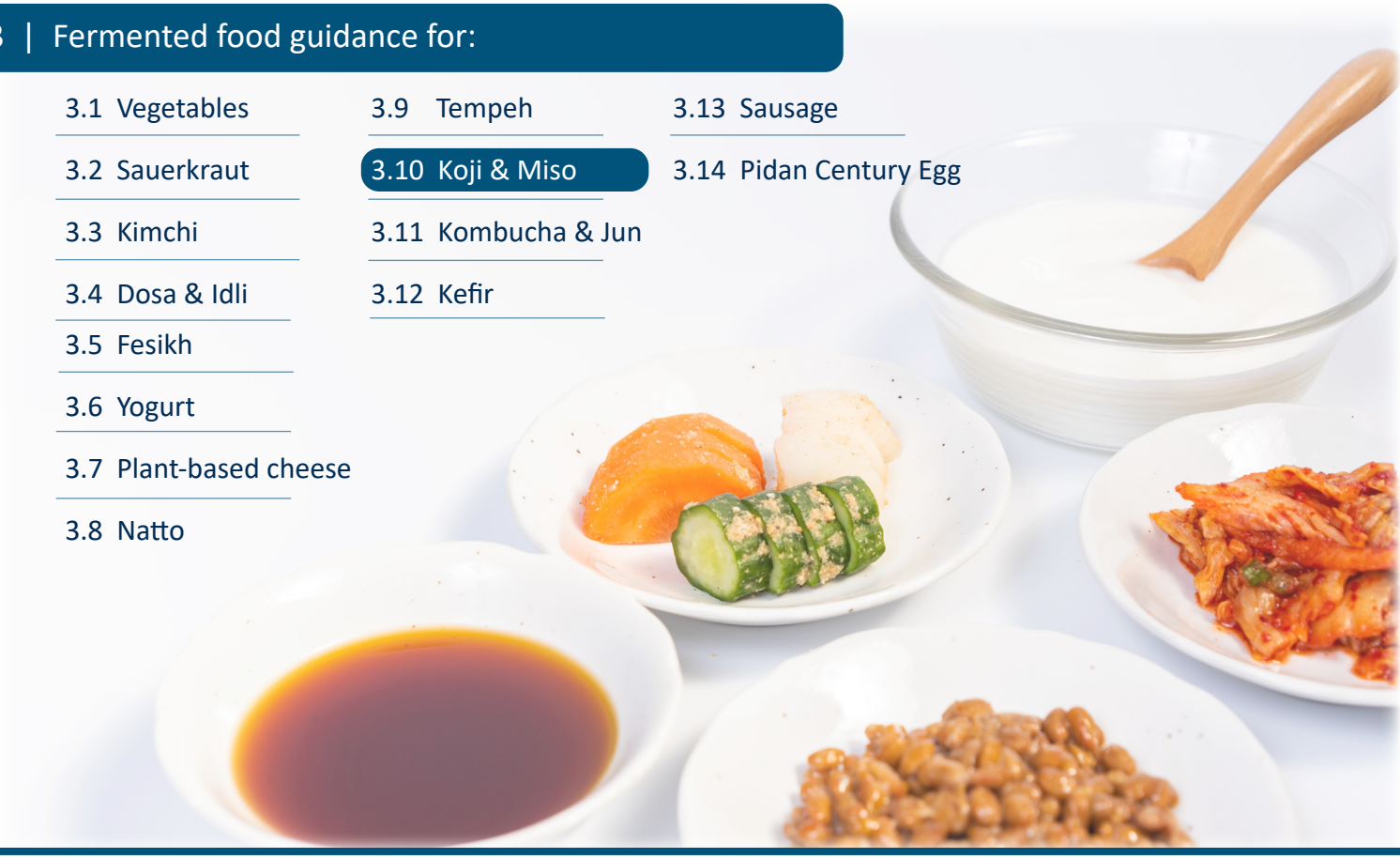
September 2023
Updated December 2024

Safety of Fermented Foods

Assessing risks in fermented food processing practices and advice on how to mitigate them



Suggested citation

L. McIntyre (author) Section 3.10 Koji foods: miso, soy sauce and amazake. *In* McIntyre, L. (editor) and the Fermented Foods working group. (2024). Safety of fermented foods. Assessing risks in fermented food processing practices and advice on how to mitigate them. Environmental Health Services, BC Centre for Disease Control. December 2024. Available from: http://www.bccdc.ca/resource-gallery/Documents/Educational Materials/EH/FPS/Food/Fermented/Fermented Foods Guideline-3.10 Koji and Miso.pdf
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Acknowledgements

Special thanks to Rosa Li for formatting the fermented food guidance and adminstrative support for the working group. The SharePoint site provided by the Federal Provincial Territorial – Food Safety Committee (FPT-FSC until May 2024) and the Canadian Food Safety Information Network (CFSIN) in July 2024 allowed all members to access files and documentation. Special thanks to Ellen Noble for administration of member access. We thank FPT Secretariat for provision of French translation and FPT-FSC members for addressing issues identified by members in fermented foods.

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Section 3 | Food safety reviews of fermented foods

A national working group of health inspectors, food safety specialists, and industry fermentation experts reviewed this food safety guidance.

Each fermented food review includes:

- background on the food,
- a description of the food preparation,
- a food flow chart,
- a review of the potential issues with the food preparation, and
- food safety control points.

Foods covered in this guidance are sorted in order of increasing complexity and fermenting agent.

Figure 1 | Fermented foods described by fermentation agent and complexity

Complexity	Foods	Fermenting Agent	Section
high ↑	Sausage	Added LAB[1], spontaneous moulds & yeasts	3.13
	Kefir, Kombucha	SCOBY[2] based: *Acetobacter*, yeast & mould	3.11-3.12
	Koji, Miso	*Aspergillus*, spontaneous or added yeast & LAB	3.10
	Tempeh	*Rhizopus*	3.9
	Natto	*Bacillus*	3.8
	Yogurt, Plant-based cheese	Added LAB	3.6-3.7
	Dosa, Idli, Fesikh	Spontaneous LAB and Yeast	3.4-3.5
↓ low	Vegetables, Sauerkraut, Kimchi	Spontaneous or added LAB	3.1-3.3

[1] – LAB-lactic acid bacteria; [2] – SCOBY-symbiotic culture of bacteria and yeast

A non-fermented, high alkalinity processed food is also included in this guidance: pidan century egg (Section 3.14).

Box 1 | How to use the information in this food safety review

The information presented here lays out best practices for a variety of fermented foods, however, it does not replace or supersede federal and provincial guidance or regulatory requirements for fermented foods. Health inspectors, food safety staff, owner and operators of food processing facilities should follow federal and provincial food safety requirements. This work intends to assist food safety staff (health inspectors) to evaluate the safety of fermented foods and fermentation processes encountered during inspections. Owners and operators of food processing facilities may also find this guidance helpful as it reviews critical control points and measures recommended to produce safe fermented foods. The best available evidence guided this work at the time of publication. The application and use of this document is the responsibility of the user.

This guidance does not include information about good manufacturing practices, labelling practices, or management control programs for cleaning and sanitation, pest control, employee training etc. It is expected that operators will follow approved guidance and seek this information elsewhere.

3.10 | Koji foods: miso, soy sauce and amazake

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Overview

Description	 Koji is a mould starter in fermented soybeans products that include miso, (brown paste, above) soy sauce, and fermented rice products, such as amazake beverages.
Starter culture	• Koji starter culture contains mould and mould spores of *Aspergillus* spp. including *A. oryzae*, *A. sojae*, *A. luchuensis*, among others. When grown on rice, barley, or wheat, starter is named rice koji or barley koji, etc. Starter culture must be purchased from commercial sources to ensure purity. • *Saccharomyces* yeast and lactic acid bacteria may occur when naturally present on ingredients and/or as deliberately added ingredients during fermentation of miso, amazake and other products.
Key features	• Koji is used in traditional Japanese recipes as fermentation starter for miso, soy sauce, amazake and other foods. This is considered the first stage of fermentation. • In most recipes, yeasts and bacteria may be added or occur in spontaneous fermentation from natural, wild strains on equipment. When koji is added to soybeans or rice (substrate) this is considered the second stage of fermentation. • This is not an acidic fermentation. The final pH of koji and other koji products are above a pH of 4.6. During fermentation, the pH of rice koji and miso will drop from a starting pH of approximately 6.0 to a final pH of approximately 5.0 but not to acidic conditions of pH 4.6 or lower. • Miso and salt koji (with added salt) should have a_w of less than 0.85; rice koji without added salt may or may not have a_w at protective levels of 0.85 or lower.
Hazards of concern	• Aflatoxin is not a concern when commercially sourced *Aspergillus* starter culture (as described above) is used. They do not contain species of *Aspergillus* that produce aflatoxin. Culture re-use (backslopping) is not recommended in these ferments as wild strains of *Aspergillus* that contain toxins may be introduced. • *Bacillus* spores may germinate and produce toxin if rice, barley, or other cereal cooling step is not controlled. Risk is lessened when cooking occurs under pressure. • Biogenic amines such as histamine have been associated with soy sauce and miso products.

Important control points for making koji starter	• Cooling of cooked rice or barley within 2 hours before inoculation
	• Inoculation at or below 35°C, optimal growth in range of 35 to 38°C.
	• During fermentation, heat is generated. Keep koji starter cooled to below 40°C for optimal growth
	• Stirring
	  o to allow uniform distribution of koji mould to enable *Aspergillus* to outcompete other bacterial strains, and
	  o for cooling as temperatures of 50°C and higher will kill the *Aspergillus* mould
	• Humidity of near 100% initially to allow mould growth. Once sufficient mould growth is achieved, air drying with reduced humidity in range of 10 to 15% will optimize mould spore formation. Note: this is an aerobic fermentation.
	• Can be stored at 10 to 15°C with humidity of 50% for one year, frozen or refrigerated. Temperature and storage conditions should be controlled to limit contamination before use in making miso, soy sauce, amazake, etc.

Important control points for miso	• Cooling of cooked rice or soybeans within 2 hours before inoculation • Physical hazard control if a mechanical chopper or metal sieve used to mash soybeans or other substrates • Koji should be added at or below 35°C with optimal growth between 27 to 38°C, depending on enzymes required for sweet or salty miso. Mixing may be required to release heat from fermentation process. • Added ingredients may include salt, halophic yeasts and/or LAB. Use of wooden equipment may have wild (residual) yeasts and LAB present. • Salt concentration should be >4%. Salt inhibits further mould, i.e., *Aspergillus* growth • Aeration to promote yeast growth. Miso mash is covered with cloth and loose lid in fermentation vessel. • Ambient temperature incubation for 1 month to more than one year. • During miso fermentation, growth of koji starter (*Aspergillus* mould) is retarded, enzymes in the koji starter break down proteins and carbohydrates. Yeasts and LAB are also present, once packed into vessel for aging, LAB pre-dominates in anaerobic environment. • Matured miso should have a_w of 0.85 or lower; finished miso usually has an a_w of approximately 0.80.
Important control points for soy sauce	• Cooling of soybeans and roasted wheat within 2 hours before inoculation. • Physical hazard control if a mechanical chopper or metal sieve is used to mash soybeans or grind wheat. • High salt of 18% required to promote growth of desired microbes.
Important control points for amazake	• Amazake may be made from rice koji and water. This is a saccarification process using amylase enyzmes produced in the koji starter, not a fermentation. • Keep incubation temp >50°C to avoid growth of *Staphylococcus.* • High levels of glucose sugar are present. Ingredient warnings are recommended for diabetics. • Amazake may also be made from the lees of sake (pressed cake after alcoholic sake is removed), water and sugar. Traditional rice-koji-based amazake does not contain alcohol. • In sake-based amazake, and in any recipe that uses yeasts, small amounts of alcohol may be present, therefore alcohol should be considered a hazard.

Background

The title of this document should be more properly called “Koji Fermented Foods” because koji is the essential starter culture for a myriad of Japanese dishes. In this section, we have chosen to focus on a few common koji fermented foods shown in figure 2 below: miso, soy sauce and amazake. Names that Canadians recognize include miso, a paste resembling peanut butter that is used in soups and broths; soy sauce, a condiment in many dishes; and sake, a brewed alcoholic liquor. Amazake is a non-alcoholic drink also made with koji. It is a sweet rice drink growing in popularity in North America, although well recognized as a traditional drink in Japanese culture.

Figure 2 | Koji fermented foods

Koji moulds creates the characteristic savoury *umami* flavours of miso and other foods, and has been registered on the UNESCO intangible cultural heritage list as a traditional dietary culture of the Japanese (termed *washoku*).[1]

Box 2 | Koji terminology

The word koji is used to describe at least three different parts (ingredients) of the fermentation process: the mould, the starter culture, and the ingredient that forms the basis of miso, soy sauce, sake, amazake etc.[1] The word koji can also be used to describe the final food being formed, such as salt koji, named “shio koji”, which is a flavouring ingredient. The Japanese language provides for this distinction but it can be confusing when translated, particularly when usage is not defined or explained in the food safety plan. Differences are described below[1]:

1. Mould *Aspergillus* is referred to in Japanese as “koji-kin”;
2. Koji starter, when the *Aspergillus* is grown onto a rice, barley, or other cereals, and used as the culture source is referred to in Japanese as “tane koji” or “moyashi”;
3. The word “koji” refers to the ingredient that is added to the fermented food such as amazake. This process is when koji starter is added to a fresh batch of rice, barley or other cereal, and then used in the final fermented food product. In English they would refer to this as “rice koji” or “barley koji”.

Japanese fermentations usually occur with koji starter as the ingredient used to make koji. In China, it is more common to use dried mould spores of *Aspergillus*, to make their fermented products.

Public health inspectors and operators need to have a common understanding of the terminology used to describe the process. If required ask the operator to explain how they are using the term ‘koji’ in the context of their food safety plan.

Fermented foods made with koji are a multi-step process. The first process is to make the koji mould spores, and this should be done by a commercial company. We do not recommend any processor grow their own spores (described below in koji mould section), these should be sourced from a commercial supplier. The second process involves making the koji starter. For every koji based food, the operator first makes the starter for the fermentation. Using miso as an example, there are two elements required to make miso: the substrate for the miso – which can be soybeans or rice or barley etc. and the "koji" or the culture starter. Allwood (2021) describes miso as a two stage fermentation process.[2] The koji itself is a separate fermentation, although it is often shown as a parallel process. Mould spores of commercially sourced *Aspergillus oryzae* are added to boiled and cooled rice, or barley etc. and used as the starter, i.e. rice koji, in a miso fermentation process. Note that koji starter may be made separately and stored until later use, or made simultaneously with the food.

Figure 3 | Miso paste

The most common type of miso is to use a rice-based koji and soybeans as the substrate for miso. Once the rice koji is prepared, soybeans are boiled and mashed, then these two components are mixed together with salt, and (sometimes but not always) additional fermenting agents, such as yeast and lactic acid bacteria (LAB). Added salt promotes the growth of LAB. Traditional fermenting vessels made of wood contain residual fermenting agents (yeast and LAB) and that is why yeast and LAB do not need to be added when wooden vessels are used in the process. In modern ferments, stainless steel or rotary mixers are used. Because they are required to be cleaned and sanitized between batches, the addition of yeast and LAB fermenting agents may be necessary.

Koji, miso and other fermented Japanese products appear to have a long history of safe consumption. One article was found that refuted associations of portion sizes of soy sauce and miso linked to elevated blood pressure (from excess salt consumption) involving more than 25,000 Japanese adults over a five year span (2012-2016).[3] In addition, a paper reviewing health impacts (based on a meta-analyses of published studies), found several general health benefits, such as reduced fasting blood glucose, and lowered total and low density lipoprotein cholesterol when plant-based fermented foods were consumed, such as miso.[4]

Outbreaks and Recalls

Very few recalls were associated with miso, soy sauce or other koji based products. As products are live, continuing fermentation may be the underlying reason for a recall of bulging packaging. Single recalls for *Bacillus* and mould occurred. A search of CDC and other outbreak databases found no outbreaks or illnesses linked to these foods. A generic search for "illness" and "miso soy sauce" in Google did not detect any articles or posts about illnesses.

Table 1 | Recalls related to koji and miso products in Canada and elsewhere

Year(s)	Hazard Category	Hazard Detail	Number Recalls	Country (s)	Product Description
2020[5]	Biological	*Bacillus cereus*	1	New Zealand	Fermented Bean Paste
2021[6]	Physical	Bulging packaging	1	Denmark	Miso paste
2013[7]	Biological	Mould	1	Canada	Noodle miso soup

Description of food preparation for koji and miso

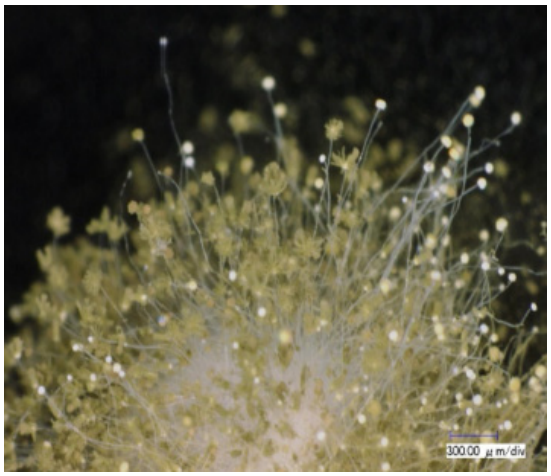
Koji mould was developed in the sake brewing industry, back in the 8th century in Japan.[1] Many refinements and changes have taken place. According to Yamashita (2021), there are twelve major manufacturers of koji mould that supply the brewing and food industry.[1] There are three species of *Aspergillus* developed and used in koji moulds: *A. oryzae* used in most fermented foods, *A. sojae* used in soy sauce, and *A. luchuensis* for an alcoholic beverage called shochu, made from a mixture of grains, sweet potato, brown sugar and other ingredients.[1] The quality of koji starter developed is impressive. Total bacterial counts (contaminants) are in the range of one thousand (1000) colony forming units (CFU) per gram, with the mould spores in excess of 5 to 30 billion (30,000,000,000) per gram.[1] We do not recommend that small scale operators manufacture koji moulds, i.e., *Aspergillus* spores. This is due to high risk of culture contamination with wild strains of *Aspergillus* and production of alfatoxin, with contaminating bacteria, the complex fermentation process and the controlled environment needed for spore production (sterile controlled air using HEPA – high efficiency particulate air, controlled temperature and environmental humidity).[1] Operators are recommended to purchase commercially made starter directly from manufacturers (in Japan or elsewhere) that can verify the number of spore counts and purity. Koji moulds are considered a national treasure in Japan, and purity of available cultures from that source is well documented.

A brief overview of how **koji mould** is made is provided here. Koji moulds are made with polished rice, barley, wheat or mixed materials of cereals.[8,9] Polishing is done to allow release of nutrients on the surface layer and to promote growth of mould.[1] Growth conditions for production of spores differs from production of koji starters, as the focus is on making spores, not production of moulds and release of enzymes into the koji starter.[1] It takes between four to six days for spore production, with humidity levels at 40% for optimal spore production and suppressed bacterial growth.[1] Before inoculation, substrates are sterilized by cooking under pressure to inactivate *Bacillus* spores, and growth occurs in clean rooms with filtered, sterile air at temperatures of 35°C to 38°C.[1] Once optimal growth of spores occurs, they are dried in 10% moisture content at temperatures of 40 to 42°C in dehumidified air environment. Dried koji spores are stored at temperatures between 5°C to 15°C.[1]

Koji starter is made with koji moulds. Koji starter processes are specific to the food being made, such as miso, soy sauce and amazake. Koji starter begins with growth of the mould in the first process step, then temperatures are decreased slightly to promote **enzyme** development (in Japanese, this is referred to as *kusumoto*). The two most important enzymes being produced are amylases and proteases. Amylases break down carbohydrates into sugars (glucose and oligosaccharides), and proteases break down proteins into amino acids and peptides, providing the savory umami taste (i.e., *kusumoto*). It is the enzymes, secreted by the moulds, that create the flavours in koji fermented foods. Koji food processes differ with respect to time, temperatures and enzymes desired by the operator for the specific foods made. The process steps for miso, soy sauce and amazake are reviewed in this guidance, including processes for making their koji starters. More detail is provided for miso in the food flow chart and food safety control points, however, safety controls are similar in these three foods.

Figure 4 | Koji starter and koji

(a) Koji starter for sake



(b) Koji mould. 400X scanning electron microscope.

This figure is reproduced with permission of author, Dr. Hideyuki Yamashita.[1]

Miso is usually made with soybeans, however rice, barley or other substrates may be used as well. There are several hundred types of miso depending on substrates used and the processing conditions.[8] Miso soup is a staple in Japanese households and made with miso paste in boiling water with a variety of ingredients added, such as vegetables, sprouts, tofu, bean curd, mushroom, sea weed and others.[8] Sweet miso has a shorter holding time (10 to 15 days)[8] and its' koji starters have higher levels of amylase, salt miso has a longer holding time (2 to 12 months)[8] and its' koji starters have higher levels of protease.[9] Both types of miso have ambient range holding temperatures. Traditional methods use wooden trays to grow the koji mould starter; modern methods (since the 1970's) use automated and ventilated equipment.[10] Kusumoto (2021) describes traditional manual tray-based koji as: loading (*hikikomi*), kneading (*kirikaeshi*), piling (*morikomi*), mixing (*teire*), rearranging (*tsumikae*), and finishing (*de-koji*).[9] There may be two types of miso produced with added koji; fermented miso and enzymatically degraded miso.[9] The latter type of miso is produced in a few days at temperatures of 50°C.

Rice-based miso. Rice miso is the most common in Japan, accounting for 80% of production.[9] In this type of miso, rice is used as the base for koji, then mixed with cooked soybeans. Rice and soybean soaking steps are reported from 8 hours to 17 hours.[2,9] Conditions for soaking are not given in recipes, however, if soaking extends for longer than 4 hours, recommendations are to soak rice or soybeans in acidified or refrigerated potable water. Cooking methods that eliminate bacteria, such as steaming, boiling or boiling under pressure are acceptable, however, cooling remains a critical control point as spore-forming bacteria (e.g., *Bacillus*) may grow and form heat-stable toxins. Once the internal temperature drops to 60°C, then the rice, soybeans and other substrates should be cooled within two hours before koji mould spores (step 1) or koji starter (step 2) is added to the substrate.[11]

Step 1. Making rice koji for miso. Cooked rice is cooled within two hours to 35°C before inoculating with *Aspergillus* spores. The mixture is placed into a culture room onto wood, covered with a food safe cloth, and incubated at approximately 30°C.[9,12] During koji manufacture, temperatures are adjusted to achieve enzyme production for the type of miso produced. For sweet flavoured miso, temperatures of 2 to 5°C above the baseline temperature of 30°C will increase amylase production; for salt flavoured miso, temperatures below 30°C (rice is kept between 27 to 30°C) are used to develop protease enzymes.[9] Temperatures must never go above 40°C or enzyme development will halt.[9] During fermentation heat begins to develop approximately 10 hr into the process. In manual methods, koji is mixed by hand at 16 to 18 hr to release heat and to separate rice grains to prevent clumping.[9,10] Mixing (or kneading) is repeated at ~26 hr, then koji is transferred to steel or wooden trays. Humidity in the room will be high at 98%, as fermentation progresses humidity and water activity of the product should decrease. The final a_w of the koji should be 0.80 when fermentation is completed in 42 to 48 hr.[9] The fermentation is complete when spores have developed, the length of time can vary dependant on the *Aspergillus* strains chosen.[9] It is not desirable for spores to age and turn black, as this will discolour miso. Automated equipment for rice koji control heat and humidity with surface and interior ventilation systems, to assist with exchange of oxygen and carbon dioxide.[9] Once koji is made, if not used immediately, it should be refrigerated or frozen.[12] Operators should be describing whether they make koji starter fresh for each batch of food, or if the koji starter is made in a large batch, then portions reserved for later use. The starter may be kept refrigerated for approximately one month, or frozen. Activity may be slightly reduced, but as long as the koji starter is protected from contamination, it is acceptable to use. Back-slopping is not recommended; operators are advised not to use the koji starter to make a new batch of starter, as risks of growing wild *Aspergillus* strains that produce aflatoxins is amplified.

Step 2. Soybeans and addition of rice koji and other ingredients. Following cooking and cooling steps, soybeans are mashed using a chopper or by using a 5 to 6mm mesh.[9] Other ingredients added are water, salt, yeast and LAB. Salt tolerant species of yeast (*Zygosaccharomyces rouxii*) and LAB (*Tetragenococcus halophilus*) are commonly used.[9] Salt concentrations are normally between 6 to 16%, as long as 4% or higher salt is used, the final water activity will be protective against *B. cereus* and toxin formation by *S. aureus*.[12] Salt, water, rice koji and the soybeans are mixed (this mixture is referred to as *moromi* in Japanese), and the mixture is placed into a fermentation vessel, then covered with parchment or fabric.[9,12] Weights are placed on top of a lid that is loosely applied (fermentation with yeast requires oxygen). When machinery is used, e.g., mechanical fermentations in tanks, the mash is inverted at least once to create aeration, release heat and facilitate yeast aerobic fermentation.[9] Temperatures using automated equipment, or in controlled environments, range between 25°C to 30°C, and the length of time to create miso ranges from one month to more than one year.[9]

Ambient temperature maturation is typical in smaller operations, is acceptable, with times to maturation of the miso likewise extended in lower temperatures. It is common for operators to base maturation completion on flavour profiles, however, the food safety control is to verify a_w is at 0.85 or lower. Water activity will decrease over time, normal final a_w is at approximately 0.80.

Figure 5 | Soy sauce

Soybean-based miso. Soybean miso has two ingredients: soybeans and salt. The first step in making miso involves washing and soaking of soybeans. Duration of soaking is important to quality and successful growth of koji mould, optimal weight of soaked and drained soybeans is 1.5 to 1.6X original weight.[9] Soybeans are boiled or steamed for three hours[2] or cooked under pressure[9] then cooled. Cooling should occur within two hours before addition of koji starter. Cooled soybeans may be mashed or chopped with a processor (5 to 6 mm mesh), salt or salt water is added prior to inoculation. Soybeans are rolled into balls (15 to 40 mm in diameter, depending on flavour desired from enzyme and LAB activity) before inoculation with koji starter, these steps occur in automated equipment.[9] Initial fermentation temperatures are reported from 27°C to 28°C and generally below 37°C during initial fermentation (to promote LAB growth), then raised to 33°C to 37°C in later fermentation stages.[2,9] Pressing may occur prior to addition of salt or salt brine and maturation steps for soybean miso. Soybean miso is fermented from 6 to 12 months.[9] Soybeans may also be mashed following fermentation.[10]

Soy sauce. Modern methods use fully automated equipment.[13] Japanese style soy sauce, described here, is made with soybeans and wheat, in comparison to Chinese style soy sauce which only uses soybeans.[13] In Japan, there are more than five types of soy sauce based on colour and flavour ranging from twice brewed, very dark and rich taste to light colour and sweet tasting.[13] In traditional soy sauce manufacture, the mash was fermented in earthen jars or wooden tanks for several years.[13] Along with soy sauce-specific koji, soy sauce fermentation ingredients include soybeans, wheat, salt, halophilic LAB and yeast.[14] This mixture is referred to as a "moromi" mash. To make the soy sauce koji, equal portions of soy beans and wheat are used. The soy beans are boiled or steamed for three hours[2] or cooked under pressure[9] then cooled, wheat is roasted at high temperatures of 160°C to 180°C and crushed.[14] Moulds added include *A. oryzae* and *A. sojae*, the latter species is mainly used in soy sauce production.[14] This dry mixture is incubated under high humidity at 30°C for 2 to 3 days.[14] Initial pH values are at pH 6.5 to 7.0, the soy sauce koji has a pH of approximately 6.0, at the end of the process, however, the finished soy sauce will have a pH of 4.7 to 4.8.[1,10] The moromi mash is added to 18% weight/volume salt and ideally up to 22% salt and the mixture is fermented for 6 to 8 months.[10,13,14] The higher salt concentration is required to promote growth of osmophilic desired microbes, including LAB.[10] LAB activity will reduce the pH of soy sauce to approximately pH of 4.8. In modern fermentation, the halophilic yeasts and LAB are added to ensure consistency of fermentation. Following fermentation the mash is pressed, and solids are filtered out as part of the refining process.[14] Filtration occurs through a cloth, or using modern horizontal filter presses. Pasteurization occurs at 70°C to 80°C, and may cause more sedimentation to occur, requiring further clarifications.[10] Sodium benzote may be added to some soy sauces as a preservative.[10] Chemical methods for manufacture of soy sauce are not reviewed here.

Amazake. This is a sweet drink made of rice and rice koji. The sweetness in this drink comes from glucose, a breakdown product of carbohydrates and amylase. The rice koji favors amylase enzyme production, and incubations at higher temperatures (35°C to 40°C) in comparison to miso rice koji (27°C to 30°C) occur.[15] Brown rice is polished, then steamed and cooled, before spores of *A. oryzae* are added.[15] The second part of this process is saccarification or creation of sugars, using the enzymes in the koji, which is not a fermentation process. The rice koji is diluted with water and incubated at temperatures of 50°C to 60°C. At this stage amylase converts rice starches into glucose, therefore this process is referred to as saccharification and not fermentation.[15] There are at least 12 oligosaccharides produced (collectively called glucose here) and concentrations of these types will vary based on temperatures from 45°C to 70°C.[16] Although citric and succinic organic acids are produced during this process, the pH can be above 4.6, requiring refrigeration of this product.[16]

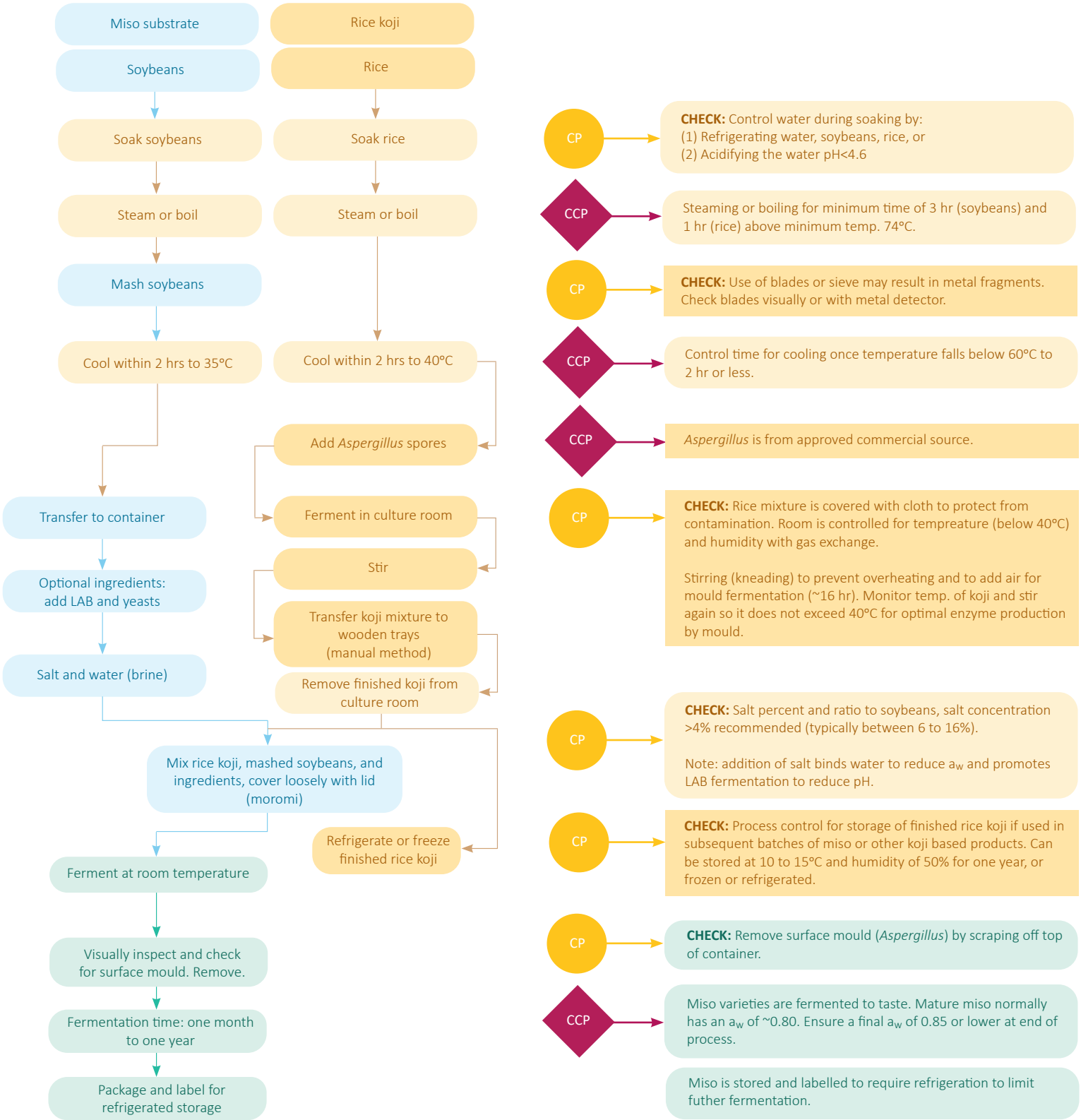
Figure 6 | Amazake

Other types of amazake:

- Sour tasting amazake, containing organic lactic acids, is produced with lactic acid bacteria-based koji starter. It is prepared similar to sweet amazake, then held for 24 hours and up to a week at ambient temperatures with a final product pH of less than 4.6.[17]
- Sake based amazake, is made from the pressed cake (lees) of sake. Water and sugar are added and mixed, and resident yeasts in the sake manufacture may make residual alcohol in this type of amazake.[15] In this type of amazake, alcohol should be considered a chemical hazard in the food safety plan.

The remainder of this document, i.e. the food flow chart and potential issues focuses on miso.

Koji and miso food flow chart | Process flow and controls



Potential issues with koji and miso food preparation

Issue	Description
Koji mould spores	Koji mould: commercially purchased *Aspergillus oryzae* should be purchased and operators are recommended to obtain a certificate of assurance (COA) for the product from a reputable manufacturer.
Koji starter and storage	When koji mould is used to create koji starter, backslopping and re-use of previous starter culture is not recommended to control for wild strains of *Aspergillus* that could be toxin producers. If koji starter is not used immediately in a recipe and is intended to be stored for later use, this is allowable, as long as the operator describes an acceptable storage method. They should include temperature control (refrigerated or frozen), length of storage before discarding and preparing a new batch and monitoring for unintended mould growth with corrective actions (discard of the batch).
Soaking of rice, barley or other ingredients	Soaking of substrates occurs when making the koji starter and making the base of miso, soy sauce, and other koji fermented foods. Soaking of substrates, e.g., rice, barley, soybeans, should be controlled to avoid risks from growth of spore-forming toxin producing organisms. Although later cook steps will destroy vegetative bacteria during the soaking step, if heat stabile toxin is formed (e.g., cereulide toxin formed from *Bacillus cereus*) these are not destroyed by boiling. Soaking is recommended in potable water, and if the soaking period is longer than 4 hours, this step should occur under refrigerated or acidified conditions. For optimal quality, it is suggested that soybeans increase in weight by 1.5 to 1.6X.
Cook step	Substrates are cooked by steaming, boiling or cooking under pressure. As long as temperatures of 74°C or higher are achieved by any of these methods, vegetative bacteria will be destroyed. Cooking methods, such as steam cooking, is typically for several hours.
Salt	Food-grade salt should be used. Salt concentrations in recipes should be calculated to ensure levels are high enough to suppress spoilage and pathogen agents. Typical salt concentration is 6 to 16%, levels of 4% or higher salt are recommended.
Mashing or blending	When ingredients are mashed or blended using knives, grinders, or other mechanical equipment this introduces the risk of physical metal fragments. The control recommended is that the operator visually inspect blades after each use to ensure not damage or to use a metal screening device.
Fermentation conditions for koji	This is an aerobic fermentation to allow spores of *Aspergillus oryzae* to germinate and fungal strands (mycelium) grow through-out the substrate. The fermentation should be ended before black spores form and discolour the koji starter. Temperature conditions vary depending on enzyme development, and are usually between 27°C and 40°C. Humidity in the room begins high (98%) and is reduced with ventilation to allow for oxygen and carbon dioxide gas exchange. Sanitary mixing by hand or using automated mechanical equipment is required once or more to prevent over-heating and to introduce oxygen during fermentation.
Fermentation conditions for miso	This is both an aerobic and an anaerobic fermentation. The principal of this fermentation is that the enzymes produced in the koji will break down the carbohydrates, proteins and sugars in the new substrate (rice, soybeans, etc.) into a complex mixture of sugars, organic and amino acids. A plastic cover and/or loosely fitted bucket ensures that the *Aspergillus oryzae* in the product does not germinate on top of the mixture. If mould does grow on top, it is scraped off and discarded when checked. This fermentation may proceed for one to 10 months or longer, depending on the variety of miso produced. Occasional mixing will promote *Aspergillus* growth, and LAB will reduce pH.
Final pH and a_w	The pH of miso will decrease from 6 at the start of fermentation to a pH of 5, and a_w will reduce from 0.98 to 0.80 in mature miso.[9]

Koji and miso food safety control points

- Commercially purchased *Aspergillus* strains are recommended to have a certificate of assurance verifying spore counts exceed approximately one billion per gram, and aerobic contaminants are less than 1000 CFU per gram.
- Soaking of rice, soy beans and other substrates in excess of four hours should occur in refrigerated conditions or in acidulated water.
- Minimum cooking and steaming temperature steps should occur at 74°C, or higher. Generally longer cooking times are recommended to inactivate *Bacillus* spores which are common contaminants of rice, soybeans and other cereal substrates. Cooking under pressure is recommended for this reason.
- Mechanical equipment for mashing of soy beans using grinders or sieves, or cracking of wheat for soy sauce, etc. may introduce a physical hazard of metal fragments. Operators can control this visually by inspecting blades or with metal detectors in the process.
- Cooling from 60°C to target inoculation temperature should occur within two hours.
- Fermentation conditions of temperature should be controlled to promote enzyme production for the desired koji, but not exceed 40°C.
- Humidity during fermentations starts high (98%) and should be ventilated to lower humidity of 15% or lower later in fermentation for spore development.
- Salt concentrations should be calculated to ensure they are in optimal range (at least 4% or higher) to limit spoilage and pathogenic microorganisms growth.
- Koji and moromi require occasional mixing to introduce oxygen for aerobic fermentation by *Aspergillus*. Sanitary handling and procedures should be in place with traditional hand mixing.
- Fermentation vessels and equipment should be cleaned and covered to prevent cross contamination.
- Visible mould growth on miso surface (*Aspergillus* or others) should be scraped off, removed and covers replaced during the maturation phase.
- End point of miso maturation should show pH of approximately 5.0 and a_w of approximately 0.80, with a target a_w of 0.85 and lower.

Potential health issues with miso and soy sauce

As with other soy-bean based fermented foods, biogenic amines can be produced during miso and soy sauce fermentation and are harmful if ingested in high concentrations. Biogenic amines occur primarily from metabolic activity by microorganisms during fermentation (see Box 3 below). The highest levels of histidine reported for miso and soy sauce were 592 mg/kg (soy sauce) and 221 mg/kg (miso) levels well above the threshold of 80 mg/kg for causing illness.[18] For tyramine, the highest levels reported were 485.9 mg/kg in soy sauce and within reported levels that cause illness between 100 and 800 mg/kg.[18] However, levels of tyramine were lower in miso, 95.3 mg/kg, indicating tyramine may be a concern in soy sauce but is not an issue in miso.[18] For phenylethylamine, the highest levels reported in soy sauce were lower, at 23.7 mg/kg and higher in miso at 42.0 mg/kg that is above the threshold of 30 mg/kg for causing illness.[18] For other biogenic amines the highest levels reported for tryptamine was 31.2 mg/kg and 762 mg/kg for miso; putrescine was 1000 mg/kg in soy sauce and 34.3 mg/kg in miso; cadaverine was 550 mg/kg in soy sauce and 201 mg/kg in miso; spermidine was 486 mg/kg in soy sauce and 35.7 mg/kg in miso; and spermine was 145 mg/kg in soy sauce and 216 mg/kg in miso.[18]

Box 3 | Biogenic amines in fermented foods

Biogenic amines (BAs) can be produced by microbes in fermented foods, such as fermented soybean products, vegetables, cheeses, sausage, and fish. Normal BA intake does not cause illness as intestinal amine oxidases break down and detoxify the BAs.[19,20] If large amounts of BA are ingested, or if amine oxidase activity is inhibited, then acute toxic symptoms can occur such as nausea, respiratory distress, hot flushing, sweating, heart palpitations, headache, bright red rash, burning sensations in the mouth, alterations in blood pressure, diarrhea and hypertensive crises.[19,21,22] The toxic effects of BA may vary between individuals depending on individual sensitivity and on the consumption of alcohol or drugs that are monoaminooxidase inhibitory.[23,24] *(continued on page 16)*

The main BAs are histamine, tyramine, β-phenylethylamine, putrescine, cadaverine and spermidine. Health Canada has set action levels for histamines in anchovies, and fermented fish sauces and pastes at 200 mg/kg and for other fish and fish products at 100mg/kg.[25] However, there are no guidelines set for other fermented food products and BAs other than histamines in Canada, or elsewhere in the world. At present, the toxic doses in food are suggested only for three biogenic amines: 100-200 mg/kg for histamines, 100-800 mg/kg for tyramine and 30 mg/kg for phenylethylamine.[20]

Operators manufacturing fermented foods are not required to test for BAs in their products. Operators are recommended to list BAs as a potential chemical hazard in their food safety plan. Operators can address risks of BAs by

(1) ensuring preventative measures are in place, the facility is clean and sanitary, handling practices are hygienic to limit bacteriophages and bacteria that interfere with the culture process;

(2) optimizing the fermentation: regulating time, temperature, moisture content, salt concentrations, and storage conditions; using good quality ingredients;

(3) purchasing commercial starter culture and/or verifying quality of the starter culture;

(4) monitoring that the expected culture activity occurs within correct timeframe; and

(5) monitoring for expected pH.

If a fermented food is linked to foodborne illness in consumers, inspectors are recommended to consider testing for BAs if symptoms and onset of illness in cases fit suspected BA illness. Further information about BAs and testing is found in Section 2 of this guidance.

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