Safety of Fermented Foods

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

BC Centre for Disease Control · Alberta Health Services · Saskatchewan · Manitoba · Public Health Ontario | Santé publique Ontario · Québec · New Brunswick | Nouveau-Brunswick · Nova Scotia · Prince Edward Island · Newfoundland & Labrador · Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada · BIO | FOOD | TECH

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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], wild moulds & yeasts	3.13
	Kefir, Kombucha	SCOBY[2] based: *Acetobacter*, yeast & mould	3.11-3.12
	Koji, Miso	*Aspergillus*, wild 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	Wild LAB and Yeast	3.4-3.5
↓ low	Vegetables, Sauerkraut, Kimchi	Wild 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.

3.9 | Tempeh

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Overview

Description	 Boiled soybeans are fermented with *Rhizopus* mould into a formed solid cake. Tempeh is served fresh, baked, boiled or fried following fermentation.
Starter culture	Lactic acid bacteria (LAB) is added during soak step, and, once soybeans have been cooked and cooled, commercial *Rhizopus* mould culture from approved supplier is added. Note: contaminated mould starter culture has been linked to *Salmonella* outbreaks. Backslopping, adding portion of previous ferment to new batch, is not recommended.
Key features	• This is a two stage fermentation: added LAB are active during soaking step, then *Rhizopus* mould fermentation and resulting enzymes digest cooked soybeans. • Soybeans are soaked and dehulled before boiling. • Mould is inoculated onto soybean cake, and incubated in moist conditions for approximately 1 to 3 days in drainable containers or perforated plastic bags. • This is an alkaline fermentation, pH increases from pH 4 to pH 7 during the process.
Hazards of concern	• *Salmonella* • *Bacillus cereus* • *Staphylococcus aureus* • *Listeria monocytogenes* • Biogenic amine formation
Important control points	• Soaking of soybeans in potable water, acidification with acetic or lactic acid, refrigeration or inoculation with LAB recommended. Lower pH will inhibit growth of spoilage microbes and pathogens during soaking. • After initial cook step, control for cooling once temperature falls below 60°C to 2 hr or less before *Rhizopus* mould culture is added. • Use of commercial *Rhizopus* culture from an approved supplier. • Fermentation conditions: temperatures during culture should not exceed 42°C, relative humidity should be maintained between 75% to 85%, and trays should drain away excess moisture.

Background

The earliest mention of tempeh was in a manuscript dating back to the 1600's in Java, Indonesia.[1] Tempeh is the only fermented soy product that did not originate in either China or Japan.[2] Tempeh is traditionally made with soybeans, variations use beans, peas, chickpeas and lower costing fruit or cassava grits, and coconut press cake as the main ingredient or substrate.[1,3] Soybean tempeh may also be made with additional ingredients such as barley, millet and brown rice.[1]

The Codex Alimentarius standard for tempeh describes this food as a compact cake-formed product which is prepared from dehulled soybeans through fermentation with *Rhizopus* spp.[4] Food quality characteristics include that tempeh should be white coloured from mould growth, have a nutty, meaty and mushroom-like flavour, with a fresh smell that is without ammonia, and be free from small stones, other beans and husks.[4] The minimum protein content should be 15% with a maximum moisture content of 65%.

Figure 2 | Fried ready-to-eat tempeh

Following fermentation and packaging, tempeh is not usually consumed as a ready-to-eat product, although it can be. A further cook step, such as steaming, baking, boiling or frying as shown in Figure 2, is normally performed. Tempeh was once considered a protein choice for those who could not afford meat, but is now increasingly viewed as a healthy protein option and preferred choice for vegetarians and vegans. Studies evaluating health benefits of tempeh found positive attributes related to vitamin B12, calcium, folate, iron, protein and isoflavone bioavailability and probiotics.[1] Enzymes released by *Rhizopus* mould, and other microbes growing during the fermentation do the work of digesting protein into amino acids, lipids into fatty acids, iron II into iron III, breaking down isoflavone glycosides into aglycones, reducting phytate content and producing vitamin B12.[1] Health benefits have been reviewed by Ahnan-Wanarno et al (2021) who report on studies examining affects of tempeh consumption on cognitive function, bone, liver, gut and cardiovascular health, malnutrition, cancer, anemia and other health impacts.[1]

Outbreaks and Recalls

In Indonesia, tempeh made with coconut milk and coconut cake contaminated with a mitochondrial toxin, bongkrekic acid caused many outbreaks and illnesses between 1951 and 2013, until public health policies were introduced.[1] The toxin was formed by a pathogen called *Burkholderia gladioli* pathovar *cocovenans* in incompletely fermented coconut tempeh. A significant safety step introduced in the public health policy was to acidify soaking water to a pH of 4.5 or lower to inhibit pathogen growth, and to require sanitary hygienic conditions in tempeh producing premises. In the U.S., contaminated *Rhizopus* culture imported from Indonesia caused a significant Salmonella outbreak in unpasteurized tempeh.[5] Tempeh has been recalled due to spoilage in Canada. Although it was not reported if this recall was due to overgrowth of *Rhizopus* mould, problems such as ammonia odour and blackened appearance when mould sporulates can occur if the tempeh fermentation period is too long, or when it continues to ferment in the package.

Table 1 | Recalls related to tempeh products in Canada

Year(s)	Hazard Category	Hazard Detail	Number Recalls	Country (s)	Product Description
2017[6]	Biological	Spoilage	1	Canada	Tempeh – various flavours
2012[7]	Biological	*Salmonella*	1	Canada	Tempeh starter

Table 2 | Outbreaks related to tempeh products

Date	Country	Pathogen causing illness	No. Ill (no. hospitalized)	Premises where outbreak occurred	Reason
2012[5]	U.S.	*Salmonella enterica* serovar Paratyphi B variant L(+) tartrate(+)	89 (8)	Restaurants supplied by manufacturer of specific brand	Contaminated starter culture; unpasteurized tempeh
≤2014[1]	Indonesia	Bongkrekic acid	9000+ and 1000+ deaths	Various premises	Coconut milk/oil substrate, unsanitary conditions and no acidification step

Description of food preparation for tempeh

Soybeans are traditionally used to make tempeh, but other substrates may be used. Soybeans may be de-hulled before or after the soaking step. The removal of the husks from soybeans is required for mould culture to penetrate the bean. That is because soybeans are left whole in tempeh, without being mashed or crushed prior to the fermentation step. Mould strands (or fungal hyphae or mycelia) are the binding agent that holds the digested soybeans in the tempeh cake together.[3] Soaking can make it easier to remove husks that float to the top of water, but this is a labour intensive process, and rarely used even with small processors. If hands are used to remove husks, hygienic handling and glove use is recommended. Most de-hulling occurs using a low cost mechanical de-huller or equipment with steel roller mills.[8,9] As de-hulling occurs by abrasion between two rotating cylinders, and no blades are used, it is unlikely for a physical hazard to occur at this step.[8] Prior to dry de-hulling, a heat step (93°C for 10 min) to shrivel the bean seed cotyledon and loosen the seed coat, may occur.[9]

Once beans are de-hulled, the next step is to soak the soybeans for 6 to 24 hours.[1] To limit microbial growth, a control step is required. It is recommended to acidify water with addition of acetic or lactic acid or with addition of LAB, specifically, *Lactobacillus plantarum*. Acidulants, such as 0.5% to 0.85% lactic acid or 0.25% acetic acid per litre of water are recommended to keep pH below 5.[2,9] Addition of acid will promote growth of native wild LAB if a culture is not added. Re-acidified water may be added if water is drained and changed during the soak process.[2] Refrigeration during soaking is also acceptable when small batches of tempeh are made.

Soak water is discarded and soybeans are boiled for 20 to 30 minutes, ranging from 10 min to 3 hrs.[1,9] Steaming for 30 min at 100°C is also reported.[9] One study suggests adding acid to the boiling water also appears to reduce occurrence of *Bacillus* spores during the cook step.[1] Cooked soybeans are emptied onto a large flat surface and cooled to between 25°C and 37°C prior to mould addition.[1,3,9] Once cooked soybeans falls below 60°C, mould should be added within two hours and no later than six hours (2 hrs=CCP; 6 hrs=critical limit). *Rhizopus* spp. moulds include *R. oligosporus*, *R. oryzae*, *R. stolonifer*, *R. microsporus* var. *chinensis* and others.[1] Recommendations are to add 6 log spores per 100 g of cooked soybeans.[9] Backslopping, the practice of adding a portion of an earlier tempeh batch into the next batch being made is not recommended, as this practice creates food safety concerns and performance concerns as the mould hyphae desiccates following log growth.[1,2] Co-inoculation with LAB, *Lb. plantarum*, *Lb. casei* or *Lb. fermentum* may also occur at this step.

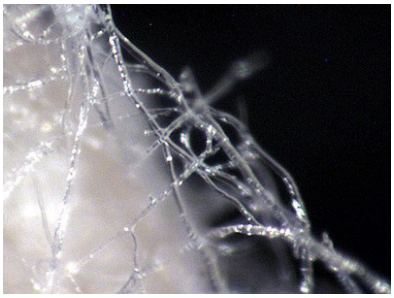
Box 2 | Interactions of microbial agents, pH and food safety considerations in tempeh

Tempeh is described as an alkaline fermentation because the main fermenting agents are *Rhizopus* spp. that release ammonia into the soybeans. The pH of starting soybeans, if acidified at pH of 4, will increase to approximately pH of 7 during the fermentation.[3] One study showed a pH a change from 4.6 to 6.6 during the first 46 hrs of fermentation, then to 7.1 at 72 hr of fermentation.[1] However, if no acidification occurs during soaking, pathogens, e.g., *Bacillus cereus*, can grow to log 6 to log 7 CFU/g after 40 hr fermentation.[1] During the soaking step, acidification of the water by adding acid or LAB will help suppress pathogenic microbial growth.[1] Other pathogens modelled in tempeh that also grow in unacidified tempeh substrates (soybean, chickpea, pea, fava bean tempeh) include *E. coli*, *L. monocytogenes* and *S. aureus*.[1] However, after boiling the LAB is largely destroyed. Co-inoculating *Rhizopus* with LAB has shown better efficacy at suppressing *B. cereus* growth, and acidification and co-inoculation has shown optimal suppression of *B. cereus*.[1]

Following addition of *Rhizopus* mould the mixture is packaged or packed into containers. Cultured tempeh will take on the shape of whatever container is used. Commonly, perforated polyethylene plastic pouches are used, however perforated plastic can also be used to cover the tops of wooden, plastic, or stainless steel containers.[1,3] Containers should drain as tempeh soybeans should not be held in standing water, optimal water activity at the beginning of the fermentation is 0.99 to 1.00.[1,9] Traditionally the soybeans would be wrapped in leaves or covered with scored banana peels, this is not recommended unless controls are present to limit contamination.[1] The fermentation conditions and packaging should allow for sufficient oxygen, but not too much oxygen, otherwise sporulation will occur.[9] Fermentation usually takes place in a separate room where temperature and humidity can be controlled, records of these conditions are recommended. Optimal humidity is between 75 and 85%.[9] Time and temperature should be controlled, conditions may vary from 1 to 3 days at temperatures between 20°C to 37°C.[3] Example conditions are 25°C for 80 hrs; 35°C for 18 hr; 32°C for 22 hr and others. Temperatures should not go above 42°C as *Rhizopus* mould may die off, permitting growth of spoilage organisms.[3] Typically fermentations are completed within 72 hr. The final water activity will be approximately 0.95 to 0.96 and final pH will be approximately 7.0, indicating tempeh is perishable with a short shelf-life.[1,10]

Fresh tempeh should be consumed within 1 to 3 days at room temperature.[9] Following fermentation, fresh tempeh may be mixed with spice or oils flavourings, vegetables or herbs. Tempeh is often dipped into sauces before frying or served as a burger patty.[9] Shelf-life can be extended by steaming, boiling, or frying. Tempeh should be pasteurized before drying, dehydrating or vacuum-packaging to extend the shelf-life.[1,9] At refrigeration temperatures, tempeh dried to an a_w of 0.48 may be stored for up to 30 weeks; vacuum-packaged tempeh may be stored for approximately 30 days.[1] Residual *Rhizopus* moulds, even when tempeh is pasteurized (immersed in 80°C for 3 min), continue to ferment in the interior of this food, over time the pH will continue to increase, the tempeh will become soft, ammonia levels will increase and the tempeh will become bitter to the taste.[11] Operators are recommended to conduct their own shelf-life testing to establish how long their tempeh may be sold and maintain optimal quality, and should label with a best before date (BBD).

Figure 3 | *Rhizopus* mould cultures

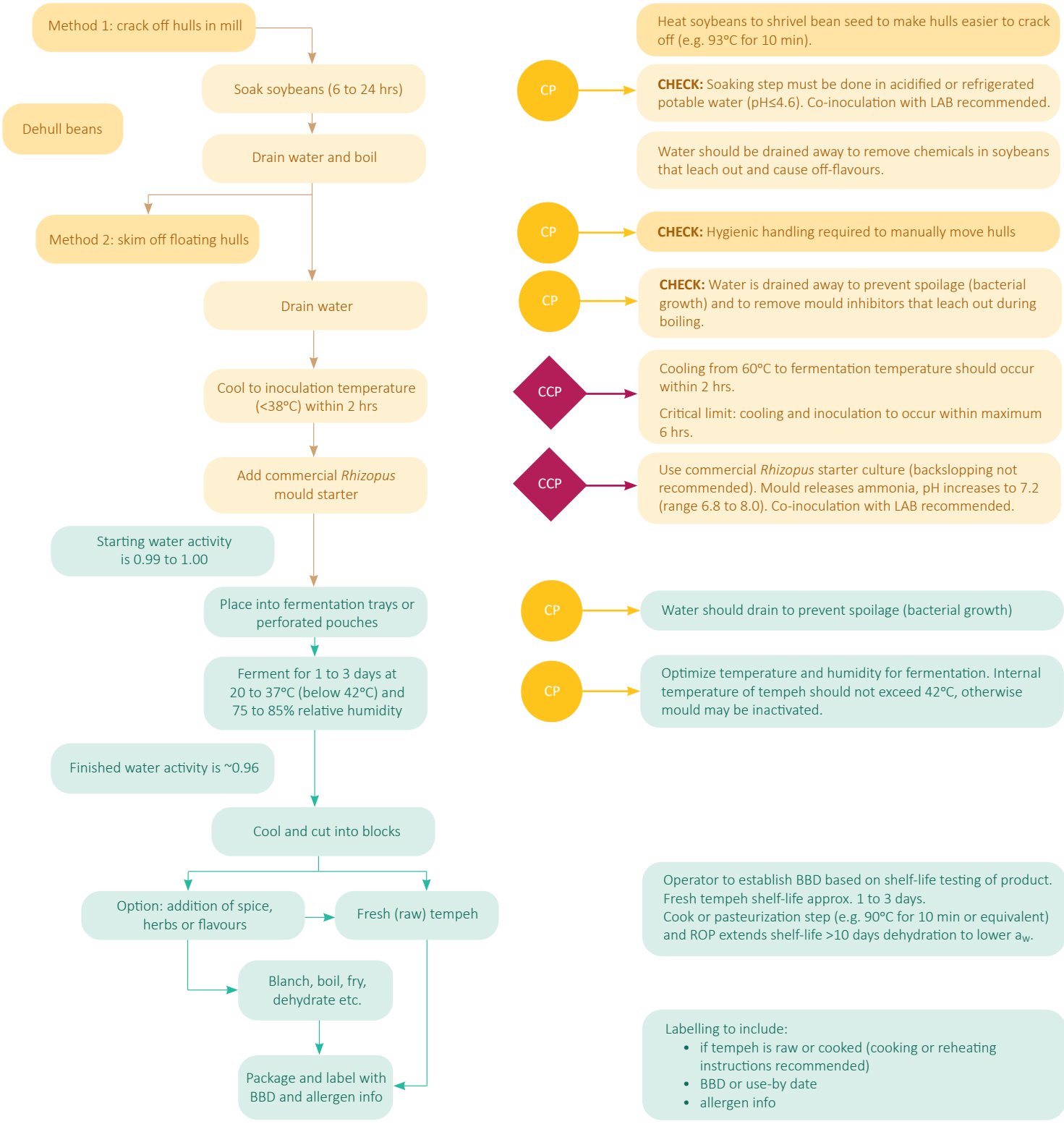


Mycelium of *Rhizopus orzae* on fresh tempeh

Sporangia of *Rhizopus oryzae* on overripe tempeh

Photos in figure 3 reproduced with permission of Top Cultures.[12]

Tempeh food flow chart | Process flow and controls



Potential issues with tempeh food preparation

Issue	Description
Starter culture	The documented tempeh outbreak in the U.S. arose from contaminated commercially purchased starter culture. Commercial starter culture is recommended, and the commercial culture should have a certificate of assurance (COA) that the culture has been tested to demonstrate absence of pathogens, specifically *Salmonella*. Absence of other potential pathogens is recommended, e.g., *Listeria* spp., *Bacillus cereus*, and pathogenic *E. coli*. Many wild strains of *Rhizopus* species can harbour species of bacteria that produce toxins. One such bacteria is *Burkholderia* known to have a symbiotic relationship with *Rhizopus*. It produces a toxin called rhizonin, which is a hepatatoxic cycloprotein. For this reason, backslopping is not recommended, as cross-contamination during successive culture cannot be monitored for toxin producing strains.
Soy bean husks	Soybeans need to be de-hulled to allow the mould, *Rhizopus*, to grow efficiently. The husking or de-hulling process does not typically confer a physical hazard if done using mechanical means using compression of the beans between rolling cylinders. However, if husks are removed using hands (traditionally using feet) then cross-contamination arising from human pathogens is possible. It is recommended that clean gloves (or boots) are worn during this step.
Soaking	When soybeans or other substrates are soaked for longer than 4 to 6 hours, bacteria capable of producing heat resistant toxins have the opportunity to grow, such as *Bacillus cereus* and *Staphylococcus aureus*. Soaking water should be potable, and soaking conditions should limit growth of microbes via acidification of the water with lactic or acetic acid.[9] *Rhizopus* mould is not inhibited until pH drops below 3.5.[9]
Boiling or steaming	Boiling or steaming is usually done for a minimum of 20 minutes but can be longer. As long as the steaming step is >74°C or higher then vegetative pathogens will be destroyed.
Cooling	Prolonged cooling periods could allow spore-forming bacteria (e.g., *Bacillus*) to grow and release toxins. Cooling to the target mould inoculation temperature (e.g., <38°C) should occur within 2 hrs after temperatures fall below 60°C (ideal) with critical limits of up to a maximum of 6 hrs.
Limiting bacterial growth and spoilage	After cooling and mould inoculation, the holding vessels and environmental conditions should be optimized for mould growth. Sufficient air circulation and oxygen, ensuring containers drain away excess liquids, and surface drying of soybeans (e.g., coating beans with wheat flour or drying with towels) are considerations.[9] If conditions during fermentation are too wet, spoilage microbes and/or harmful bacteria may grow. If too much oxygen is available, mould sporulation and darkening of tempeh may also occur.
Cooling during fermentation	*Rhizopus* mould is inhibited above 42°C, during fermentation tempeh temperatures should be monitored and incubation temperatures adjusted to ensure optimal fermentation. As temperature may rise between 5°C to 7°C, fermentation temperatures at or below 37°C or lower is recommended)[3].
Over-fermentation	*Rhizopus* mould growth will bind the tempeh into a compact cake-like form. If the alkaline fermentation goes too long, surface discolouration will result from the white mould on the surface turning black due to formation of spores, and an over-production of ammonia odour. While this is not a safety issue, it is an undesirable food quality issue.
Storage and shelf-life	Prolonged refrigerated storage of fresh tempeh can result in undesirable odour from the ammonia smell and growth of black coloured *Rhizopus* mould when it sporulates.[9] Fresh tempeh has a shelf-life of 24 to 72 hrs. To store tempeh for longer periods, the finished tempeh should be fried, boiled (blanch pasteurized) or steamed. Packaging in reduced oxygen packaging would also extend shelf-life.[3,9] Operators are responsible to establish shelf-life through testing. Tempeh may also be dried and dehydrated.

Tempeh food safety control points

- Commercial culture of *Rhizopus* is recommended. A COA should verify the culture is free of *Salmonella* and other pathogens, e.g., *Staphylococcus aureus* and *Bacillus cereus*. Backslopping using previous tempeh and *Rhizopus* mould is not recommended.
- Control undesirable bacterial growth during soaking of soybeans by one or more processes:
    - Acidification of water with acetic or lactic acids to pH of 4.6 or lower, but not below pH of 3.5
    - Co-inoculation with LAB strains
    - Refrigeration
- Fermentation conditions should limit bacterial growth and optimize mould growth, such as:
    - Internal tempeh temperatures should not exceed 42°C otherwise mould will die off
    - Containers to hold tempeh should allow excess water to drain, sufficient oxygen allows mould and mycelium (fungal strand) growth
    - Relative humidity should be controlled to between 75% to 85%.
- Consumer packaging should include adequate labelling to inform:
    - if the tempeh is sold as a raw or cooked (pasteurized product). If the product is raw, labelling on packaging should indicate a cook step is required,
    - if the tempeh includes an allergen risk (e.g., wheat flour used to dry soybeans)
    - a pull-by or use-by date is included on the packaging to establish shelf-life
- Operators should establish a shelf-life for their product by conducting quality testing of the product.

Potential health issues with tempeh

As with other soy-bean based fermented foods, biogenic amines can be produced during tempeh fermentation and are harmful if ingested in high concentrations. Biogenic amines occur primarily from metabolic activity by microorganisms during fermentation (see Box 3 below).[13] The highest levels of histidine and phenylethylamine reported for tempeh were 100 mg/kg and 30 mg/kg, respectively, both at the threshold for causing illness. Levels of tyramine were 575 mg/kg and within reported levels that cause illness between 100 and 800 mg/kg. Putrescine (3200 mg/kg), cadaverine (225 mg/kg), spermidine (105.5 mg/kg) and spermine (21.9 mg/kg) were also detected.

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.[14,15] 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.[14,16,17] 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.[18,19]

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.[20] 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.[15]

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; *(continued on page 12)*

(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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