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INVITED REVIEW

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Food safety considerations in the production of traditional fermented products: Japanese rice koji and miso

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Abstract

While established in Asia, rice koji and miso are fermented foods that are becoming more popular in western countries. They have been shown to contain a variety of microorganisms, consisting of bacteria, yeasts, and fungal species. Many contemporary miso varieties are not pasteurized as consumers are looking for more natural products, and/or have the desire to consume fermented foods containing live microorganisms. While correctly prepared fermented foods are rarely associated with food safety outbreaks, incidences have been recorded. On these occasions, pathogenic, or spoilage microorganisms were introduced into the products from external sources such as the raw material or the processing environment. Consequently, hygiene and fermentation conditions need to be carefully monitored to ensure food safety. Furthermore, many of the production steps during koji and miso manufacture do not fit into contemporary food safety guidelines for foods. Although pH is a required food safety hurdle for fermented foods, this does not apply to nonacidic foods such as koji or miso. This review focuses on control of microbial pathogens and discusses the processes of miso fermentation, and how fermentation of rice koji and miso fits with current food safety hurdles in western countries.

1 | INTRODUCTION

Fermentation is an ancient form of food processing. The fermented product will typically be more shelf stable than its constituents and will have quite different chemical and physiological properties. Historically fermentations were largely spontaneous, while a more modern definition of fermentation, "foods made through the desired microbial growth and enzymatic conversions of food components" (Marco et al., 2021) places an emphasis on "desired" microbes. These desired microorganisms may be either inoculated onto or found naturally on the food or in the environment. It is important that the desired fermentation microbes grow and undesired microbes do not. To ensure this happens, correct environmental conditions, such as temperature and oxygen requirements that are appropriate for the type of fermentation need to be maintained (Adams & Moss, 2008). A ferment that has desired microbes and optimal conditions brings about a change in the food which preserves, increases digestibility (Sanlier, Gokcen, & Sezgin, 2019), and results in the creation of new flavors.

1.1 | Microbial outbreaks in fermented foods

Fermented foods that are made correctly are rarely associated with food safety outbreaks (Marco et al., 2021). However, fermentation still needs to be monitored to ensure safety, as microorganisms such as *Clostridium botulinum*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* are potential food safety hazards due to contamination from the raw materials. Outbreaks from the consumption of contaminated fermented foods that have been reported: *Bacillus cereus* was identified as the cause of illness in douchi (fermented black beans) in China (Zhou et al., 2014); *C. botulinum* was the cause of botulism from the consumption of home fermented tofu in New York (Centers for Disease Control and Prevention [CDC], 2013); *Salmonella* Weltevred as found to be the cause of illness from a fermented cashew nut cheese in Canada (Schmitt, Yu, Greve, & McIntyre, 2018); and *E. coli* was associated with two outbreaks in middle schools in South Korea (Shin et al., 2016) where gastroenteritis was caused by consumption of tainted cabbage kimchi and young radish kimchi.

1.2 | Intrinsic safety of fermented foods

Although outbreaks have occurred in fermented products, they are rare. Undesirable microorganisms are kept in check by several processes. During fermentation, for example, desirable microorganisms may produce antimicrobials that reduce the risk of contamination from both spoilage and pathogenic organisms, or the production of acidic metabolites reduce the pH and make conditions unfavorable for many unwanted organisms (Adams & Mitchell, 2002). Maintaining optimal fermentation conditions also contributes to food safety, by allowing prolific growth of the desired microorganisms, which will then out-compete undesirable microorganisms (Hutkins, 2019). To successfully ferment foods specific parameters including pH, temperature, and water activity (A_w) need to be carefully monitored to encourage target microorganisms and inhibit spoilage and pathogenic microorganisms (Adams & Moss, 2008). Any variations in fermentation parameters can influence which microorganisms proliferate, and if a specific parameter changes, other parameters may also need to be modified to ensure that food safety and quality of the product is maintained.

1.3 | Traditional miso fermentation

Miso is a traditional fermented soybean paste from Japan, where it has been made for thousands of years, and is assumed to be safe for consumers (Kusumoto et al., 2021; Tanaka, Kushiro, & Manabe, 2006). It has recently become increasingly popular in western countries, where it is used as a versatile flavoring for miso soup, marinades, and desserts. Miso is different from many fermentations in that its production is a two-stage process (Allwood, Wakeling, & Bean, 2021). The first step is the production of koji, where a substrate (typically rice) is inoculated with a filamentous fungus, such as *Aspergillus oryzae*, and fermented at 30°C for 48 hr. Second, the koji along with salt are then added to a second substrate, which is fermented by yeasts and bacteria for between 2 and 24 months (Shurtleff & Aoyagi, 2018).

Both finished koji and miso have been shown to contain a variety of microorganisms, consisting of bacteria, yeasts, and mold species (Hui et al., 2017; Kim, Lee, Park, & Kim, 2010). Many contemporary miso varieties are not pasteurized as consumers are looking for more natural products, and/or have the desire to consume fermented foods containing live microorganisms. This may be due to recent research suggesting a beneficial link between fermented foods and human health (Marco, 2020; Marco et al., 2017; Peters et al., 2019; Sanlier et al., 2019; Wastyk et al., 2021). Therefore, to be safe for consumers, each step of koji and miso preparation and fermentation needs to be risk assessed for potential microbial hazards (Tables 1 and 2). Food safety hurdles such as pH, temperature, and A_w are used to preserve the product, and therefore a detailed understanding of the role of each, alone and in combination, is required to assure food safety.

1.4 | Koji and miso food safety guidelines

Koji and miso are established products in Asia. Having a long history of consuming these products, they are generally regarded as safe in these regions. Although these foods have little alignment with standard food safety guidelines, they are sold globally and are not considered a critical food safety hazard (Marco et al., 2021). However, in western countries the production of these products needs to fit within existing food safety requirements. western risk assessments were not developed with traditional fermented products in mind, and standards from European Union, Australia, Canada, and United States (European Commission, 2019; Food Standards Australia New Zealand, 2021; Government of Canada, 2020; U.S. Food & Drug Administration, 2017) do not include any guidelines for fermented foods such as rice koji and miso, that have a pH above 4.6. This is an issue for the many small businesses who want to make traditional unpasteurized rice koji and miso, as business operators have the responsibility to ensure food produced is safe.

This review discusses the processes of miso fermentation, and how fermentation of rice koji and miso fits with current food safety hurdles in western countries. Miso made using rice koji, salt, and soybeans, is the most common type of miso made, at around 80% of total production (Japanese External Trade Organization, 2020); therefore, the focus here will be on ingredients and hurdles specific to this type of miso. Other variations would require risk assessments contingent on their respective recipes. As miso production is effectively a two-stage process, we have presented the rice koji and miso fermentation operations separately. This review limits its scope to microbial pathogens. While many toxic byproducts of microbial activity may also be considered food safety risks (e.g., mycotoxins, biogenic amines) these are considered outside the scope the current review and have been considered elsewhere (Shukla et al., 2011; Sivamaruthi, Kesika, & Chaiyasut, 2018; Tanaka et al., 2006).

2 | FOOD SAFETY AND FERMENTATION OF RICE KOJI

2.1 | Rice koji production

Koji is used in many Asian ferments, such as sake, soy sauce and miso. Koji is made when mold spores are inoculated onto a substrate, such as rice, barley, wheat, or soybeans, and fermented at 30°C for

TABLE 1 Validation chart for rice koji production.

Process	Potential hazards	Microbiological validation	Validation details and comments
Source Aspergillus oryzae spores	Presence of microbial pathogens	Evidence of authentication from supplier	*Aspergillus oryzae* is a permitted enzyme. (Food Standards Australia and New Zealand, 2022; U.S. Food & Drug Administration, 2018)
Soak rice 8–12 hr at room temperature	Contamination with microbial pathogens Growth of microbial pathogens		Soak at 4°C for 24 hr (instead of room temp; Food Standards Australia New Zealand, 2018) Rice will be steamed in Step 2 to kill any microorganisms
Steam rice	Survival of microbial pathogens	Products cooked to at least 75°C and held for at least 2 min (Food Standards Australia New Zealand, 2018)	Steaming of rice for 30 min reduces or kills pathogenic bacteria to safe levels (Sun-Yuong, Hyun-Jung, Joong-Han, Dougherty, & Dong-Hyun, 2006)
Cool rice to 35°C	Contamination with microbial pathogens		Rice to be placed in sterilized cloth, on sanitized tray. All tools used are sanitized (Food Standards Australia New Zealand, 2018) Studies on use of wooden trays compared with stainless steel trays required, as wooden trays are traditionally used and may be a natural reservoir for fermentation microorganisms (Bodor et al., 2020; György & Laslo, 2021)
Inoculation with A. oryzae Rice inoculated at 35°C Wrapped in cloth, and placed into incubator set at 30°C, for 24 hr After 24 hr spread koji onto trays, temperatures within 30–40°C	Contamination with microbial pathogens Growth of microbial pathogens	Ensure rice is cooled to target temperature (5°C) within 2 hr (Food Standards Australia New Zealand, 2019) *Aspergillus oryzae* outcompetes spoilage microorganisms when fermentation conditions optimal (Adams & Mitchell, 2002) A_w of Rice koji 0.92 (0 hr 0.98 46 hr 0.92; Bechman, Phillips, & Chen, 2012) Good hygiene processes to be in place—Especially hand washing	A challenge study looking at *Bacillus cereus* inoculated into steamed rice, with *B. cereus* at 10^2, 10^4, and 10^6 spores/g of steamed rice, making small batch rice koji in triplicate, found that *B. cereus* did not germinate, grow, or produce toxins during the rice koji making process (Takahashi, Kita, Minakami, & Mukai, 2021a) Check temperature every 6–12 hr to ensure within range (30–40°C) for 48 hr (Nakaji, 2020) The water activity of rice koji limits the contamination of microbial pathogens, such as *B. cereus* (*B. cereus* optimal A_w 0.93–0.99; U.S. Food & Drug Administration, 2011) More data to verify rice koji A_w required *Staphylococcus aureus* A_w water activity 0.83 > 0.99, pH 4.0–10, temperature 7–48°C (U.S. Food & Drug Administration, 2011), if contamination occurs after steaming step, A_w, pH, and temperature are all within range for growth of *S. aureus*
Storage Refrigerate or freeze	Growth of microbial pathogens	Check storage temperatures regularly	Maintain storage temperatures and times. Refrigerator and freezer storage requires Microbial validation to demonstrate the process will not permit pathogen growth (unless a further step for example, pasteurization, will mitigate pathogens)

TABLE 2 Validation chart for soybean miso production.

Process	Potential hazards	Microbial validation	Validation details and comments
Rice koji	Presence of microbial pathogens		Check use by date, and that storage conditions have been maintained
Soak soybeans 8–12 hr at room temperature	Contamination with microbial pathogens Growth of microbial pathogens		Soybeans will be boiled in Step 2 to kill any microorganisms
Boil soybeans Approximately 3 hr, until soft	Survival of microbial pathogens	Products cooked to at least 75°C and held for at least 2 min (Food Standards Australia New Zealand, 2018)	Boiling of soybeans is the kill step microorganisms
Mash and cool soybeans to 37°C	Contamination with microbial pathogens	Ensure rice is cooled to target temperature within 2 hr (Food Standards Australia New Zealand, 2019)	Soybeans placed into sanitized stainless-steel tray. All tools used are sanitized (Food Standards Australia New Zealand, 2018)
Add salt to rice koji	Contamination with microbial pathogens	Addition of salt reduces risk of mold and pathogenic microorganisms	Ensure correct salt percentage are calculated – According to ratio of koji to soybean, and expected fermentation time and temperature
Mix Rice koji/salt, mashed soybeans and boiled soybean water	Contamination with microbial pathogens		All tools used are to be sanitized (Food Standards Australia New Zealand, 2018)
Place into fermentation vessel Cover miso with parchment or fabric, and then place weights on top of miso Place lid loosely onto fermentation vessel Ferment miso at room temperature	Contamination with microbial pathogens Growth of microbial pathogens	Line vessel with salt to reduce mold growth Eliminate air pockets to reduce unwanted molds and aerobic bacteria pH of miso 4.9–5.2 (Onda, Yanagida, Tsuji, Shinohara, & Yokotsuka, 2003; Shukla, Park, Kim, & Kim, 2011) A_w of low salt miso 0.835–0.843 (Tanaka, Kovats, Guggisberg, & Meske, & Doyle, 1985) Good hygiene processes are required to be in place—especially hand washing	All tools used are to be sanitized (Food Standards Australia New Zealand, 2018) Studies on use of wooden fermentation vessel compared with stainless steel/glass vessel required, as wooden vessels are traditionally used and may be a natural reservoir for fermentation microorganisms (Bodor et al., 2020; György & Laslo, 2021) *Staphylococcus aureus* optimal A_w 0.83 > 0.99, pH 4.0–10, temp 7–48 (U.S. Food & Drug Administration, 2011), note that if contamination occurs after steaming step, A_w, pH, and temperature are all within range for growth of *S. aureus*

40–48 hr. Koji is further named according to the substrate that is used, for example, rice koji. *Aspergillus oryzae*, a filamentous fungus, is mostly used for this fermentation, which is low moisture and known as a solid-state fermentation. The most important feature of koji fermentation is the type and range of catabolic enzymes produced by the mold. Different strains of *A. oryzae* are utilized depending on whether higher proteolytic enzymes (a more savory flavor), or higher amylolytic enzymes (for a sweeter result) are preferred (Wicklow, McAlpin, & Peterson, 2002).

2.2 | Starter culture and raw ingredients

Aspergillus oryzae is the most commonly used species of *Aspergillus* in the production of koji (Shurtleff & Aoyagi, 2018). Wild spores are no longer used, with modern processes using a pure culture of a food safe (nontoxigenic) strain to make koji. *Aspergillus oryzae* is authorized as "generally recognized as safe" and is classified as a permitted source for enzyme production for use in food processing (U.S. Food & Drug Administration, 2018; Food Standards Australia and New Zealand, 2022). *Aspergillus oryzae* is a prolific mold, and when conditions are optimal will outcompete unwanted microorganisms (Yamashita, 2021). To produce a safe ferment, it is essential to ensure that uncontaminated starter cultures are used. For example, in 2012, a fungal tempeh starter was linked to a *Salmonella* Paratyphi outbreak where 89 cases were identified (Griese et al., 2013). Therefore, sourcing spores from an accredited supplier which conducts checks for purity is essential. If unsure, it is recommended that producers request a Certificate of Assurance that ensures that the starter culture used is free from contaminants or pathogens (BC Centre for Disease Control, 2017).

For small businesses and home producers of koji, texts are available that include information on how to sporulate koji at the end of fermentation to produce spores that can be used in future koji fermentations (Katz, 2012; Nakaji, 2020; Shurtleff & Aoyagi, 1980). This process may have associated risks as it has been seen that the spores may change over repeated generations of sporulation. Research by Evans and Lorimer (2021) found *A. oryzae* cultures grown repeatedly for 25 generations underwent spores changes in both color and furriness. The spores changed from white to green by generation 10, and then to black by generation 16, and after 20 generations it was noted that the growth was furrier (Evans & Lorimer, 2021). If or how these changes may affect the food safety of the koji spores has not been studied, and it is not known how easily *A. oryzae* spores can be contaminated if not produced in a sterile environment (Yamashita, 2021). It is believed that *A. oryzae* may have been domesticated from *Aspergillus flavus* (Chang, 2019; Chang & Ehrlich, 2010). Further research has shown that the effect of domestication is relatively recent, likely occurring well after species divergence (Watarai, Yamamoto, Sawada, & Yamada, 2019). Despite the close genetic identity (99.5%; Rokas et al., 2007) *A. oryzae* and *A. flavus* have an important phenotypic difference in that *A. flavus* produces aflatoxins and *A. oryzae* does not (Barbesgaard, Heldt-Hansen, & Diderichsen, 1992). Aflatoxins are carcinogenic mycotoxins known to contaminate agricultural crops, and are a global threat to food safety (Chang & Ehrlich, 2010). Therefore, to ensure safe rice koji production, *A. oryzae* spore stocks should be routinely tested for contamination. Some have speculated that mutants of *A. oryzae* may revert to aflatoxin production; however, investigation of the biosynthetic gene clusters suggests the risk of this occurring is low (Kiyota et al., 2011).

Raw materials used may be the source of pathogens included in fermented foods prior to processing, and while the processes to make the fermented food can reduce microbial hazards, steps to identify any pathogens and reduce risk need to be followed (Adams & Mitchell, 2002; Tables 1 and 2). To reduce the chance of an unsafe or inferior ferment, it is essential the raw ingredients are of good quality, and undamaged (Department of Health and Human Services, 2017). Rice is often found to contain *Bacillus* spp., including pathogenic *B. cereus* (International Commission on Microbiological Specifications for Foods, 2005) with higher concentrations of the pathogen found in less processed rice, such as brown rice (Rodrigo, Rosell, & Martinez, 2021).

Along with quality, the type of rice is also important to consider when making rice koji. The type of rice will dictate the preparation of the rice, as varieties of rice differ in the amount of water absorbed during soaking and steaming preparation steps. It is necessary to ensure the rice is soaked to the stage where water reaches the core and the grains of rice can be crushed easily, and that the rice is then drained and steamed until it is soft on the inside and firm on the outside (Nakaji, 2020). This preparation ensures that correct moisture conditions can be achieved for adequate *A. oryzae* growth.

2.3 | pH

The growth of microorganisms is affected by pH of the environment as all organisms have an optimal pH for growth. Many food fermentations are considered acidic ferments, where the fermenting microorganisms produce organic acids that lower the pH of the food to a level considered suitable to prevent pathogens (Adams & Moss, 2008). Food safety standards require that food businesses producing acidified and fermented foods keep records of pH measurement of the food to ensure they are safe for retail or food service. Standards for the pH of fermented foods state foods are below pH 4.6 to ensure safety against pathogenic bacteria (U.S. Food & Drug Administration, 2020; Food Standards Australia New Zealand, 2018). This pH level protects against many pathogens, including *B. cereus*, and *C. botulinum*. As rice is steamed to make rice koji, spore-forming pathogens such as *B. cereus* and *C. botulinum* are the greatest risk, but as rice koji fermentation is an aerobic ferment this will prevent germination of *C. botulinum* spores, which are anaerobic. Pathogens of concern other than *B. cereus* would be the result of cross-contamination during production. The conditions during fermentation of rice koji are suitable for pathogens such as *S. aureus*, which is found in the environment and is part of the normal human flora, highlighting

TABLE 3 Physiochemical analysis of rice koji and miso samples.

Sample	Salt %	pH	A_w
Rice koji (USA) Fermentation time 0 hr (Bechman et al., 2012)	na	6.37	0.98
Rice Koji (USA) Fermentation time 46 hr (Bechman et al., 2012)	na	5.86	0.92
Japanese low salt miso A (Tanaka et al., 1985)	3.75	5.26	0.843
Japanese low salt miso B (Tanaka et al., 1985)	5.79	5.30	0.835
Japanese rice miso (Shukla et al., 2011)	nd	5.0	nd
Japanese red rice miso (Shukla et al., 2011)	nd	4.9	nd
Japanese miso (Onda et al., 2003) Fermentation time 0 weeks	11.9	5.8	nd
Japanese miso (Onda, Yanagida, Uchimura, et al., 2003) Fermentation time 15 weeks	nd	5.2	nd

Abbreviations: na, not applicable; nd, no data.

that good manufacturing process (GMPs), and hygienic conditions are important for the home fermenter, along with small and large businesses.

Rice koji made using *A. oryzae* as a starter culture is not an acidic ferment, and as such does not have a pH below 4.6 (Bechman et al., 2012; Tanaka et al., 1985; Table 3). This indicates that to enable the production of koji for commercial sale, alternate hurdles need to be assessed and validated.

2.4 | Temperature

The initial step in the production of rice koji involves steaming of the substrate, which effectively pasteurizes it. For example, rice is steamed for around 1 hr, which will eliminate any vegetative pathogens. This is supported by a study in 2006, which found that steaming rice cakes for 30 min, killed pathogenic bacteria (*E. coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, and *S. aureus*), and reduced *B. cereus* to levels below which toxins were able to form (Sun-Yuong et al., 2006).

After steaming the rice, the rice is cooled to around 35°C, and inoculated with the spores of *A. oryzae*. Conventional wisdom might suggest this is unsafe, food safety regulations typically require producers to keep food out of what is known as the "danger zone" between 5 and 60°C (U.S. Department of Agriculture, 2017). Food businesses are advised to follow strict temperature control guidelines, particularly for potentially hazardous foods such as cooked rice, ensuring they are either held at ≥60°C, or cooled to 21°C within 2 hr, and then cooled to ≤5°C within 4 hr for storage. This is due to pathogens, including *B. cereus*, growing fastest between 30 and 40°C (U.S. Food & Drug Administration, 2017; Food Standards Australia New Zealand, 2019). *Bacillus. cereus* is a spore-forming pathogen that is found in the environment, and when found in foods the spores are able to survive normal cooking temperatures (Food Standards Australia New Zealand, 2018). Cooking can activate the spores to germinate and become vegetative cells, which are then able to grow within the food. This requirement to cool rice quickly is not feasible when making rice koji, as the optimum temperature for *A. oryzae* when inoculated onto cooked rice, with some variations dependent upon the strain, is between 30 and 40°C, and the rice needs to be held at these temperatures for up to 48 hr (Nakaji, 2020; Shurtleff & Aoyagi, 2018).

Bacillus cereus, due to its ability to produce heat-resistant spores, would be considered the greatest risk in rice koji manufacture. Because of this, a challenge study where *B. cereus* was inoculated into steamed rice, at 10^2, 10^4, and 10^6 spores/g, which was then made into batches of rice koji ($n = 3$). This study found that *B. cereus* did not germinate, grow, or produce toxins during the rice koji-making process (Takahashi et al., 2021a). It should be noted however, that the fermentation of rice koji in this study was completed in a production facility under optimal conditions, with target temperatures and humidity maintained throughout the fermentation (Takahashi, Kita, Minakami, & Mukai, 2021b). Further research to determine if *B. cereus* grows during small-scale home fermentation or in the environment of a small producer fermentation, where it may be difficult to maintain completely optimal conditions is needed.

Although temperatures achieved during fermentation of rice koji are not suitable as a food safety hurdle, temperature control is still a critical parameter to make safe koji. *Aspergillus oryzae* is a prolific mold and will outcompete unwanted microorganisms (Shurtleff & Aoyagi, 2018) when appropriate concentration of spores are used as per manufactures directions, and optimal temperatures according to strain of *A. oryzae* are maintained.

2.5 | Water activity

Water activity (A_w) is the measure of free water in food is and ranges between 0 and 1.0 where pure water is 1.0. A_w is considered an important hurdle for the inhibition of pathogenic bacteria (Adams & Mitchell, 2002). The A_w of koji has only been determined in one study where it was shown to be 0.92 (Bechman et al., 2012; Table 3). This level is below the required optimum level of *B. cereus* (0.93–0.99) the pathogen of concern in cooked rice. However, is above 0.83, therefore is not considered sufficiently low to inhibit toxin formation or growth of pathogenic *S. aureus* (Food Standards Australia New Zealand, 2018). *Staphylococcus aureus* is found in the environment and is part of the normal human flora. This highlights the importance of GMPs and hygienic techniques during rice koji fermentation, especially hand washing.

3 | FOOD SAFETY AND FERMENTATION OF MISO

3.1 | Miso production

The most commonly prepared miso is made when rice koji is mixed with salt and cooked soybeans (Japanese External Trade Organization, 2020). The addition of salt inhibits mold and undesirable bacteria, and when left at suitable temperatures yeasts and target bacteria continue the fermentation process (Tamang, 2012). It is believed that the composition of microorganisms vary according to the recipe used and the environmental conditions of the fermentation (Shurtleff & Aoyagi, 2018). There is no single recipe to make miso, and recipes can be handed down from generation to generation, sourced from local miso makers, or found in the many books available commercially (Katz, 2012; Shih & Umansky, 2020; Shockey & Shockey, 2019; Shurtleff & Aoyagi, 2018). Recipe variations include the type of substrates, the ratio of these substrates, the salt concentration, and the amount of liquid added. The physical conditions that can affect the fermentation are temperature, oxygen availability, the type of fermentation vessel, and the length of fermentation (Lee et al., 2014; Villares, Rostagno, García-Lafuente, Guillamón, & Martínez, 2010).

3.2 | Rice koji and raw ingredients

Three ingredients are used to make the most common miso: rice koji, soybeans, and salt. Rice koji may be used fresh, or from storage as a dried or frozen product. If rice koji either made in house or is purchased for use, as with all ingredients, it is important to ensure the product meets specifications, such as use by dates and that recommended storage conditions have been maintained. Although no published studies have been undertaken that test the microbiological safety of storage conditions of rice koji, Nakaji (2020) suggests semi-dried koji can be stored for 2 weeks at room temperature, 6 months in the fridge, and 1 year in the freezer, and if the rice koji is dehydrated it is expected to last over 1 year at room temperature. Studies to determine any unwanted microbial growth during various storage methods would assist in ensuring the safety of rice koji during storage and the safety of downstream fermentation that uses rice koji, such as miso.

Soybeans for miso are soaked for 8–12 hr, and then boiled until soft for around 3–4 hr (Shurtleff & Aoyagi, 2018). The boiling step is sufficient to remove any vegetative pathogenic microorganisms that are present (Food Standards Australia New Zealand, 2019). However, spore forming bacteria may still be present after boiling, which may include pathogenic *B. cereus* and/or *C. botulinum*.

The purchase of quality salt is also important, to ensure no spoilage microorganisms are present. One study has shown salt to be a carrier of contaminants. When seven commercially available sea salts in the United States were examined, they were found to contain many fungi, including spoilage fungi (Biango-Daniels & Hodge, 2018). As discussed by the authors of this study, it would be beneficial to establish microbiological standards for sea salt, which would then reduce any spoilage risk where sea salt is used in fermentations.

3.3 | pH

Generally, the pH of fermented foods should be below pH 4.6 to ensure safety against pathogenic bacteria (U.S. Food & Drug Administration, 2020; Food Standards Australia New Zealand, 2018). As with rice koji, miso is not an acidic ferment. Previous studies (Table 3) have shown the pH of purchased Japanese rice miso's to be between 4.9 and 5.3, with a rice miso at 5.0, a red miso at 4.9 (Shukla et al., 2011) and low salt miso at 5.26 and 5.30 (Tanaka et al., 1985). A study that monitored pH of Japanese miso during fermentation found the pH starting at 5.8 and dropping to 5.2 after 15 weeks of fermentation (Onda, Yanagida, Uchimura, et al., 2003). These are considered suitable pH levels for miso, as this pH is optimal for the yeasts in miso to produce some ethanol, which combines with organic acids in the miso to produce esters, required for the distinctive flavor and odor of miso (Yamabe, Kaneko, Inoue, & Takita, 2004). This indicates that pH levels are not a suitable hurdle for miso, and other parameters, including salt concentration and A_w, are more important for ensuring food safety.

3.4 | Salt concentration and water activity

Salt concentration in miso traditionally ranges between 6 and 16%, dependent on the type and length of fermentation of the miso (Shih & Umansky, 2020; Shockey & Shockey, 2019; Shurtleff & Aoyagi, 2018). Lower salt miso's have become popular due to the health advice of reducing salt in our diet. The reduction of salt may alter the microbial communities found in miso, as has been found in studies on the fermented vegetable suancai (a brassica vegetable; Liang et al., 2020), and doubanjiang (broad bean paste; Yang et al., 2021). No studies investigating how low-salt concentrations affect the microbial community during fermentation of Japanese miso, especially spoilage or pathogenic microorganisms have been published to date.

Salt in miso contributes to its food safety. Salt binds free water molecules, thereby reducing the A_w of the miso (Tanaka et al., 1985) and inhibiting unwanted mold and bacteria (Tamang, 2012). Research completed by Tanaka et al. (1985) assessed the survival and growth or toxin-producing abilities of four bacterial pathogens, *S. aureus*, *S. typhimurium*, *Yersinia enterocolitica*, and *C. botulinum* when added to lower salt (2.36–5.79%) miso. None of the pathogens grew in any of the low-salt miso samples, and *C. botulinum* did not produce any toxins, when the samples were held at either 10 or 25°C for 18 weeks. Although each miso was made with low salt concentrations, they also had relatively low A_w below 0.85 (Tanaka et al., 1985; Table 3). FSANZ Microbiological criteria for food (Food Standards Australia New Zealand, 2018) states that A_w < 0.85 would control growth of pathogens. It should be noted that in Tanaka et al. (1985) study, the pathogens were inoculated into the miso samples at the end of fermentation. Therefore, further challenge studies would be required to confirm whether the pathogens may adapt to conditions and survive in a low salt environment if contamination occurred in the early stages of miso production.

The A_w required to inhibit pathogens of concern is <0.92 for *B. cereus*, <0.83 for *S. aureus* growth, <0.85 *S. aureus* toxin formation, and <0.97 for *C. botulinum* (U.S. Food & Drug Administration, 2011). Data from published studies determine A_w of Japanese low salt miso to be 0.835 (5.79% salt), 0.843 (3.75% salt; Tanaka et al., 1985; Table 3). Therefore, this data indicates that miso made to recipes with salt concentration 4% and above would be adequate to inhibit *B. cereus* and *C. botulinum*, and would inhibit toxin formation but not growth of *S. aureus*. Further research to determine A_w for a wider variety of miso types and salt concentrations is needed to verify this.

4 | CONCLUSION

Many of the production steps during koji and miso manufacture do not fit into contemporary food safety guidelines for foods. Although pH is a required food safety hurdle for fermented foods, this does not apply to non-acidic foods such as koji or miso. Use of a certified safe mold as the starter culture and monitoring appropriate temperatures to ensure prolific growth of the mold (and to minimize growth of unwanted bacteria) should be considered appropriate to ensure safe rice koji. A_w is an important food safety hurdle (Adams & Moss, 2008) and investigation into salt content along with A_w as an appropriate combined hurdle for miso, would assist in ensuring safe production processes. Focusing on quality ingredients and hygienic practices, along with the development of standardized optimal fermentation conditions would also assist in safe production of these ferments.

Research on unpasteurized koji and miso, to determine appropriate temperatures, pH, A_W, and salt concentrations during the fermentation process is needed. This information may be used to assist in optimizing parameters during koji and miso production, and to ensure a product is produced that will comply with food safety requirements.

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CONFLICT OF INTEREST STATEMENT

The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

Not applicable, as this is a review article.

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