and outcompete the mycotoxigenic fungi at the early stage of fermentation can help prevent mycotoxin contamination. An example of this is *Aspergillus oryzae*, which has been used in the production of soybean paste (doenjang, miso), soy sauce, and sake. Interestingly, *A. oryzae* is recognized as domesticated from *A. flavus*, which produces carcinogenic aflatoxins (Steensels et al., 2019). A study of starter cultures on the quality and safety attributes of doenjang revealed that mixing selected *Rhizhopus nigricans* in the starter culture could effectively improve the nutrition and safety of doenjang and reduce AF level and hazardous microbial count in the final products (Shukla et al., 2020). Introducing biocontrol strains into the natural fermentation process may also prevent mycotoxin contamination. Many biocontrol microorganisms showing great potential for food protection have been isolated from fermented foods. An *A. oryzae* strain (M2040) isolated from meju could effectively suppress the aflatoxigenic *A. flavus* growth and AFB1 production in peanuts at an inoculation level of 1% (Alshannaq et al., 2018). The cell-free culture filtrate of this fungi could also effectively inhibit both the growth and AF production of *A. flavus*, indicating the existence of antifungal and antiaflatoxigenic compounds in the filtrate. Introducing mycotoxin-degrading strains during soybean fermentation is another strategy to control mycotoxin contamination in the final products. A group of AF-degrading *A. oryzae* strains isolated from meju can suppress *A. flavus* growth and effectively degrade more than 90% of AFB1 in culture broth in 2 weeks (Lee et al., 2017). Such *A. oryzae* strains have potential applications in the control of AF contamination in soybean-based foods and other fermented foods.

Bacteria, such as *Bacillus* spp., *Lactobacillus* spp., and *Staphylococcus* spp., also play important roles during the fermentation of soybean foods, which include but are not limited to meju, tempeh, and furu. Two *Bacillus* strains isolated from a traditionally fermented soybean product in Thailand (thua nao) were able to inhibit the growth of both *A. flavus* and *Aspergillus westerdijkiae* and decrease AFB1 and OTA production in both solid and liquid media by up to 74% and 92.5%, respectively (Petchkongkaew et al., 2008). A *Bacillus subtilis* strain (KU-153) isolated from kimchi, a Korean traditional fermented cabbage, exhibited the ability to reduce the OTA content by approximately 90% in red wine (Shukla et al., 2018). Elimination of mycotoxins in fermented foods using LAB is a common practice in food industries. Many LABs from the genera *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Staphylococcus* have been studied for their ability to bind or inhibit the biosynthesis of major mycotoxins, including AFs (Ahlberg et al., 2015), FBs (Niderkorn et al., 2009), ZEA (El-Nezami et al., 2002a), and trichothecenes (El-Nezami et al., 2002b). Introducing these bacterial biocontrol strains could also contribute to the decrease in bioavailability of mycotoxins in the final fermented products and their toxic effects on human health (Sadiq et al., 2019).

In addition, genome-editing, by inserting, removing, or replacing certain DNA sequences from a microbial genome, can be used to manipulate important biological functions of the filamentous fungi or biocontrol strains, including the biosynthesis of mycotoxins, antibiotics, molecules involved in crop infection, or enzymes required for food fermentation. Advanced genetic tools are already available to activate or repress specific genes without leaving exogenous DNA in the edited microbial genome. Among them, CRISPR-Cas9 technology is one of the most popular genetic tools and has been successfully applied to different filamentous fungi (Shi et al., 2017). Such technologies open up new frontiers in the improvement of the performance of fermentation strains and biocontrol agents to meet the increasing demand for safer, healthier, and tastier fermented food products. However, such technologies have not yet been practically applied in food fermentation. The applicability of these technologies will be determined by public acceptance as well as legislative support or constraints (Sarrocco & Vannacci, 2018).

Safety issues still need to be considered before such antimycotoxigenic- and mycotoxin-degrading strains can be employed for biocontrol purposes in food fermentation. In the EU, microbial species that have a record of safe use in food manufacturing are listed by the European Food and Feed Cultures Association (EFFCA) (Sundh & Melin, 2011) and are allowed to be used in traditional food manufacturing. In the United States, a microbe used in food production can be applied for listing as "Generally Recognized As Safe" (GRAS) by the US FDA, which testifies that a safety assessment of the microbe has been performed and filed. Microorganisms considered GRAS can be used in traditional food fermentation without the legal requirements from or communications with the FDA, such as LABs used in bread, rolls, and buns manufacturing, *L. bulgaricus* and *Streptococcus thermophilus* used in yogurt fermentation, *P. roqueforti* used for blue cheese production, and *A. oryzae* used for soy sauce fermentation, and the main responsibility for the safety of the resulting food product is borne by the producers and the suppliers (Mattia & Merker, 2008). However, the deliberate addition of selected microbes into food fermentation for the technical purpose of controlling mycotoxin contamination cannot be considered traditional use. Appropriate risk assessment and authorization may be conducted by different regulatory bodies.

4.3.3 | Applying exclusive inhibitors of mycotoxin biosynthesis

Many natural compounds have shown much potential to be used as safe antifungal agents for food protection (Ahmed et al., 2022). However, natural antifungal agents often have broad-spectrum activities against multiple fungal species. Thus, they may alter the microbial communities if applied during food fermentation and may result in the unacceptable quality of the final product. Natural compounds that exclusively inhibit mycotoxin biosynthesis without affecting other fungal growth could be highly useful for mycotoxin control during food fermentation without disrupting the original microbial communities or causing their replacement by other undesired mycotoxigenic fungi. In a previous study, plant-based natural compounds, namely, alisol A, 5-*O*-demethylnobiletin, meso-dihydroguaiaretic acid, kaempferol, 20(S)-protopanaxatriol, and syringaresinol, were found to strongly inhibit AFB1 production (>85%) without inhibiting fungal growth in the culture medium (Tian et al., 2021). Caffeic acid reportedly inhibited AF biosynthesis by *A. flavus* in a fat-based growth medium by more than 95%, with no effects on the growth of the fermenting fungi (Kim et al., 2008). Similarly, fennel essential oil suppressed OTA production by *Aspergillus carbonarius* by up to 89%, but it only reduced the growth of desired fungal species by 14% (El Khoury et al., 2016). The exact mechanism of action of these exclusive inhibitors is still unclear. However, it might be associated with the inhibition of the expression of mycotoxin biosynthetic genes, interruption of the synthesis of related enzymes, and interaction with related transcription factors, as well as the disruption of mitochondrial functions (Figure 3). Furthermore, because most of these inhibitors exhibit antioxidant activity, it has also been suggested that their inhibitory effect is associated with oxidative stress alleviation (Sakuda et al., 2016). Soybeans are commonly rich in phenolic compounds and other antioxidants. Fermenting soybeans with filamentous fungi can increase their phenolic content and enhance radical scavenging activity (Ghanem et al., 2020). These compounds may also be able to contribute to the suppression of mycotoxin production. The addition of these inhibitors against mycotoxin biosynthesis could be the most effective strategy for preventing mycotoxin contamination during food fermentation while maintaining good quality and favorable characteristics of the final products.

5 | CONCLUSIONS

Mycotoxin contamination in fermented soybean foods poses risks for human health. Both the raw materials and the manufacturing environment are sources of mycotoxigenic fungal and mycotoxin contamination. AFs, DON, FBs, OTA, and ZEA are among the most commonly detected mycotoxins in fermented soybean products. However, most countries have not yet set regulations regarding the mycotoxin content in fermented foods. To guarantee the safety of fermented soybean products, specific guidelines and regulations on mycotoxin contamination should be established (Figure 4). Preventing mycotoxin contamination in raw materials is considered a more effective strategy than subsequent control methods. In addition, good agricultural practice is one of the most effective approaches of preventing mycotoxigenic fungal and mycotoxin contamination. Appropriate storage practices and optimized fermentation procedures are also key to controlling mycotoxin contamination. Biocontrol strategies using non-mycotoxigenic fungi, mycotoxin-absorbing or mycotoxin-degrading bacteria, or natural antifungal and antimycotoxigenic compounds could also reduce mycotoxin contamination in the fermented final products. With the growing awareness of food safety and environmental protection among consumers, a combination of good agricultural and fermentation practices together with appropriate and efficient quality-monitoring programs and eco-friendly and biosafe antimycotoxigenic or detoxification procedures throughout the production process could be the main measure for future mycotoxin control in fermented foods.

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AUTHOR CONTRIBUTIONS

Fei Tian: Investigation; Methodology; Software; Writing—original draft; Writing—review & editing. **So Young Woo**: Data curation; Investigation; Methodology; Writing—original draft; Writing—review & editing. **Sang Yoo Lee**: Data curation; Investigation; Software; Writing—original draft; Writing—review & editing. **Su Been Park**: Investigation; Software; Validation; Visualization; Writing—review & editing. **Ju Hee Im**: Data curation; Investigation; Software; Validation; Writing—review & editing. **Hyang Sook Chun**: Conceptualization; Funding acquisition; Project administration; Supervision; Visualization; Writing—review & editing.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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