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Review article

Enhancing food safety in soybean fermentation through strategic implementation of starter cultures

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A B S T R A C T

Fermented soybean products have played a significant role in Asian diets for a long time. Due to their diverse flavours, nutritional benefits, and potential health-promoting properties, they have gained a huge popularity globally in recent years. Traditionally, soybean fermentation is conducted spontaneously, using microorganisms naturally present in the environment, or inoculating with traditional starter cultures. However, many potential health risks are associated with consumption of these traditionally fermented soybean products due to the presence of food pathogens, high levels of biogenic amines and mycotoxins. The use of starter culture technology in fermentation has been well-studied in recent years and confers significant advantages over traditional fermentation methods due to strict control of the microorganisms inoculated. This review provides a comprehensive review of microbial safety and health risks associated with consumption of traditional fermented soybean products, and how adopting starter culture technology can help mitigate these risks to ensure the safety of these products.

1. Introduction

Soybean, a legume that is native to East Asia, has been widely incorporated into Asian diets due to its high protein content and nutritional properties. Soybean is one of the most valuable, versatile, and nutritionally important legumes globally. A high demand of soybean arises from the use of soybean oil in the food industry, and soybean meal as livestock feed [1]. In Asia, soybean is also commonly consumed in the form of fermented products, such as soy sauce, miso, and tempeh [2]. Fermentation of soybean involves the breakdown of complex substrates through the action of microorganisms, such as moulds, yeasts, and bacteria, to either generate desirable flavors or act as a preservation technique [3]. However, studies have shown that consumption of soybean products that have undergone traditional spontaneous fermentation presents several potential hazards, such as the presence of food pathogens and high concentrations of mycotoxins and biogenic amines (BAs), which can lead to health problems [4].

Foodborne diseases are defined as the illnesses arising from consumption of foods contaminated with pathogenic bacteria, viruses and parasites [5]. Common pathogenic microorganisms found in fermented foods include *Escherichia coli, Staphylococcus aureus* and *Salmonella,* in which contamination arises from poor sanitation practices and processing under unhygienic environments [6]. *Clostridium botulinum* has also been deemed as a hazard in fermented foods, especially in fermented vegetables, as they can grow in oxygen-free environments and when insufficient acids are produced during the fermentation [7]. Apart from foodborne diseases, there are concerns regarding the high levels of BAs and mycotoxins present in fermented foods. These compounds are commonly produced

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by some microorganisms during fermentation and consumption in high amounts can negatively impact human health [8]. BAs are produced by the decarboxylation of amino acids catalysed by enzymes produced by specific microorganisms while mycotoxins are secondary metabolites that are synthesized by adventitious fungal strains during fermentation. Consumption of high concentrations of these compounds can negatively impact human health, as some of these compounds are carcinogenic and can lead to severe hepatotoxic effects, kidney damage and gastrointestinal issues.

Approaches have been adopted to control microbiological hazards in soybean fermentation. Sanitation practices emerge as an indispensable component of a comprehensive food safety strategy. This involves the sterilization of raw materials and fermentation equipment such as pots and spatulas to ensure no pathogenic microorganisms are present to contaminate the final product. However, these sanitation practices are often not strictly followed, and the lack of such guidelines for preparing food-safe fermented soybean products is alarming [9]. Rai et al. (2014) studied the traditional model of producing *kinema*, a fermented soybean product in Nepal and isolated *Escherichia coli, Salmonella enteritidis, Shigella sonnei, Staphylococcus aureus* and various other pathogenic bacteria in the final product following the traditional preparation of *kinema*. The authors reported high levels of *Bacillus* spp. in the tap water used, which illustrates the high levels of food safety risks associated with traditional soybean fermentation practices [10]. In addition to the lack of strict safety guidelines for preparation of fermented soybean products, traditional fermentation often uses spontaneous inoculation, which relies on the microorganisms present in the environment or on/in the surface of the raw ingredients to initiate the fermentation process [11]. This poses a risk of introducing microorganisms that have the ability to produce toxins, such as biogenic amines and mycotoxins, which poses a threat to human health if ingested [12].

In conjunction with ensuring strict sanitary practices being followed to produce safe fermented soybean products, starter culture technology serves as an additional strategy to establish a robust defense against potential contaminants, thereby elevating the overall safety profile of soybean fermentation. Using starter cultures for fermentation enables controlled addition of selected microorganisms into the raw soybean to bring about desired and predictable changes in the final product. This is inherently important to food safety as it allows for the selection of strains that do not produce toxins, mycotoxins, and biogenic amines. In addition, selecting strains that have robust growth in soybean offers a competitive advantage over spoilage/pathogenic microorganisms that are present in the raw material or equipment, hence enhancing overall food safety even when the sanitary environment is compromised during traditional fermentation. In this review, we address the microbiological hazards related to traditional fermentation and how starter culture technology can improve the safety of fermented soybean products.

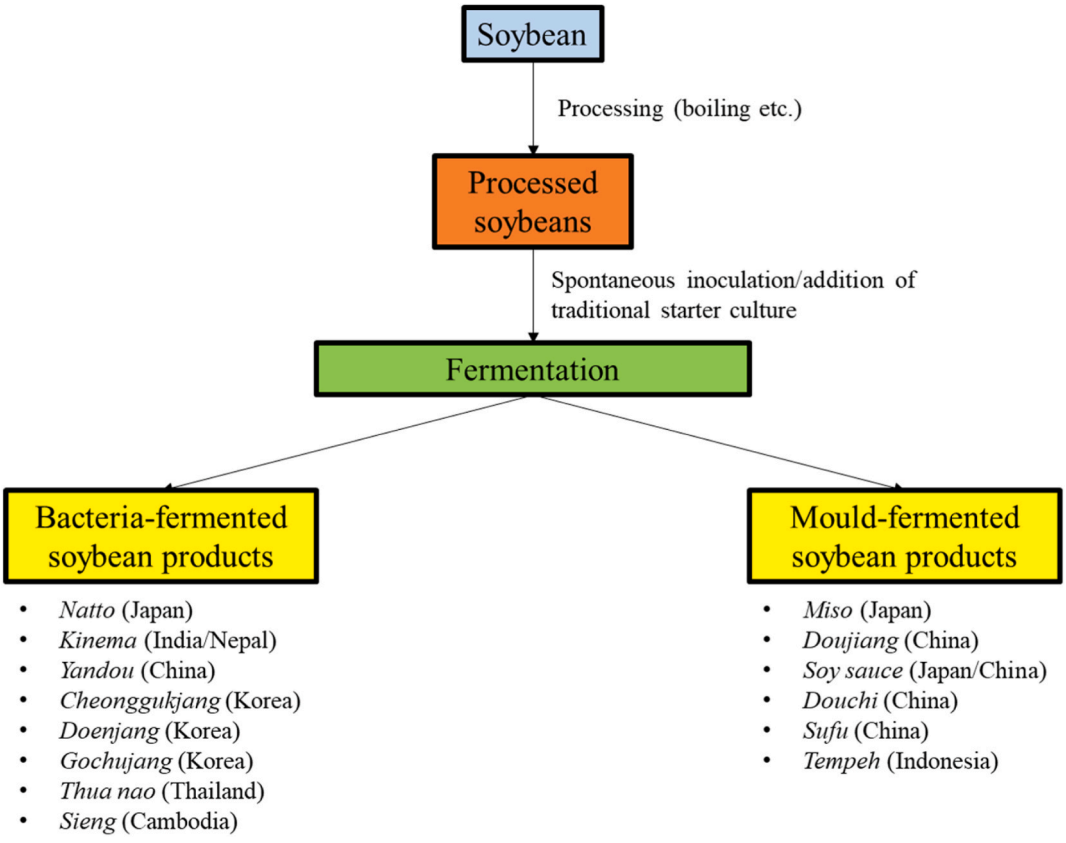

Fig. 1. Summary of traditional fermented soybean products.

2. Commonly consumed fermented soybean products

Fermented soybean products (Fig. 1) are commonly consumed, mainly in Asia, due to their high nutrition value, functionality, and low cost [2]. Soybean fermentation is typically carried out in either solid-state, submerged conditions or a combination of both [3]. Each fermented product is defined by their distinct flavor profile and texture, which is contributed largely by the microorganisms involved in the fermentation and post-processing steps used to make the product.

Bacteria-fermented soybean products are commonly consumed in Asia. Popular soybean fermented products include *natto* from Japan, *kinema* from India/Nepal and *yandou,* which is a salted soybean product from China. The dominant bacterial species found in these fermentations are often *Bacillus* spp., which gives each fermented product its characteristic aroma and texture. *Cheonggukjang*, a soybean pastes commonly found in Korea, is made by spreading boiled soybeans on rice straw, which naturally contains *B. subtilis* and is fermented for 2–3 days in solid-state conditions [13]. *B. subtilis* also plays an important role in the production of *doenjang* and *gochujang*. However, the preparation involves the addition of *meju*, which are soybean blocks that have been aged for approximately 1–2 months in a natural environment, to boiled soybeans to initiate the fermentation [14]. For *doenjang*, brine is added to the fermenting mixture while for *gochujang*, ingredients such as rice powder, salt and red pepper are added instead to make a red pepper paste [13]. Apart from Korean fermented soybean pastes, *B. subtilis* is also dominant in other fermented soybean products such as *thua nao* from Thailand and *sieng* from Cambodia, which are less well-known but remain widely consumed in their countries. They are produced in a similar manner to *tempeh* from Indonesia, where boiled soybeans are wrapped in banana leaves and left to ferment and the final products have a brownish appearance and slightly sticky texture [15,16]. The differences in substrate preparation and fermentation conditions gives rise to varied aroma profiles formed in the final product, even though all products are dominated by *B. subtilis.*

Mould-fermented soybean products are also commonly consumed in Asia. These include *miso*, *doujiang*, and soy sauce, which have a characteristic umami taste and roasted aroma. The first step involves producing *koji*, an inoculum of *Aspergillus* spp. grown on a substrate such as soybean [17]. Following this, miso is produced by adding *koji* to a salt and soybean mash and left to ferment for up to 2 years in solid-state conditions. Conversely, soy sauce production involves the immersion of *koji* in a brine solution and left to ferment for several months to 4 years in a submerged fermentation system [18]. *Aspergillus* spp. is also involved in the fermentation of soybean products found in China. *Douchi*, a solid fermented soy product that uses black soybeans as a substrate is fermented with *Aspergillus* spp. and kept at 30 °C for 3–4 days to form koji. The koji is then washed with water and mixed with 16 % salt, 10 % water, ginger and various mixed powdered spices, and stored in closed jars for several weeks at 35 °C [19]. Another Chinese fermented soybean product, known as *sufu* involves the use of a mixture of moulds, *Actinomucor, Mucor* and *Rhizopus* for fermentation of soybean curd [20]. Another commonly known mould-fermented soybean product that has gained global popularity is *tempeh*, a traditional fermented soybean product from Indonesia. *Rhizopus oligosporus* is the dominant strain in the fermentation, which is found on the banana leaves that are used to wrap cooked soybeans, and the soybeans are left to ferment. The resulting product is a compact soybean 'cake' tightly packed together and bound by a dense cottony mycelia [21].

3. Safety risks from consumption of traditionally fermented soybean products

3.1. Pathogenic microorganisms

Disease outbreaks due to the consumption of fermented soybean products are few. However, incidences have been reported from the consumption of fermented soybean contaminated with foodborne pathogens [22,23]. Traditional spontaneous fermentation presents a higher risk of contamination, due to poor handling of raw soybeans and equipment, or improper pretreatment of substrates before fermentation. In addition, spontaneous fermentation relies on using natural microorganisms present in the environment, and contamination can occur as a result of unhygienic surroundings that can introduce contaminants into the fermentation [24]. An outbreak of food poisoning was reported from the consumption of *douchi* in Yunnan, China, and isolation of the strains from vomit and unprepared/unprocessed *douchi* revealed the presence of three different *Bacillus* strains. Molecular analysis revealed that two strains were characterized to produce the emetic toxin cereulide while the other strain was characterized to produce enterotoxins [23].

Generally, fermented food products seek to achieve a pH below 4.5 to inhibit the growth of pathogenic bacteria. However, many fungal fermented soybean products such as miso and tempeh achieve pH 5 at the end of fermentation, potentially creating an environment that supports the proliferation of pathogenic microorganisms [25]. Traditionally soybean fermentation is most often contaminated with *B. cereus* and *B. thuringiensis*, which are ubiquitous in soil and closely related to *B. subtilis*, a strain that is commonly used in soybean fermentation. *B. cereus* can produce an enterotoxin known as cereulide, which causes emesis after consumption. Ingestion of an infective dose of *B. cereus* spores or vegetative cells causes diarrhea [26]. Inatsu et al. (2020) reported that *B. cereus* strains N11, N41 and O21 were isolated in 90 % of Lao-fermented and 78 % of Thai-fermented *thua nao,* and genetic analysis revealed the presence of the cereulide coding (*crs*) gene, suggesting a capability of producing the emetic toxin [27]. Similar results were also found in *sieng,* where strain isolation showed that 49 out of 120 samples obtained from the traditional market were confirmed to contain *B. cereus*, and 12 strains were reported to contain the *crs* gene [16]. Destruction of *B. cereus* spores and enterotoxins produced using adequate thermal treatment is difficult, as they are heat resistant [28]. In addition, due to the thick viscosity of many fermented soybean products, ensuring thorough heat treatment is a challenge, especially with the lack of equipment and technology in home-based fermentations.

Other foodborne bacterial pathogens including *Staphylococcus aureus*¸ *Clostridium perfringens, Salmonella enterica,* and *Listeria monocytogenes* were also reported in fermented soybean. *C. perfringens* is a spore-forming bacterium that proliferates in anaerobic conditions and can cause foodborne disease through the production of the enterotoxin Cpe [20], where consumption of the

contaminated food can lead to watery diarrhea. Han et al. (2001) conducted a screening study for food pathogens in 23 *sufu* samples produced mainly in China and the Netherlands using traditional fermentation techniques. High levels of *C. perfringens* of approximately 10^5 CFU/g were detected in one of the samples. *S. aureus* is one of the most infamous and widespread bacterial pathogens that is responsible for many foodborne illness outbreaks every year [29]. Han et al. (2001) also reported that although no *S. aureus* was detected, enterotoxin A, which may be produced by *S. aureus*, was found in several samples [20]. *Salmonella,* another bacterium commonly responsible for foodborne illness outbreaks, has also been isolated from traditionally fermented soybean products. Khaq and Dewi (2016) reported high counts of *Salmonella* isolates (approximately 10^4–10^6 CFU/g) from traditionally fermented tempeh wrapped in banana leaves when the samples were cultured on Salmonella Shigella Agar (SSA). The presence of *Salmonella enterica* serotype Parathyphi B was also reported in unpasteurized tempeh, which led to an outbreak of gastroenteritis in North Carolina, USA [22]. Investigations revealed the contamination of the *Rhizopus oryzae* starter culture used in the fermentation, which is known to have slower growth, and likely could not have outcompeted the growth of *S. enterica* as compared to the more commonly used *Rhizopus oligosporus*. Table 1 presents a summary of microorganisms that have been involved in foodborne disease outbreaks from contamination of traditionally fermented soybean products.

These findings reveal that uncontrolled fermentation increases the exposure risk of fermented soybean products to foodborne pathogens present in the environment. Challenge studies, which is the inoculation of food pathogens into a food medium and monitoring of their behavior during the manufacturing process, have also revealed that soybean is a suitable medium for the

Table 1
Summary of health risks from consumption of spontaneously fermented traditional soybean products microorganisms.

	Food hazard	Intake limit	Commonly contaminated fermented soy products	References
Bacillus cereus (emetic-type)	Contamination of cereulide in fermented soybean causing vomiting or more severely, multi-organ damage and failure	*B. cereus*: $<10^5$ CFU/g in food	*Douchi* *Thua nao* *Sieng*	[16,23,27,65]
Staphylococcus aureus	*S. aureus* can produce enterotoxin A in fermented soybean foods. Consumption of contaminated products can lead to gastroenteritis.	*S. aureus*: $<10^3$ CFU/g in food	*Sufu*	[20,66]
Clostridium perfringens	*C. perfringens* can produce enterotoxin Cpe in fermented soybeans. Consumption of contaminated products can lead to watery diarrhea.	*C. perfringens*: <10 CFU/g	*Sufu*	[20,67]
Salmonella enterica serotype Paratyphi B	Consumption of fermented soybean contaminated with *S. enterica* could cause gastroenteritis.	Consumption of $>10^8$ CFU/mL in skim milk induced clinical illness in humans	*Sufu* *Tempeh*	[20,22,68]
Bacillus subtilis, Staphylococcus pasteuri, Staphylococcus capitis	Consumption of products containing excessive amounts of histamine can lead to "scombroid fish poisoning", which results in the flushing of the face, neck, and upper arms, oral numbness and or burning, heart palpitations, asthma attacks, hives, and the disruption of the gastrointestinal system.	Histamine: <500 mg/kg	*Sufu* *Douchi* *Chunjang* *Doenjang* *Natto* *Ganjang*	[36–40,42,69]
Staphylococcus carnosus, *Candida* spp., *Enterococcus faecium*	Consumption of products containing excessive amounts of tyramine can lead to dietary-induced migraine, increased cardiac output, nausea, vomiting, respiratory disorders, and elevated blood glucose	Tyramine: <800 mg/kg	*Sufu* *Chunjang* *Doenjang* *Ganjang*	[31,31,39,40,42]
Bacillus licheniformis, *B. methylotrophicus*, *S. carnosus, E. faecium*, *Lactobacillus* spp., *Veillonella* spp., *Sphingobacterium* spp., *Flavobacterium* spp.	Ingestion of an excessive amount of putrescine and cadaverine could lead to oversaturation of enzymes in the human body that can break down BAs, and can lead to food intoxication	Lack of human studies; intake of 2000 mg/kg body weight in rats for both putrescine and cadaverine caused toxicity	*Douchi* *Soy sauce*	[38,42,70]
Aspergillus flavus, A. parasiticus, A. nominus, A. tamarii, A. pseudotamarii	Consumption of products containing high amounts of AFs can have carcinogenic, hepatotoxic, teratogenic, and mutagenic effects on the human body	Aflatoxins: <40 ppb	*Doenjang*	[51–53,71]
Aspergillus spp., *Penicillium* spp.	Consumption of products containing high amounts of ochratoxin A can lead to toxicity in the kidney and kidney tumors	Ochratoxin A: <50 ppb	*Doenjang* *Dwangjang* *Kanjang*	[53,59,61,71]
Rhizopus spp., *Aspergillus* spp.	Consumption of products containing high amounts of zearalenone can result in disruptions to estrogen receptors, leading to disruption to the physiological and metabolic system in the human body	Zearalenone <1000 ppb	*Tempeh*	[63,71,72]

proliferation of these pathogens. Ashenafi (1991) studied the growth of *L. monocytogenes* in traditionally fermented tempeh and observed growth from 300 CFU/g to 10^6 CFU/g over a 40-h fermentation period. In a similar study, the growth of *Salmonella infantis, Enterobacter aerogenes and E. coli* was evaluated during *tempeh* fermentation through intentional inoculation of the strains. The results showed that *S. infantis*, *E. aerogenes* and *E. coli* grew to 10^6–10^7 CFU/g in 40 h [30]. These studies suggest that the raw soybean material prepared for fermentation can act as a suitable reservoir for these pathogenic microorganisms to grow. Consuming contaminated fermented soybean products will likely put the public health at high risk and result in foodborne disease outbreaks, depending on the nature and extent of post-fermentation processing.

3.2. Biogenic amines

Biogenic amines (BAs) are commonly found in fermented soybean products and are mainly formed through the decarboxylation of free amino acids or through the transamination process of aldehydes and ketones by microorganisms during fermentation [31]. Consumption of moderate amounts of BAs has no adverse effects as they undergo detoxification by acetylation and oxidation reactions catalysed by intestinal amine oxidases, such as monoamine oxidase, diamine oxidase and polyamine oxidase [32]. However, ingesting an excessive amount can lead to oversaturation of these enzymes and may cause food intoxication [33]. Out of all the BAs, histamine and tyramine present the highest risk in inducing severe acute effects in humans. Excessive histamine consumption can lead to "scombroid fish poisoning", which results in the flushing of the face, neck, and upper arms, oral numbness and or burning, heart palpitations, asthma attacks, hives and the disruption of the gastrointestinal system [34]. Currently, the European Standard Commission Regulation (EC) 1441/2007 only regulates histamine levels in fish products [31] and no specific standards are set in place for the control of histamine in other food products. Tyramine is associated with a vasoconstrictor effect and excessive intake can cause dietary-induced migraine, increased cardiac output, nausea, vomiting, respiratory disorders and elevated blood glucose [34]. According to the European Food Safety Authority (EFSA), the daily intake of tyramine should not exceed 800 mg/kg of food?, while concentrations above 1080 mg/kg have been known to induce toxicity [31]. Formation of BAs occurs typically through a decarboxylation process of amino acids [31]. They naturally occur in most plant material as the process is often carried out by endogenous plant enzymes that are activated as a response to stress. However, the presence of high levels of BAs in fermented soybean products is often attributed to the decarboxylating activity of microorganisms [34]. Histidine and lysine can be converted to histamine and cadaverine by histidine decarboxylase (HDC) and lysine decarboxylase (LDC), respectively, while tyrosine is converted to tyramine via tyrosine decarboxylase (TDC). During fermentation, bacteria harboring genes that encode these decarboxylases can initiate the conversion of amino acids to BAs to increase intracellular pH and to maintain pH homeostasis, and secrete them into the environment [35]. Fermented soybean products contain abundant proteins and free amino acids, which act as precursors for the formation of BAs. Hence, the contamination of these microorganisms during spontaneous fermentation can promote the formation of excessive BAs.

High levels of BAs are more commonly found in fermented soybean products that are traditionally fermented, as spontaneous fermentation presents the risk of contamination by BA-producing microorganisms that exist in the environment. Very often, these products contain histamine levels above 500 mg/kg, which is the allowable limit suggested by the US Food and Drug Administration. Guan et al. (2013) studied 38 different *sufu* samples purchased from markets in different regions of China and analysed the samples for BAs. The study reported high concentrations of putrescine and tyramine in most of the samples, with 5 samples containing histamine levels over 50 mg/kg, posing histamine intoxication risk when consumed [36]. Similar results were also reported by Li et al. (2019), where 6.3 % of traditionally fermented *sufu* contained total BA contents over 1000 mg/kg. In addition, 26.6 % of the samples had high levels of histamine that could induce histamine poisoning, while 23.4 % of the samples had high levels of tyramine [37]. In traditionally fermented *douchi* samples, high levels of putrescine (10.89 mg 100 g^{-1}), cadaverine (12.14 mg 100 g^{-1}) and histamine (20.24 mg 100 g^{-1}) were reported, which individually do not exert toxic effects but collectively can indirectly aggravate the toxic effects of tyramine and histamine, by competing with detoxifying enzymes in the body [38]. Traditionally fermented Korean soybean pastes such as *chunjang* and *doenjang* were also to found to have dangerously high levels of BAs. Twenty-three traditionally fermented *doenjang* samples had average concentrations of histamine and tyramine levels of 267.9 mg/kg and 1266.6 mg/kg, respectively [39], while traditionally fermented *chunjang* had histamine levels of up to 273 mg/kg and tyramine levels of up to 131 mg/kg [40]. Commonly consumed fermented Japanese soybean products such as *natto* have also been reported to contain high levels of histamine of up to 1000 mg/kg [41]. These findings suggest that traditionally fermented soybean products can contain high levels of BAs, and excessive consumption can have health implications.

Metagenomic approaches have successfully identified microbes responsible for producing BAs during soybean fermentation. Analysis of BAs during *ganjang* fermentation revealed a steady increase in cadaverine, putrescine, tyramine, and histamine concentrations from the start of fermentation. Metagenomic and metatranscriptomic analyses revealed the presence of a total of 16 putative BA-producing decarboxylase sequences in *ganjang* samples. The results showed that LDC and HDC genes were each derived from a single genus, *Staphylococcus* and *Tetragenococcus* respectively; ornithine decarboxylase (ODC) genes were derived from *Bacillus* and *Halomonas*, while TDC genes were derived from *Tetragenococcus, Virgibacillus, Bacillus* and *Enteroccocus* [42]. Similar metagenomic techniques were used to analyse *sufu* samples with high BA contents, and the results showed that *Enterococcus, Lactobacillus* and *Lactococcus* were responsible for histamine production as they had high expressions of HDC genes. *Bacillus, Chryseobacterium, Kurthia, Lysinibacillus, Macrococcus* and *Streptoccocus* were found to decarboxylate tyrosine to produce tyramine. *Lactobacillus, Veillonella* and *Sphingobacterium, Flavobacterium* were found to have high expressions of ODC, and agmatine deaminase respectively, which contributed to the production of putrescine [43]. Adopting a metagenomic approach could provide a better understanding of which microorganisms contribute to BA production during fermentation at a genus level. However, studies have shown that BA-producing abilities of bacteria are strain specific. qPCR analysis of *Bacillus* species isolated from soybean fermentation showed that only

B. subtilis H'J53-3 had a markedly high expression of HDC and TDC genes, while *B. subtilis* HJ0-6, *B. subtilis* D'J53-4 and *B. idriensis* RD13-10 showed no expression of these genes [44]. The ability to produce BAs by isolated strains can be evaluated by culturing strains using respective amino acid precursors. Li et al. (2018) studied the BA-producing ability of strains isolated from traditionally fermented *douchi* in a medium containing 1 g/L of histidine, tyrosine, tryptophan, phenylalanine, ornithine monohydrochloride, lysine or agmatine sulfate salt and reported that *B. licheniformis* DC3-1, *B. licheniformis* JD18 and *Candida* sp. 4 TMS-2011 were able to convert histidine to histamine; *Staphylococcus carnosus* a23, *Candida* sp. 4 TMS-2011 and *Enterococcus faecium* RK 204 were responsible for tyramine production; *B. licheniformis* L5, *B. methylotrophicus* HB25, *S. carnosus* a23 and *E. faecium* RK 204 were responsible for putrescine production [45]. In addition, many decarboxylase genes associated with BA production are encoded in plasmids and can be transferred between microbes through horizontal gene transfer. Amplification of *Tetragenococcus halophilus* isolated from *doenjang*, which had high concentrations of tyramine, revealed that the strain harbored a homolog of the TDC gene *tdcA* in the plasmid pTDC-A [46]. Therefore, strains that are not known to produce BAs may acquire the ability to do so through horizontal gene transfer from BA-producing strains, which can contaminate the fermentation medium under uncontrolled fermentation processes.

These findings suggest a risk of exposing the fermentation medium to BA-producing microorganisms in traditional soybean fermentation techniques, resulting in the production of high levels of BAs that exceed recommended consumption levels. Furthermore, strains that initially do not produce BAs can potentially obtain the decarboxylase genes through horizontal gene transfer from BA-producing bacteria that exist in the environment. Metagenomic analysis of the contaminated samples can accurately identify strains that possess decarboxylase genes at the genus level. However, the ability of microorganisms to produce BAs seems to be strain specific. Hence, metagenomic analysis should be coupled with culturing the strains in an amino acid-rich medium to further understand their BA-producing abilities.

3.3. Mycotoxins

Mycotoxins are secondary toxic metabolites that are produced by certain fungal species which mainly contaminate agricultural produce such as cereals, and legumes [8]. The most common mycotoxins that are present in fermented soybean products are aflatoxins (AFs), ochratoxins and zearalenone. Among these three mycotoxins, AFs are of great concern due to their more severe harmful effects on human and animal health [47]. They have been classified by WHO as genotoxic and carcinogenic as they can induce carcinogenic, hepatotoxic, teratogenic and mutagenic effects when consumed or absorbed through the skin even in minute concentrations [48]. On the other hand, ochratoxins are classified as potential human carcinogens and have been shown to cause kidney toxicity in different animal species and kidney tumors in rodents [49]. While zearalenone is not classified as a carcinogen, studies show that it can bind to estrogen receptors due to its estrogen-like structure and interfere with the physiological and metabolic system in the human body [50].

The group of microorganisms that are mainly responsible for producing AFs belong to the genus *Aspergillus*, which include *A. flavus, A. parasiticus, A. nominus, A. tamarii* and *A. pseudotamarii*. These fungi can make their way into the fermentation by infecting the soybean crop or are introduced at the start of fermentation because of poorly controlled traditional spontaneous fermentation. Lee (2022) investigated the mycotoxin levels in 1436 samples across a period of three years in traditionally fermented soybean paste to confirm if AFB_1, AFB2, AFG1 and AFG2 were present in these samples. The AF concentrations ranged from 5.88 μg/kg to 281.92 μg/kg. In addition, the study reported that the margin of approach vales (MOE) of $<10,000$ for all age groups, indicating that there was a risk of excessive exposure to AFs for all age groups through the dietary intake of homemade soybean paste [51]. Other studies also reported similar results. Woo et al. (2019) reported significantly higher concentrations of AFB_1, AFG1, ZEN, ZAN, β-ZEL, FB1, and FB in homemade *doenjang* samples compared to commercial samples [52]. A comparison of AF concentrations in homemade *doenjang* and starter culture fermented *doenjang* revealed that contamination rates and levels of AF detected in the homemade samples were significantly higher than those detected among the starter culture fermented samples. In one of the homemade samples, a concentration of 273.3 μg/kg of AF was detected, which is 18 times higher than the permitted limit [53].

The most common and potent AFs, B1, B2, G1 and G2 are synthesized through the polyketide biosynthetic pathway. Mycotoxin production is strain specific, as the presence and expression of specific mycotoxin gene and gene clusters are responsible for the type and amount of mycotoxins synthesized [54]. Even though the AF gene clusters between *A. flavus* and *A. parasiticus* have a 90–99 % similarity in homology, *A. flavus* produces B-type AFs (AFB_1 and AFB_2) while *A. parasiticus* produces B- and G-type AFs (AFB_1, AFB_2, G1 and G2) [55]. Studies showed the cluster of 30 AF pathway genes (*aflR* antisense gene, 2 sugar utilization genes, 1 ORF gene) in the 80 kb DNA region in *A. flavus*, while in *A. parasiticus*, only 24 AF pathway genes (3 sugar utilization genes, one ORF gene) were found clustered within the 80 kb DNA region [56]. Evidence suggests that the accumulation of AFs is most severe when soybean fermentation is contaminated with *A. flavus* and *A. parasiticus* at the starting phase. Kumar et al. (2022) studied the growth of *A. flavus* during different stages of *doenjang* fermentation by inoculating with *A. flavus* at each stage of fermentation. The results showed high growth of *A. flavus* and large amounts of AF production at the *meju* preparation stage after incubation. However, when *A. flavus* was inoculated in the primary and secondary fermentation stage, the fungus was absent and no AF production was found at the end of fermentation [57]. Competitive interactions with the native microorganisms in the *doenjang* medium that have proliferated during the initial stages of fermentation may have inhibited the growth of *A. flavus* during fermentation. In addition, even if *A. flavus* were viable during the fermentation process, the presence of mycotoxin-degrading microorganisms may reduce the levels of mycotoxins produced [58]. However, if the fermentation is heavily contaminated with mycotoxins from the start of the process, insufficient degradation of mycotoxins may occur, and the final fermented product may still contain high levels of mycotoxins. Jeong et al. (2019) studied the natural occurrence of AFs and ochratoxin A (OTA) in soy sauce and soybean paste, and reported that even after degradation of AFs and OTA during fermentation, the levels of AFB_1 and OTA exceeded the recommended allowable limit, and had concentrations of 11.9 μg/kg and 190.4 μg/kg, respectively [59].

Another mycotoxin that is commonly found in fermented soybean foods is ochratoxin, with the more potent toxic form being OTA. It has been detected in *doenjang* and is especially prevalent in samples that have been traditionally fermented [60]. Kang et al. (2023) reported higher concentrations of OTA in homemade *doenjang* compared to starter culture fermented samples. Jeong et al. (2019) also reported that 5 samples out of 45 traditionally fermented soybean paste in South Korea were found to have OTA concentrations above the permissible limits set by the Korean Food & Drug Administration. *Aspergillus* spp. and *Penicillium* spp. were found to be the main group of microorganisms contributing to OTA production in fermented soybean products such as *meju, dwangjang and kanjang* [61].

The third mycotoxin that is commonly found in fermented soybean foods is zearalenone (ZEN), which is produced by *Fusarium* species such as *F. roseum* 'Graminearum' and *F. sporotrichiodes* [62]. These fungi frequently infect the soybean crop and produce ZEN, which can interact with estrogenic receptors ERα and Erβ, and cause hormonal disorders. Recent studies have shown that the toxicity of ZEN mainly arises from the transformation of ZEN to its reductive metabolites α-ZEL and β-ZEL, where α-ZEL is assigned a potency factor 60 times higher than β-ZEL [63]. The conversion of ZEN is mainly carried out by *Rhizopus* spp. and *Aspergillus* spp. [64], which are the main fermenting microorganisms in tempeh and soy sauce production. Analysis of tempeh products from Indonesian markets showed contamination of the products with ZEN and α-ZEL. Borzekowski et al. (2019) studied the monoculture fermentation of tempeh with *A. oryzae* and *Rhizopus* spp., and reported that samples fermented with *Rhizopus* spp. had a significant increase in α-ZEL concentrations, and a decrease in ZEN concentrations, while *A. oryzae* showed no conversion of ZEN to α-ZEL [63]. However, Brodehl et al. (2014) reported that culturing *A. oryzae* in ZEN media showed a 50 % conversion of ZEN to its metabolites, suggesting that the conversion of ZEN to its metabolites might differ between different strains, and the mechanisms and ability of strains to carry out this conversion should be studied at a genomic level.

These studies show that the soybeans are an excellent medium for the proliferation of mycotoxin-producing fungi. Spontaneous fermentation increases the risk of contamination with these microorganisms, which leads to continuous production of mycotoxins throughout the fermentation and high concentrations of mycotoxins in the fermented soybean product.

4. Using starter culture to mitigate food safety risks in traditional fermented soybean products

Currently, common processing methods for fermented soybean products include heat treatment and the addition of antimicrobial substances to eliminate spoilage and pathogenic microorganisms. However, consistent and thorough heat processing of fermented soybean foods remain as a challenge due to the thick viscosity of many products, such as fermented soybean pastes. Lee et al. (2012) reported that continuous heat pasteurization of *doenjang* and *gochujang* at 75–85 °C for 30 min could only deactivate most of the fungi present, but heat-resistant microorganisms and spores such as *Bacillus* remained to be isolated [73]. In addition, spoilage microorganisms often produce heat-stable toxins such as *cereulide*, *Staphylococcus* toxins and mycotoxins that cannot be eliminated with heat treatment [8]. Several new processing techniques have been studied, such as the application of high pressure and ohmic heating. Cho and Song (2021) studied the use of ohmic heating on *doenjang* and its effects on microbial reduction, reporting that the temperature rise of ohmic heating was 2–4 times faster than that of conventional heating methods, and minimized damage to the taste, flavour, colour, and viscosity of the product [74]. However, fermented soybean products are rich in bioactive compounds such as antioxidants and vitamins [75], and heat processing risks degrading these compounds to ultimately reduce the nutritional value of the product.

Control of BAs in fermented soybean foods mainly involves the addition of food additives and natural antimicrobial compounds at the start of fermentation, such as nicotinic acid, potassium sorbate, sodium benzoate and sodium chloride. Addition of nicotinic acid, or niacin, has been proven to be the most effective, where research shows that addition of 0.20 % in *cheonggukjang* samples resulted in an approximately 83 % reduction in BAs as compared to samples without these additives, through inhibiting the growth of BA-producing LAB [33]. However, even though nicotinic acid is added in small amounts, it may cause the potential side effects of consumption, such as 'niacin flush', which is the burning, tingling sensation in the face and chest and red or flushed skin. Hence, other natural compounds should be considered to replace these additives [76].

Reduction and elimination of mycotoxins in fermented soybeans have been widely researched, mainly consisting of the use of fungicides to control mycotoxin contamination in the raw soybean material. This includes the use of azoles and strobilurins, which are the most widely used fungicidal chemicals in agriculture, to eliminate mycotoxin-producing *Aspergillus* spp [77]. However, there are strict regulations with regards to the addition of synthetic chemicals to food crops and this practice presents the risk of the fungal strains eventually developing resistance to these fungicides which will render them ineffective. Heating of soybean paste models to reduce mycotoxins has also shown promising results, for instance, Lee et al. (2015) reported a 97.9 % and 33.6 % reduction of AFs in soybean milk and steamed soybeans, respectively, when the products were subjected to heat treatment at 150 °C for 90 min. However, as mentioned above, most fermented soybean foods are highly viscous, and heat treatment can result in the degradation of nutrients in the product [78].

4.1. Elimination of indigenous contaminants and harmful pathogens

Outbreaks arising from consumption of traditional soybean fermented foods are not common, but they still occur. Inoculating a robust starter culture at the start of fermentation can reduce the risk of pathogen growth, as it can outcompete indigenous contaminants and harmful pathogens through rapid substrate utilization. In addition, starter cultures can produce antimicrobials such as acids, mycosins and bacteriocins that can inhibit or even eliminate indigenous contaminants present in the starting material.

Studies have successfully used selected *B. subtilis* strains as a biocontrol agent in a fermentation starter culture in producing fermented soybean products. *Cheonggukjang* inoculated with *B. subtilis* SN7 together with intentional contamination of *B. cereus* ATCC 14579 achieved *B. subtilis* SN7 counts of 8.50 log CFU/g after 24 h of fermentation, while no growth of *B. cereus* ATCC 14579 was

observed. In addition, the presence of *B. cereus* ATCC 14579 was not detected from *cheonggukjang* stored at 4 °C after 6 months. In comparison, spontaneously fermented *cheonggukjang* showed high growth of up to 8.62 log CFU/g of *B. cereus* ATCC 14579 after 24 h without *B. subtilis* SN7 inoculation [79]. Similar results were found when *B. subtilis* was used as a starter culture in other traditionally fermented soybean products. Dajana et al. (2012) reported that *thua nao* fermented with *B. subtilis* TN51 exhibited antimicrobial activity against Gram-positive pathogens, while Sopheap et al. (2019) demonstrated high concentrations of bacteriocins to be produced during *sieng* fermentation, and successfully eliminated *B. cereus* to ensure the safety and the stability of the product. The ability of *B. subtilis* in inhibiting the growth of undesirable native microorganisms could be attributed to the production of bacteriocin-like substances (BLISs), in which the type of BLISs produced are strain specific [80]. Yang & Hae (2007) reported that the production of BLIS in the analysis of *B. subtilis* MJP1 isolated from *meju* displayed antimicrobial activity against various species of Gram-positive bacteria, yeasts, and moulds. Further analysis revealed the presence of two bacteriocin-like substances that possess both antifungal and antibacterial activities, which had heat stable properties and could withstand proteolytic enzymatic treatment but only remained effective in the pH range of 6.0–10.0 [81]. Although bacteriocins or BLIS produced by *Bacillus* spp. that are stable at low pH have been little studied in soybean fermentation, bacthuricin F4 has been industrially used [80]. This is a bacteriocin produced by *B. thuringienesis* subsp. *kurstaki* BUPM4z that is reported to be active against *Bacillus* species, heat stable, and resistant to acidic pH but susceptible to protease degradation. Further verification is thus needed to determine the efficacy of this bacteriocin in a soybean fermentation medium.

It is important to note that a myriad of microorganisms often participate in the fermentation of a soybean product. Even though the production of antimicrobials such as bacteriocins seems like a natural and effective way to reduce or eliminate pathogenic and spoilage microorganisms, this may also prevent the growth of desirable microorganisms that play an important role in the fermentation. Hence, other methods of contamination control should be considered. Biosurfactants are another class of natural compounds produced by some strains isolated from soybean fermentation shown to be effective against pathogenic microorganisms. Cao et al. (2009) reported *B. subtilis* subsp. *natto* TK-1 produce lipopeptides that can act as a biosurfactant with anti-adhesive and antimicrobial properties. *In vitro* testing of the isolated biosurfactant produced by *B. subtilis* TK-1 showed that it can significantly inhibit the adhesion of *S. typhimurium, E. coli* and *S. aureus*. Addition of the biosurfactant onto an agar plate showed zones of inhibition with large diameters on plates amassed colonies of with *Botrytis cinerea*, *Fusarium verticillioides* (*formerly F. monoliforme*), *Micrococcus luteus* and *S. typhimurium*, with the antimicrobial activity increasing with biosurfactant concentration [82].

Another effective natural antimicrobial compound is β-glucan, which is a soluble fibre found in the cell walls of bacteria, fungi, yeasts, and certain plants. They have been commonly used in the industry as an alternative to antibiotics due to the development of antibiotic resistance [83]. Studies show that β-glucan can stimulate phagocytosis and suppress pathogen invasion of *Salmonella* spp. in the gut intestine, while maintaining the integrity of the mucous protective layers for the expulsion of enteric pathogen invasion [83]. It can also be applied to soybean fermentation, where production of β-glucan can reduce the growth of pathogenic microorganisms. Rizal et al. (2021) reported that co-inoculation of *S. cereivisiae* and *R. oligosporus* in *tempeh* fermentation resulted in an increase in β-glucan concentrations and antibacterial activity against *E. coli* compared to fermentation with *R. oligosporus* alone [84].

Hence, it is effective and cost-efficient to use starter cultures to eliminate indigenous and adventitious contaminants and pathogenic microorganisms in the fermentation of soybean products. This provides an alternative to traditional soybean substrate processing methods such as the application of heat and the addition of antibiotics.

4.2. Elimination or reduction of biogenic amines

Using starter cultures to ferment soybeans has been reported to have significantly lower BA contents as compared to spontaneous fermentation. *Shuidouchi* naturally fermented at 37 °C for 4 days showed BA concentrations higher than 280 mg/kg. However, inoculation of *B. subtilis* T2 resulted in a 82.13 % reduction in total BA content [85]. Similar results were found in *miso* fermentation, where Lee et al. (2016) reported that the addition of *Lactobacillus (now Lactiplantibacillus) plantarum* D-103 to the starter culture lowered the histamine content to 58 % and total BA content to 27 % of the control [86].

Adopting starter culture technology to eliminate or reduce BAs can be achieved. This entails the selection of strains that can rapidly proliferate in the soybean substrate and do not have the ability to produce BAs, as these strains can outcompete BA-producing microorganisms through the rapid use and depletion of nutrients and/or hinder their growth through the production of antimicrobials. Li et al. (2018) reported that inoculation of *B. subtilis* HB-1 and *S. pasteuri* JX-2 at the start of spontaneous fermentation of *douchi* significantly reduced total BA concentrations. Even though the strains did not exhibit BA-degrading abilities, their growth during fermentation can inhibit the growth of BA-producing strains naturally occurring in the fermentation medium, by competing for nutrients [45]. As reported previously, some *Bacillus* strains can produce antimicrobials that eliminate pathogenic and spoilage microorganisms. In addition, they can reduce the growth of BA-producing microorganisms during fermentation. Inoculation of bacteriocin-producing *Bacillus* spp. DB047, *B. licheniformis* DB612 and *B. subtilis* DB821 resulted in significantly decreased cell counts of BA-producing bacteria such as *E. faecium* D12 and *E. faecalis* D51 when inoculated together during fermentation. A reduction of 36.07 % and 39.6 % in histamine and tyramine was reported when compared to the control [87].

Even though the use of starter cultures in soybean fermentation has been shown to effectively control BA concentrations, traditional fermentation methods that eschew their use may still be preferred, as spontaneous fermentation can introduce microorganisms that produce desirable compounds that contribute to the distinct flavour profile of the food product, even if such microorganisms can produce BAs. Recent studies have shown that replacing 'traditional' strains with a combination of strains that do not produce BAs can produce fermented soybean products with superior flavour profiles. Shukla et al. (2005) used a consortium of *B. subtilis*, *A. oryzae* and *Mucor racemosus* to ferment *doenjang* with negligible BA production and the highest sensory scores compared to traditionally

fermented *doenjang*. Similar results were also reported in *douchi* fermentation, where rapid fermentation of *douchi* using *A. oryzae* 2339, *A. oryzae* 41380, *A. oryzae* 40188, *M. racemosus*, *Actinomucor elegans*, and *M. wutungqiao* resulted in low levels of total BA content (40.95 mg/kg) and the low presence of ornithine and tyrosine decarboxylases [88].

When BA-producing strains cannot be eliminated from the starter cultures, co-inoculation with species that harbour BA-degrading abilities may be considered. Some microorganisms contain genes that encode for amine-degrading enzymes, such as monoamine oxidases, diamine oxidases and multicopper oxidases, which can degrade BAs into non-toxic products [89]. Studies have successfully detected amine oxidase genes and their expression levels with transcriptional and translational analyses. Eom et al. (2015) investigated the ability of *B. subtilis* HJ-06, *B. subtilis* D'J53-4 and *B. idrensis* RD13-10 to express amine oxidase genes. The strains showed the ability to degrade histamine and tyramine when grown in LB broth containing 0.25 % the BAs. However, the three strains showed different degradation rates. Both *B. subtilis* HJ0-6 and *B. subtilis* D'J54-3 showed lower histamine H3 receptor expressions compared to *B. idrensis* RD13-10, leading to higher levels of histamine in both *B. subtilis* strains [44]. It is also important to examine both BA-degrading and BA-producing abilities, as some microorganisms have been shown to harbour both capabilities. *B. subtilis* GD-4 and *Candida* sp. JX-3 were reported to have high BA-degrading abilities, with a 50.73 % and 56.25 % degradation rate of histamine respectively. However, inoculation of the respective strains in spontaneous fermentations of *douchi* for 7 days showed significantly higher total BA contents, as these strains were also capable of producing large amounts of BA [45]. Hence, selecting strains without BA-producing abilities seems to be a more effective way to control BA production during fermentation. Table 2 shows a selection of strains that can be used in starter cultures to control BA production in manufacturing traditional fermented soybean products.

4.3. Elimination and reduction of mycotoxins

Fermented soybean products made using starter culture technology have lower mycotoxin contents as compared to traditionally fermented products. Lee et al. (2022) reported that commercial Korean soybean paste fermented with a starter culture had significantly lower AF concentrations compared to traditional homemade soybean paste. The microorganisms selected for starter culture fermentation can reduce and eliminate mycotoxins in at least one of the three possible ways. Firstly, the selected microorganism does not have the ability to produce mycotoxins; secondly, the selected microorganism can hinder the growth of mycotoxin-producing

Table 2
Desirable strains used as starter cultures for fermentation of traditional fermented soybean products.

Food product	Strains in starter culture	Benefits in ensuring food safety	Reference
Cheonggukjang	*B. subtilis* SN7	Prevents the growth of *B. cereus*	[79]
Thua nao	*B. subtilis* TN51	Exhibits antimicrobial activity against Gram-positive bacteria	[96]
Sieng	*B. subtilis*	Produces bacteriocins that impede growth of *B. cereus*	[16]
Meju	*B. subtilis* MJP1	Produces bacteriocin-like substances displaying antimicrobial activity against Gram-positive bacteria, yeast, and moulds	[81]
Natto	*B. natto* TK-1	Produces lipopeptides with anti-adhesive and antimicrobial properties that can inhibit adhesion of *S. typhimurium, E. coli* and *S. aureus*	[82]
Tempeh	*S. cerevisiae, R. oligosporus*	Increases β-glucan content and antibacterial activity against *E. coli*	[84]
Shuidouchi	*B. subtilis* T2	Reduces total BA content by 82.13 % as compared to spontaneously fermented samples	[85]
Miso	*L. plantarum* D-103	Reduces total histamine content by 58 % and total BA contents by 27 % as compared to control	[86]
Douchi	*B. subtilis* HB-1, *S. pasteuri* JX-2	Inhibits the growth of BA-producing strains and significantly reduces total BA concentrations as compared to uninoculated samples	[45]
Fermented soybean (unspecified product)	*Bacillus* spp. DB047, *B. licheniformis* DB612, *B. subtilis* DB821	Significantly decreases cell counts of BA-producing bacteria such as *E. faecium* D12 and *E. faecalis* D51. Reduces 36.07 % and 39.6 % of histamine and tyramine when compared to uninoculated samples	[87]
Doenjang	*B. subtilis, A. oryzae, M. racemosus*	Negligible BA production with higher sensory scores compared to traditionally fermented *doenjang*	[97]
Douchi	*A. oryzae* 2339, *A. oryzae* 40188, *A. oryzae* 40188, *M. racemosus, Actinomucor elegans, M. wutungqiao*	Low levels of BA content and rapid fermentation process	[88]
Thua nao	*B. licheniformis*	Inhibits the growth of mycotoxin-producing *Aspergillus* spp., reduces AFB_1 and ochratoxin concentrations by 74 % and 92.5 %, respectively.	[98]
Meju	*A. flavus* M2040	Inhibits the production of AFB_1 when co-cultured with mycotoxin producing *A. flavus* 3357	[91]
Chinese fermented soybean (unspecified product)	*A. niger* FS10	Results in shrinkage of spores and conidia, and severe damage and collapse of the cell wall of mycotoxin-producing *A. flavus* when incubated together	[92]
Meju	*A. oryzae* MAO 103 *A. oryzae* MAO 104	Reduces the mutagenic effects of AFB_1 over 14 days	[94]
Doenjang	*B. albus* YUN5	Cell-free supernatant of *B. albus* YUN5 extensively degrades AFB_1, even after heat treatment	[95]

strains; thirdly, the selected microorganism can produce enzymes that eliminate the mycotoxins.

The identification of strains with mycotoxin-producing abilities can be carried out with molecular methods. Non-aflatoxigenic *A. flavus* and *A. oryzae* in *meju* fermentation had high mutation frequencies in the AGC region, and strains with deletions in the AGC region did not have AF-producing capabilities [90]. However, contamination with mycotoxin-producing *A. flavus* often occurs during the crop growth or harvesting stage, and strategies to inhibit or reduce the production of mycotoxins during fermentation should be considered. Strains that can inhibit the growth of these contaminants and/or strains that harbour the genes encoding for enzymes that can degrade mycotoxins can be ultimately used to control the level of mycotoxins in the fermented samples. Studies have successfully identified genes that encode for toxins or enzymes that when expressed, can inhibit the growth of mycotoxin-producing *Aspergillus* strains during fermentation. Petchkongkaew et al. (2008) isolated some mycotoxin-degrading strains from *thua nao* and reported the ability of *B. licheniformis* to inhibit *Aspergillus* growth and reduce AFB_1 and ochratoxin concentrations by 74 % and 92.5 %, respectively. *A. flavus* M2040 isolated from *meju* was reported to inhibit the production of AFB_1, when co-cultured with the mycotoxin-producing *A. flavus* 3357 and had higher inhibition rates compared to Afla-Guard, a common natural biocontrol agent used to reduce mycotoxin contamination in food products. In addition, autoclaved cell-free culture filtrates of *A. flavus* M2040 did not show the same inhibitory effects, suggesting that the growth inhibition of *A. flavus* 3357 was a result of strong competition for nutrients in the fermentation medium. Genome sequencing revealed the presence of the gene *g11617*, which was identified as "*Clostridium* epsilon toxin ETX/*Bacillus* mosquitocidal toxin MTX2", and genes *g1677* and *g1678*, which were identified as "mycotoxin biosynthesis protein UStYa-like", all of which could encode for enzymes that produce toxins inhibiting the growth of AFB_1-producing strains [91]. Other non-mycotoxin producing *Aspergillus* species have also been reported to inhibit the growth of *A. flavus*. Xu et al. (2013) reported that analysis of the 18S rDNA of *A. niger* FS10 isolated from Chinese fermented soybean showed the presence of genes that when expressed, could inhibit spore germination, mycelial growth, sporulation and AFB_1 production of *A. flavus*. Optical microscopy results revealed the loss of germ tube and shrinking of *A. flavus* spores, after incubation with *A. niger* for 2h, followed by shrinkage of the conidia after 6 h and severe damage and collapse of the cell wall after 10 h [92]. Yang et al. (2020) used transcriptome sequencing to study the interactions of non-AF producing *A. oryzae*, commonly used in soybean fermentation, and *A. flavus*, and reported that the treatment with cell filtrate of *A. oryzae* downregulated the specific AF-producing pathway regulator (AflS). In addition, comparative transcriptomics revealed that in the presence of *A. oryzae* filtrates, a significant reduction of *brlA* and *abaA* expressions were reported, resulting in the downregulation of conidiation-specific genes of *A. flavus* and suppression of asexual development [93]. More genomic studies to identify genes that inhibit the growth of *A. flavus* should be conducted, to identify more strains to be used in starter cultures that can act as a biocontrol agent to reduce and eliminate mycotoxins.

Apart from the inhibition of growth, selecting strains with genes that encode for mycotoxin degradation or mycotoxin-detoxifying enzymes should be considered. Lee et al. (2017) showed a 90 % reduction of AFB_1 over 14 days in *A. oryzae* MAO 103 and *A. oryzae* M 104, both isolated from *meju*. They reported a reduction of the mutagenic effects of AFB_1. This suggests that the strains likely possessed enzymes that metabolized AFB_1 to biodegradation products with different structural and chemical properties, resulting in the loss of mutagenicity of AFB_1, as no AFB_1-degradation products were present in the samples inoculated with the two strains [94]. Apart from

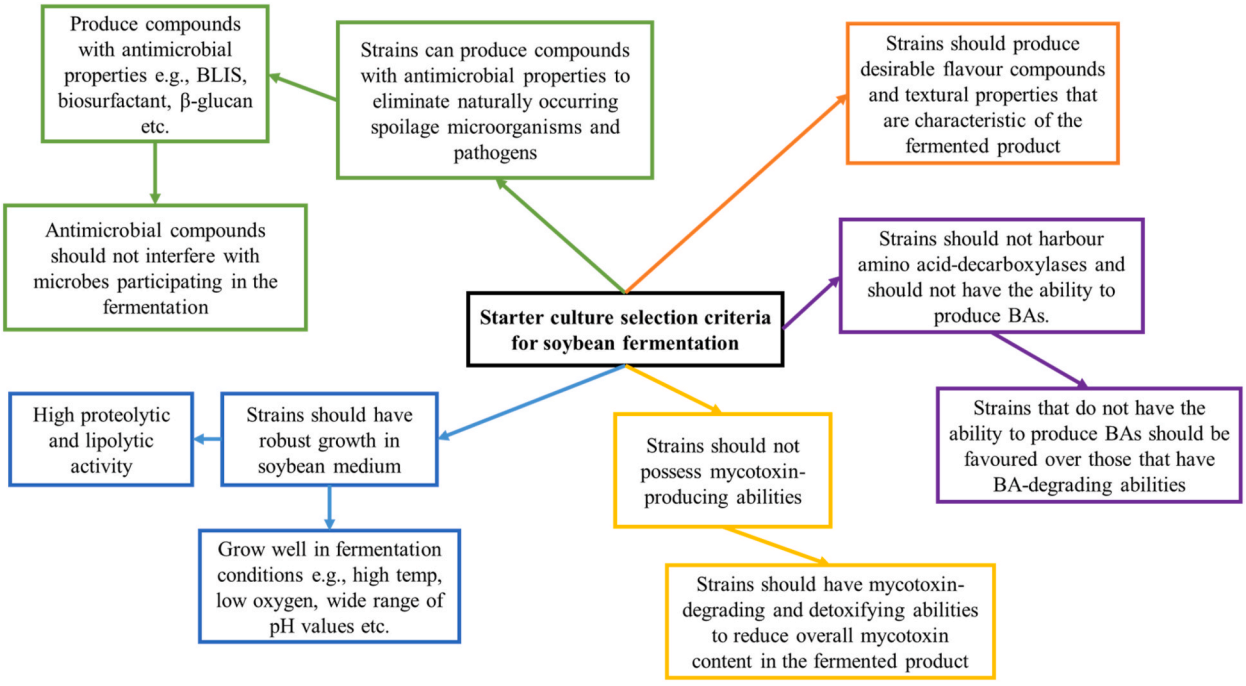

Fig. 2. A summary of the criteria for starter culture selection for a robust soybean fermentation process.

inoculating with mycotoxin-degrading and mycotoxin-detoxifying strains, it is also possible to inoculate fermentation cultures with the cell-free supernatant of mycotoxin-degrading strains to lower mycotoxin levels, as this eliminates the need to introduce new strains into the fermentation culture, which can potentially alter the flavour and texture of the final product. Kumar et al. (2023) studied the degradation mechanism of AFs by *B. albus* YUN5 isolated from *doenjang* and reported that the cell-free supernatant (CFS) of *B. albus* YUN5 had the highest degradation of AFB_1, as compared to insignificant degradation observed in the intracellular fraction, viable cells, and cell debris. Heating the CFS to 100 °C retained the AFB_1 degradation ability, suggesting that components other than proteins and enzymes were responsible for the degradation. Analysis of the degradation products suggested either the difuran or lactone ring of AFB_1 and the lactone ring of AFG_1 to be the main target site for CFS of *B. albus* YUN5. In addition, inoculation of the CFS of *B. albus* YUN5 and *B. albus* into AF-spiked doenjang showed a significant reduction of AFB_1 and AFG_1 compared to autoclaved samples [95].

These studies illustrate the advantages of adopting starter culture technology to produce fermented soybean products. As the soybean crop is often susceptible to infections by mycotoxin-producing *Aspergillus* strains during the harvesting stage, it is difficult to avoid having high levels of mycotoxins in the raw material used for soybean fermentation. Hence, selecting strains that have mycotoxin-degrading and mycotoxin-detoxifying activities seems to be the most effective in reducing and eliminating mycotoxins in the final fermented product to ensure that it is safe for consumption. Table 2 illustrates a summary of desirable strains that can be used in starter cultures for the fermentation of traditional fermented soybean products.

5. Selection criteria of starter cultures for assuring safety of fermented soybean products

The main goals of a food fermentation process are to eliminate safety hazards, preserve food quality and deliver a safe product for consumption. As illustrated above, introducing undesirable microorganisms into the raw ingredients and through human contact is unavoidable during food manufacturing, as these microorganisms are ubiquitous in the environment. Using starter cultures for fermentation serves as an effective and cost-efficient approach to minimizing food safety risks and preserving nutrients found in the fermented products which may otherwise be lost in traditional processing.

Consideration of the data discussed above identifies some desirable criteria in selecting strains to design starter cultures for soybean fermentation (Fig. 2). The criteria for designing a strong starter culture for successful soybean fermentation are listed below:

a. The strains should have robust growth in the soybean medium during fermentation. Strains that are used in starter culture fermentation often have high proteolytic and lipolytic activity due to the high fat and protein content of soybeans. For example, in *tempeh* fermentation, *R. oligosporus* and *R. oryzae* were chosen as the starter cultures as they had high proteolytic activities that could release significant amounts of amino acids that contribute to the characteristic texture and flavour of *tempeh* [99]. Selecting the desirable candidates is highly dependent on the type of fermentation chosen (submerged, solid state, mixed) and the overall conditions in the medium (e.g., high salt, low pH). These strains can be characterized in the soybean fermentation medium under different conditions such as temperature, oxygen level, pH values, and salt concentrations to assess their ability to proliferate as the fermentation progresses.

b. The strains can produce compounds with antimicrobial properties

- Raw soybean material is often contaminated with foodborne pathogens and undesirable filamentous fungi. Strains that can produce compounds with antimicrobial properties (e.g., BLIS, biosurfactants, and β-glucan) can eliminate these naturally occurring microorganisms or reduce their growth. This will ensure more successful fermentation with lower risks of pathogenic and spoilage microorganisms dominating the fermentation. Screening for antagonistic activity and characterization of the generated antimicrobial compounds should all be performed [100–103].

c. The strains should not harbour amino acid decarboxylases and should not be able to produce BAs. In addition, the strains should possess genes encoding for amine-degrading enzymes.

- As mentioned earlier, strains that do not have the ability to produce BAs should be favoured over those that have BA-degrading abilities, as these strains have a more significant impact on the final levels of BA. Detection of the BAs and their degradation can be performed with different methods, [104–107].

d. The strains should not possess mycotoxin-producing abilities. They should also possess genes that encode for mycotoxin-detoxifying or mycotoxin-degrading enzymes.

- Contamination with *Aspergillus* spp. that produce mycotoxins in the raw soybean material is common. Hence, selecting strains that have mycotoxin-degrading and detoxifying abilities is important for reducing the overall mycotoxin content. An overview of traditional and advanced analytical techniques for screening and detection of mycotoxins, and their biocontrol in foods can be found in Refs. [106,108–110].

e. Advanced technology applications

Designing new and improved strains using modern genetic and metabolic engineering tools to remove the toxic end products of metabolism or produce desired fermentation metabolites by the selected strains are promising strategies for the future. The availability of the complete genome sequence of many food-grade microorganisms (generally recognized as safe, GRAS) facilitates this process. For example, genome-wide screening using clustered regularly interspaced short palindromic repeats with Cas9 (CRISPR-Cas9) to identify the AFB_1 in f fungal, and bacterial toxins, as well as their target tissue has been reported [111–113]. These CRISPR tools can remove undesirable genes, and both inhibit and activate gene expressions. Similarly, the application of *in silico* investigations such as omics and metagenomic studies allows for the screening of microbial diversity in the fermentation mass, identifying the predominant species, and understanding the metabolism, genetics and physiology of selected microbial strains [114,115]. Furthermore, advanced molecular

techniques can also be used to modulate the metabolic pathways of microorganisms for enhancing the sensory quality of the final products, for instance, by removing lactate dehydrogenase (LDH), the enzyme directly responsible for reducing pyruvate to lactate and indirectly generating flavour compounds such as diacetyl in LAB [116]. These techniques will significantly improve the characteristics of starter cultures.

6. Conclusion

Using starter cultures is an effective and cost-efficient technique for producing fermented soybean products without the food safety risks inherent in traditional fermentation methods. The selection of desirable microorganisms for a robust fermentation can ensure the elimination of pathogenic microorganisms that can cause foodborne illness outbreaks. In addition, it presents a natural way to reduce BA and mycotoxin production during the fermentation process and can serve as a suitable alternative to traditional processing methods such as heating and the addition of food additives. However, changing the microbial population will inadvertently result in alterations to the texture and flavour of the traditional product. Hence, further research and development on starter cultures, with the incorporation of sensory studies, should be considered, to ensure that the product is not only safe for consumption, but also organoleptically desirable.

Data availability

Data included in the article/supplementary material/referenced in article.

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CRediT authorship contribution statement

Xin Hui Chin: Writing – review & editing, Writing – original draft. **Hosam Elhalis:** Writing – review & editing. **Yvonne Chow:** Writing – review & editing, Supervision. **Shao Quan Liu:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

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