

# Safety of Fermented Foods

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

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Additional fermented food guidance can be accessed at:

<http://www.bccdc.ca/health-professionals/professional-resources/fermented-foods>

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## Section 2 | Starter cultures and fermented food standards

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Inspection practices differ between provincial jurisdictions. Readers of this guidance are advised that information provided on best practices for fermented foods may not fit with all provincial requirements. Examples of provincial differences include:

- requirements for food safety and sanitation plans that identify critical control points (CCPs) and control points (CPs),
- labelling and refrigeration practices for foods considered potentially hazardous (PHF),
- addressing alcohol as a chemical hazard in fermented beverages like kombucha, jun and kefir,
- compositional and naming conventions for dairy products made with milk or plants.

Provincial differences may be based in legislation. Information in this section will identify issues of concern for inspectors with a food safety focus. Recommendations and best practices will be identified, for example, what microbial tests are appropriate to take when sampling a fermented food, when samples should be taken (by the inspector or operator), when testing should be requested, and others. This section will also provide depth to topics covered in guidance provided for specific foods, such as consistency in controls and critical controls for times and temperatures, starter culture management for fermented foods, further information about biogenic amines and alcohols as chemical hazards in some fermented foods, and how to address shelf life testing questions.

Differences may also arise at the operator level. Each fermented food covered in section 3 of the guidance will provide a description of how the food is made, a food control flow chart describing the process flow and controls, a description of hazards and potential issues and food safety control measures recommended for the food. Variations in how to make fermented foods are too numerous to describe, and where they are not described, it is crucial that inspectors review and operators document their process. Three likely errors when assessing micro-safety in foods include overlooking a step on a process flow diagram, failing to recognize a potential hazard at the process step (biological, physical or chemical), and incorrectly evaluating the significance and severity of the hazard.<sup>1</sup>

### Requirements for sanitation plans and food safety plans at premises

Sanitation plans and food safety plans are recommended for all fermentation operations. Fermentations can range from simple to very complicated. All fermentations require strict adherence to good manufacturing practices (GMPs) and sanitary operational controls. Even when fermentations result in high acid content, i.e., pH of the food product is less than 4.0, poor sanitary practices can affect food quality and shelf life. Poor sanitary practices may result in contamination of ingredients, such as starter cultures. A food safety plan will assist the operator and inspector in determining whether critical limits and critical control points are achieved during the fermentation process. A sanitation plan and GMPs will control issues with incoming ingredients, storage, equipment (sanitary and hygiene design) and building maintenance. This guidance does not include information about designing food safety plans, sanitation plans, prerequisite programs for premises, preventative control plans (PCPs) or hazard analysis critical control point (HACCP) plans. It is expected that inspectors and operators will find this information from their local health department or most appropriate agency.

### 2.1 Ingredients

In Section 1 fermentation was described as the process in which foods are modified by the action of microorganisms. The microorganisms digest the food, transforming and preserving the food components into something with desirable taste, texture, quality and safety. In this section we explore how starter cultures work with food ingredients, referred to as 'the substrate' in this context. For other unfamiliar terms in the fermentation guidance, consult the glossary of fermented food terms available on the website.

## **Starter cultures**

### ***Initiation of fermentation***

Fermentation will not begin without microbes (e.g., fermentative bacteria, yeasts, moulds) first being present to break down the food substrate. Starter culture in this guideline refers to how the fermentation process is initiated using fermentative microbes. Fermentations may begin (i) spontaneously with microbes naturally present in ingredients, (ii) with the addition of commercial starter cultures, (iii) from backslopping, (iv) from contact with equipment or utensils that were used in a previous fermentation and contain residual live culture or (v) through addition of some other ingredients, such as probiotics (not recommended for initiation of fermentation). Each of these different types of starter culture are reviewed, along with food safety risks.

### ***Spontaneous fermentations without starter culture***

A spontaneous fermentation, sometimes referred to as “wild fermentation” or “natural fermentation” is any fermentation that occurs through natural processes without starter culture, or introduction of added microbes. The main driver of spontaneous fermentation are microbes introduced into the fermentation system from (1) raw ingredients, (2) vessels, tools and equipment used in the fermentation, and (3) the environment in which the fermentation is being performed. Other factors may also influence the course of a fermentation. For example, addition of salt drives spontaneous fermentation towards specific salt-tolerant, acid-producing fermentative microbes (lactic acid bacteria).

Temperature is an important factor in most spontaneous fermentations. The term ambient (room) temperature is vague and does not define the optimal temperature range for a fermentation. A sauerkraut ferment conducted during the summer and winter may require differing lengths of time to achieve the same end result. Cooler temperatures generally result in longer, slower fermentations. Because there is limited control over the type and number of microbes entering the fermentation process, as well as over physical and chemical conditions under which the fermentation is performed, there is an inherent unpredictability associated with spontaneous fermentation. For example, sanitary handling and cleanliness of incoming ingredients, sanitation of vessels, tools and equipment, and general hygiene in the process have a direct effect on fermentations.

Spontaneous fermentations have inconsistent quality and often fail.<sup>2</sup> Microbes on ingredients will be a mixture of beneficial bacteria that can start the fermentation, spoilage bacteria that may cause off odours, off flavours or change the texture of the ingredients, and pathogenic microbes capable of causing illness. Spontaneous cultures have also been linked to production of toxins, including mycotoxins, biogenic amines and other issues.<sup>3</sup> Problems can occur at the beginning of a fermentation as well as during and at the end of the fermentation. A successful fermentation will have the correct concentration and composition of microbes during the dynamic activity of the fermentation process. The unpredictability of spontaneous fermentation may reduce the control over the sensory, nutritional, and functional quality of the final product, as well as the safety of it. Spontaneous fermentation activity should be confirmed visually through observation, for e.g., by bubbles forming indicating gas production, by measuring a pH drop within the expected timeframe to more acidic conditions, or by some other acceptable measure.

### ***Commercial starter cultures***

Commercial starter cultures are preparations of live bacteria, yeasts or moulds whose metabolic activity creates the desired effect on the fermentation substrate. Commercial starter culture preparations contain one or more microbial species and/or strains together with media components from production of starter culture and other components which are necessary for their survival during storage and activity during use. Commercial starter cultures are produced by specialized manufacturers who implement rigorous quality control to ensure performance, composition, and safety of the culture. The International Dairy Federation (IDF), in conjunction with the European Food Safety Authority (EFSA), have created a list of acceptable food cultures for fermented foods that have undergone examination and testing for safety.<sup>4,5</sup> As of 2022, 314 species are listed in this inventory.<sup>5</sup> Should inspectors or operators question the validity of a commercial culture for its intended use, the inventory defines the use of microorganisms and separates them into seven fermented food categories: bakery items, alcoholic beverages, dairy, meat, plant-based, seafood and vinegars.<sup>5</sup> In addition to food usage and microorganism identification (by kingdom, phylum, family, genus, species and sub-species), the inventory provides reference of the food usage within the category identified. For example, *Lactobacillus delbrueckii* subsp. *bulgaricus*, has historic provenance in dairy foods (1979), wines (1966), meat (2008) and plant-based culture (2009).<sup>5</sup>

If the food culture appears on the inventory, then it has been verified as an acceptable food culture in fermented foods. This system provides assurance that commercial starter cultures used are established with a history of safe use.

Besides substrate composition and environmental conditions, starter culture is the most important factor that will determine the characteristics of the final fermented product. Starter cultures minimize inherent unpredictability of spontaneous fermentation and allow operators to take better control of the fermentation when additional steps are taken to prevent contaminant microbes from substrate, vessels, tools, equipment, and environment from entering the fermentation process. Starter culture introduces a high number of beneficial microbes into the fermentation process selected and tested for that specific fermentation (specific substrate at specific conditions). The selection process ensures that the final starter culture strains have sufficient acid production rate, a short lag phase to growth, produce desirable flavour and texture, and are resistant to phages (phages are viruses that kill or limit the activity of bacteria).<sup>2</sup> Commercial starter cultures normally come with instructions for use, operators should follow manufacturer guidance on quantity to add under specific environmental conditions.

### ***Backslopping***

Backslopping is the practice of using finished fermented product that contains live microorganisms to inoculate a new batch of product substrate. The culture from the original fermentation is often referred to as the “mother”. There are two ways to add previous culture to a new batch. One is to do so directly by adding portions of a previous fermentation into a new batch. Examples of this can be found in the production of kombucha. Kombucha culture, i.e., “Symbiotic Culture of Bacteria and Yeast” (SCOBY) or the liquid, is not available as a commercially available dehydrated culture powder. There are a limited number of commercial suppliers for live kombucha culture, and these suppliers can be used to initiate new kombucha when a SCOBY becomes contaminated or less effective. Another practice is by re-use of containers that allow survival of live microorganisms from batch to batch.<sup>2</sup> When this method of backslopping is used, the equipment cannot be cleaned and sterilized between batches, which raises obvious contamination and food safety concerns. An example of this form of backslopping includes kefir, a traditional fermented milk beverage made from kefir grains. Kefir grains contain lactic acid bacteria and yeasts and addition of them to a batch would be another example of direct backslopping. Traditional methods that add fresh milk to a cloth, skin bag would also be an example of backslopping, where the residual culture on the surface of the skin bag restarts in a continuous fermentation process.<sup>6</sup> Use of skin and cloth bags to make commercial kefir is not recommended, because of difficulty in cleaning and sanitizing of these bags.

### **Food safety considerations when backslopping is used in food fermentations**

If an operator is backslopping, questions to ask the operator to assess food safety include:

#### ***1. Is backslopping being used to promote acid fermentation?***

Backslopping should only be used in acid fermentations since acid producing microorganisms and acidified product minimize risks of acid-sensitive pathogens from contaminating backslopped culture. However, acid-tolerant spoilage agents and pathogens can persist and may amplify in backslopped culture when cross-contamination occurs. Further, backslopping is not recommended for alkaline types of fermentations. For example, backslopping koji is not recommended since the process can introduce undesirable moulds into new substrate that backslopping could potentially propagate. Another example would be smear bacteria on cheese. As smear bacteria are consuming the lactic acid and de-acidify the surface of the cheese, this may allow the growth of potential pathogens on the smear layer that could penetrate the cheese surface. If the smear was backslopped (old to new smearing), it could transfer and propagate growth of the pathogens. In summary, backslopping in non-acid fermentations or in fermentations where the culture creates low acid conditions are not recommended.

#### ***2. Is the backslopped culture compatible with the fermentation performed?***

In general terms, use of backslopped culture will initiate fermentation faster in comparison to spontaneous fermentation. The backslopped culture should originate from the same type of food that is being made. For example, sauerkraut juice from a fermented batch can be used to initiate fermentation in a new batch of sauerkraut. We should not recommend sauerkraut juice be used to initiate fermentation in a different food product, such as plant-based cheeses or water kefir.

### **3. How is the operator managing the backslopped culture?**

Is a fresh batch of culture taken from the previous batch, or is the same starter maintained in a refrigerator from an older batch (for weeks, months, or longer periods). In general terms, a product in process of active fermentation should be used as backslopped culture. Any storage is reducing fermentation activity, promoting contamination and undesirable microbial activity. For example, growth of oxidative yeast and moulds that are using acid as energy source may reduce the pH, potentially allowing for acid sensitive microbes to grow. Some fermentations are accompanied by growth of oxidative yeasts that form a film on the top of product; this part should be avoided when collecting product for backslopping. The operator should be able to describe how the starter culture is maintained and managed for the intended food process; for example, they should be able to explain what a 'good' (optimal) backslopped culture looks like and how it differs from a 'bad' one. Information about rotation, storage temperature, and duration of storage should be included in the food safety plan.

### **4. How is the operator assessing the quality and safety of the backslopped culture?**

The operator should be able to describe critical limits to assess the backslopped starter culture in terms of its appearance, performance during the fermentation, and in terms of the final fermented product. Defining the optimal characteristics could include colour of the starter, performance (bubbles should start within defined period of time), size or growth of culture (e.g., size of kefir grains, or quantity of growth) and absence of visible contaminants. For food safety, the backslopped culture should be free of pathogens and should be able to initiate fermentation rapidly, i.e., within 24 hours. For food quality, if the fermented food did not result in the desired flavour, colour or texture, that could be an indication that the starter culture is not performing well. Control of the product food safety for fermented foods means the operator should know what the typical fermentation process 'looks' like. This can include direct visual observation: such as bubbles becoming visible in the fermentation liquid within 8 hours; or can include objective criteria, such as total acid development within a specific timeframe, or reduction in total acidity. For example, starting pH for the food is usually between 5.8 and 6.5 and the pH should drop by 1.5 units within 12 hours.

#### **Recommendations for assessing safety of backslopped culture:**

- The operator should check pH has dropped (or changed) in an established expected time, such as within 24 hours of adding backslopped culture. If the process of taking samples and measuring the pH would disrupt the fermentation process (e.g., miso or yogurt fermentation) a smaller side fermentation could be set up that would give an indication of active fermentation.
- Operator should assess food safety of backslopped culture by submitting a sample to the laboratory for hygiene/sanitation testing. Frequency of testing should be established based on production volume, i.e., number of lots with larger operations testing more often. Recommended tests: *E. coli* should be absent from all starter cultures; *Staphylococcus aureus* may be indicated for foods with higher salt content, or in foods handled with direct hand contact (food worker contamination). Aerobic colony count is not recommended as healthy fermenting starter cultures will have actively growing bacteria, moulds, or yeasts.

During an inspection, if health inspectors observe issues or infractions, such as those associated with premises sanitation, employee hygiene or fermentation process, then requesting food samples, including starter cultures for microbiological assessment is recommended. Examples of problem hygiene and sanitation issues can range from visible pests (rodent activity), fermentation vessels and foods are inadequately covered, food handlers do not follow hygiene procedures, regular cleaning and sanitation of equipment is not performed, or poor hand-washing and other practices are observed. Process issues may include findings on log sheets demonstrate fermentation process is consistently poor, temperature and time controls are not followed, backslopped cultures are not used fresh, batches that appear to fail are not discarded or managed correctly according to corrective action plan defined for the process, and other issues noted on inspection.



## Probiotics

**Probiotic cultures** are live microorganisms which when administered in adequate amounts confer a health benefit on the host.<sup>7,8</sup> Probiotic culture and fermented cultures for foods are not the same, because the former (probiotics) are created for human health benefit and the latter (fermented food starter culture) is optimized for food fermentation. Although production of acid is one of the mechanisms by which probiotics confer health benefit, it is not a specific characteristic for probiotics selection. Probiotic strains are frequently isolated from microbial populations in gastrointestinal tract (GI tract) environments because most of the health benefits come from mechanisms connected to the GI tract. Probiotics are rarely isolated from food sources. Probiotics are by definition live microbes that need to keep their viability during transition through the GI tract. Characteristics for probiotic selection include ability to resist low pH of stomach acids, the inhibitory effects of bile salts and digestive enzymes, the ability to attach to epithelial cells, to transitionally colonize GI tract, thereby inhibiting pathogens via competitive exclusion from intestinal walls, or direct pathogen inhibition from bacteriocin production.<sup>7</sup>

Fermentative ability of probiotics is rarely included in selection process, in fact, probiotics are usually not very good at growing in food substrates. For example, probiotics in dairy and other food products rarely grow to desired numbers, additional probiotics are added in concentrations required in the final product or even higher to account for die-off during shelf life. Probiotics that appear on the market in the form of pills, capsules and powders are even less selected to grow in milk or other food products even though they belong within the group of lactic acid bacteria. The primary reason these probiotic strains were selected are the health benefits they confer (e.g., control of traveler's diarrhea). The media to cultivate them is tailored to meet their requirements. Although many probiotics are produced by growing the strains in dairy containing media this is not true for all probiotics that are produced. Some of the components used in dairy and non-dairy media include yeast extracts, enzyme digests, amino acids, vitamins, salts, and buffers.

A very common feature in fermented and non-fermented probiotic products is microencapsulation of probiotic strains. Microencapsulation is a process of coating individual cells or cell groups to introduce a protective barrier that protects the cells from the outside environment during storage (e.g., desiccation, oxygen), food production (e.g., production of yogurt), and transition through GI tract (e.g., stomach acid, bile salts). Microencapsulation is usually designed for targeted release of cells in GI tract, initiated by either stomach acid or specific enzyme activity in the intestine. Microencapsulation is limiting the availability of nutrients to the cells as well as limiting the space available for cells to grow and replicate.

### Box 1 | Rationale for why probiotics should not be used as starter cultures for fermented foods

**Fermented food working group members provide a consensus opinion that probiotics are not suitable as starter cultures for fermented foods.** We believe that adding commercial probiotic products in reasonable amounts to any food matrix is not a food safety concern. However, it is not suitable to rely on probiotic mixtures meant for human consumption and GI tract health to consistently perform fermentation; key reasons described above are summarized here:

1. Probiotic cultures are made to keep the strains alive in the matrix they were designed for and rarely include strains that can grow and perform active fermentation. Probiotics are bad at competing with other fermentative microbes, spoilage organisms, or even pathogens, and if they are able to grow, this growth is slow with slow or insufficient acid production.
2. Probiotic products like pills, capsules and powders are designed to keep probiotic strains alive in the product and during transition through the GI tract. The probiotic strains in these products are not selected to grow in food or perform fermentation.
3. Probiotic products usually contain strains that are microencapsulated which represents a physical barrier between the bacterial cells and the outside environment. The space and nutritional availability limitations presented by microencapsulation limit the strains' ability to grow and initiate the fermentation.
4. Even if probiotic products contain a strain that is capable of performing fermentation, there is no guarantee that this strain will perform fermentation consistently from all lots of probiotic product, in all food fermentation batches, or that it will remain part of the probiotic product formula over time.

**Evaluating ingredients.** The safety of fermented foods relies on the safety of the ingredients used to make them. When evaluating a fermented food recipe and process it is important to gather details about their type and origin, that ingredients are appropriate and come from approved sources, and that ingredients can be managed for their inherent risks.

**Quality of the ingredient.** Many fermented food recipes describe that fresh fruits, vegetables, nuts, meats or other ingredients should be clean, free of debris, fresh and of good quality. There are several good microbiological explanations for this: naturally occurring microbes provide the basis for starter culture bacteria in spontaneous fermentations (i.e., fermentations without added commercial starter culture). When food ingredients are stored for long periods (old), the number of spoilage and pathogenic organisms can increase over time due to opportunistic growth; this is elevating the risk either directly by increasing pathogen numbers on the ingredients or indirectly because of a higher likelihood of failed fermentation. The undesirable microbes can compromise fermentation success by slowing the start-up of spontaneous fermentations, can impart off-flavours and odours, may drive formation of chemical hazards, such as biogenic amines, or change conditions in the fermentation that allow additional growth of harmful foodborne pathogens.

**Risk of the ingredient.** Always evaluate risk of ingredients in a recipe list. What do you know about the ingredient? The name of an ingredient does not always tell you about the risk. Risk can be based on where the ingredient was from, if it is raw or processed, who the supplier is, and how they've sourced the ingredient, and other factors. For example, in this list of ingredients to make plant-based cheese, what additional information is needed to evaluate risk of the ingredients?

- Water
- Hazelnuts
- Apple cider vinegar
- Lemon juice
- Coconut milk
- Starter culture

Questions to ask the operator might include:

- **Water.** Is the source of water potable? Do they boil the water and cool it before use? Do they use 'alkalinized water', and if so, do they have a cleaning and sanitizing or maintenance program for the device used to make this water?
- **Hazelnuts.** Are the hazelnuts raw or roasted? Who is the supplier? Is there a manufacturers' specification sheet that provides information about the ingredient? Does the information include testing information about the lot, for example, the nuts show absence of *Salmonella*. If the operator claimed the hazelnuts were from a local farm, other questions/concerns that may arise would include how the hazelnuts are prepared, if they are raw, roasted, or jarred in a puree, etc.
- **Apple cider vinegar.** Is this product purchased from the store or made by the operator? What is the vinegar concentration? Cider vinegar made by the operator would need to be evaluated separately.
- **Lemon juice.** Is the lemon juice freshly squeezed, and what are the handling steps to be assessed or is it commercially prepared and purchased from the store? Is it pasteurized?
- **Coconut milk.** Is the coconut milk canned or fresh? Canned coconut milk is lower risk than extracting fresh milk from a coconut or squeezing milk from rehydrated coconut flakes. Use of fresh coconut milk would require more details about the handling and process.
- **Starter culture.** What are they using as starter culture? The risk would be very different between a commercially purchased lactic acid bacteria (LAB), in comparison to a backslotted culture unsuitable for the nut substrate (e.g., fermentative microbes from sauerkraut is based on a substrate of cabbage, natto on soybeans, kombucha on tea or kefir on milk).

**Ingredient documentation.** In the example above, a manufacturers' specification sheet was mentioned. These may be named or referred to as:

- Certificate of Analysis (CoA)
- Product Specification Sheet (PSS)
- Suppliers' Quality Assurance (SQA)
- Ingredient Specification Sheet (ISS or Spec Sheet)
- Technical Data Sheet (TDS)

A CoA should provide information about the ingredient and the manufacturer. It should include what the ingredient is, name and address of the manufacturer, storage and shelf life, and test results for product quality and safety.<sup>9</sup> Asking for CoA certificates from suppliers of ingredients is one method of control and assurance that ingredients are meeting standards. Our fermented food guidance will often request CoA documentation demonstrating the ingredient is free of a hazard, such as *Salmonella*. Obtaining a CoA does not guarantee the ingredient's safety or remove requirements for safe food handling and processes. However, it can be included as a requirement for the overall quality assurance program for the business.<sup>10</sup> A CoA that indicates absence of a pathogen does not mean that the risk of this pathogen being present in the ingredient is zero; you have to still follow the appropriate processing procedures that would inactivate the potential pathogen even if a CoA was available for the ingredient.

### **Differences between acidified and fermented foods and evaluating food safety and quality**

Fermented foods become acidified through microbial activity of starter cultures and development of organic acids, such as lactic acid. However, these foods should not be confused with acidified foods, when this term is used to describe the process of direct acidification of foods using vinegar (acetic acid) without any fermentation process. Another layer of potential confusion is the term used to describe both of these foods, pickling. To most consumers, pickling and pickles refer to acidified cucumbers hot-packed into a vinegar solution and pasteurized in glass jars. But it can also refer to lacto-fermented pickles, for example, kosher dill pickles are partially or wholly fermented as sour pickles. In this guidance, we avoid using the word pickle as it refers to both processes and pickles (see [Section 3.1 on fermented vegetables](#) for more information).

This guidance will focus on the **process** for fermented foods. When evaluating food safety and food quality characteristics of fermented foods, it is important to understand the distinction in the aims thereby leading to differences in recommendations. Food safety aims to protect consumers from harms, specifically from biological, chemical, and physical hazards that may arise during processing. An example of a biological hazard of concern in fermented foods is botulism. Botulism occurs when spore-forming bacteria of *Clostridium botulinum* grow and release toxins into ready-to-eat (RTE) foods. Examples of chemical hazards in fermented foods include unintentional formation of alcohol (ethanol) and biogenic amines, described later in this section. Physical hazards might include fragments of metals from mixing blades or blenders.

Process tests for fermented foods almost always involve checking the acidity or pH of the food. Microbiological assessment for hazards recommends tests for pathogens (e.g., *Listeria monocytogenes*, *Salmonella*), indicators of pathogens (e.g., *E. coli*, *Enterobacteriaceae*), or evaluation of pH and time out of temperature control (e.g., *S. aureus* degree-hour calculations when making fermented sausage). Chemical hazard assessments may recommend calculating and recording measurements of ingredients to ensure correct percentages are added (e.g, salt, nitrite). For example, physical hazard assessment may recommend use of metal detectors if possible or visual observations to ensure foods are free from metal fragments arising from knives, blenders or equipment used to mix foods.

By contrast, food quality aims to assess the shelf life and optimize consumer preferences. Evaluation could include colour, texture, and odour (sensory characteristics), changes in chemical parameters of the food affecting quality (pH,  $a_w$ ) and possibly microbiological tests to determine spoilage or elevated bacterial growth (e.g., moulds, yeasts).

Fermentation is generally described as a 4 step process<sup>11</sup>:



Vegetables, grains, meats, and other substrate ingredients will have a mixture of Gram-positive and Gram-negative bacteria, yeasts, and moulds. Primary fermentation begins when the mixture of native or wild sources of microbes including bacteria (e.g., LAB), spore-formers (e.g., *Clostridium botulinum*, *Bacillus cereus*), yeasts, moulds, Enterobacteriaceae (e.g., *E. coli*, *Salmonella* ssp.) and other microbes are released from the substrate. Depending on the type of fermentation, commercial starter culture may be added, ingredients are added, for example, salt concentration is a critical control point (CCP) to drive the primary fermentation towards specific species of LAB groups in spontaneous fermentations, and specific environmental conditions are created to optimize the fermentation process, such as humidity for *Rhizopus*-mould fermentations. In the secondary fermentation stage, a more homogenous population of culture occurs in the fermentation. Fermented products contain live microbes. Depending on the environment, containers and process, packaging, temperature, etc. spoilage may occur during the primary and secondary portions of fermentation if the process and good manufacturing practices (GMPs) are poor. Spoilage will occur in the post-fermentation period at some point, depending on product process controls. Pasteurization of finished fermented foods will inactivate microbes allowing longer product storage.

### Box 2 | Key items to consider for safety and quality of fermented foods

From a quality and food safety perspective, there are two key items to consider:

1. Are pathogens controlled in the product?
  - a. If pathogens are not destroyed, how are they controlled?
  - b. Is the product formulated or controlled to the end of the shelf life of the product, for example, to ensure pH will not rise above 4.6 to allow spore-forming microbes, e.g., *C. botulinum* to grow?
2. Is spoilage a concern in the product?
  - a. Does the process during primary and secondary fermentation prevent spoilage microbes from growing?
  - b. Is the product formulated or controlled so that pH will not rise above 4.6 to allow moulds and yeasts to grow?

Controls include pasteurization, refrigeration, holding times to allow acidification to destroy acid-tolerant pathogens, addition of preservatives and additives.

**Optimizing processes for log reduction.** Log reduction in this guidance refers to decrease in microbial populations for safety and quality (i.e., pathogen and spoilage log reduction). Generally, if a pathogen risk exists on an ingredient or in the prepared fermented food, then a 5-log reduction is required. A 1-log reduction removes 90% of the target microbes from the population of microbes in the food, a 2-log reduction removes 99% of target microbes, and a 5-log reduction removes 99.999% of the target microbes.

The process may be linked to how a specific ingredient is handled, holding times, or multiple controls that work to limit microbial growth in the food product. Examples in the fermented food guidance include cook steps to remove vegetative bacteria, cooling steps to limit microbial growth, and holding times, as shown in Table 1 on page 13.

**Table 1 | Examples of log reduction processes and controls in the manufacture of fermented food**

Food	Process for log reduction
Kimchi	This is a raw food with no cook step. Once fermentation is complete, and a pH of 4.6 or lower is achieved, a holding time of two weeks before distribution and sale to consumers is recommended. This time is required to allow acids that have developed to inactivate any <i>E. coli</i> present. This is based on studies showing 5-log reduction in acid-packed vegetables. <sup>12</sup>
Plant-based cheese made with nuts	Nut ingredients have been identified as the source of many outbreaks. <sup>13</sup> Before the fermentation begins, processes to reduce bacterial levels on nuts are recommended in the plant-based cheese guidance. This control point is stated as 90°C for ≥2 min and is derived from an Industry Handbook for the Safe Processing of Nuts (Dec 2020). <sup>14</sup>
Sausage	Some types of ready-to-eat sausages are made with raw meat that does not undergo a cook step during the fermentation. Smoking is often done at lower temperatures, less than 30°C. Several process controls are in place to reduce the risks of pathogens. A minimum amount of 100ppm nitrite is added to reduce risk of <i>C. botulinum</i> . A degree-hour calculation for the time sausages are held over 15.6°C is required to control the development of <i>S. aureus</i> toxin. And freezing of pork sausage meat is required before or after the fermentation to ensure <i>Trichinella</i> control. A special guideline is in place to reduce risk of <i>E. coli</i> O157:H7 in sausages made with beef. Health Canada’s guideline No. 12 requires manufacturers of sausages to comply with one of 5 process control options to ensure a 5-log reduction of <i>E. coli</i> O157:H7 occurs. <sup>15</sup>

## 2.2 Consistency for fermented food requirements

When creating guidance, members aimed for consistency on the requirements for check points (CPs), critical control points (CCPs) and critical limits (CLs) for process steps that were similar in different fermented foods. Decisions were justified based on evidence-based science, historical illnesses linked to the food, overall process risk, industry standards, and general practice. For example, any fermented foods that required a chopping, blending, or mechanical grinding step for various ingredients e.g., cabbage, nuts, soybeans, etc. invoked a CP for the process step. Operators are expected to check visually that blades are not damaged or to use a metal detector on the final product to ensure absence of this physical hazard, i.e., metal fragments.

Definitions for CPs, CCPs and CLs are described in the box and in the fermented foods glossary.

### Box 3 | Definitions for critical control and control points

**Critical control point (CCP):** A point at which a specific hazard must be controlled and managed if present because there is no other step in the process to control it; after this point a specific hazard cannot be controlled. There can be different Critical Control Points to control different hazards.

**Check or control point (CP):** A point at which a potential hazard may be additionally managed or controlled. For example, a CP could be a time/temperature control before a heating step is applied as the CCP. A specific hazard can have a check or control point only after it has a Critical Control Point.

**Critical limit (CL):** An upper or lower limit of a measurable parameter that defines conditions that control the hazard at the Critical Control Point or Control Point. When the limit is reached, the hazard is controlled, and when limit is not reached the hazard is not controlled and a corrective action should be initiated. Limits should be measured, recorded, and tracked by the Operator and corrective actions initiated when limits are not reached.

**Corrective Action (CA):** Action that regains control over a hazard in food after Critical Limits of a Critical Control Point were not reached.

Table 2 describes decisions for assigning a process step as a CP or CCP for fermented foods reviewed in this guidance.

**Table 2 | Consistency across fermented food guidance for control and critical control points**

Process Step	Guidance	Control	Critical Control Point (CCP)	Check (Control) Point (CP)
<b>Mechanical blending, chopping, grinding or use of blades</b>	All guidance	This process step typically occurs before fermentation, however, operators can verify the hazard is absent after blending, chopping, etc., at packaging, or other process points, using different methods. For this reason, we gave this as a control or check: ensure it is included somewhere in the process.  Others may choose to list this as a CCP. Because our guidance is focused on biological and chemical hazards we chose to always list this as a CP.		CP. Check blades visually or use a metal detector on finished product.
<b>Cooling time after cook step (before starter culture added)</b>	Natto	When the process includes a cook step, the substrate must be cooled before the starter culture is added. Prolonged cooling creates a hazard because, although the cook step kills vegetative cells, spore-forming microbes can still grow.  Control for this process step is to limit time in the danger zone (between 20°C and 60°C) to ≤2 hr.	CCP. 2 hours (critical limit is 6 hrs)	
	Koji & Miso		CCP. 2 hours (critical limit is 6 hrs)	
	Tempeh		CCP. 2 hours (critical limit is 6 hrs)	
	Yogurt		CCP. 1 hour (critical limit is 2 hrs) because industry standards/best practices recommend to limit this step to 1 hr.	
	Vegan milk kefir		CCP. 2 hours (critical limit is 6 hrs)	
<b>Commercial starter culture a requirement</b>	Koji & Miso	Adding a viable, healthy starter culture is important to the fermentation process. Commercial culture should be used whenever spontaneous fermentation, or backslopping has led to foodborne illnesses in the past.	CCP. Toxigenic <i>Aspergillus</i> from wild strains an unacceptable risk.	
	Plant-based cheese			CP. Strongly recommended.
	Yogurt		CCP. Backslopping not recommended in small-scale operations.	
	Natto			CP. Strongly recommended.
	Tempeh		CCP. Outbreaks linked to contaminated starter culture.	
	Sausage			CP. Strongly recommended. Stringent controls required when backslopping used.
<b>Cooling time after fermentation step</b>	All guidance	Prolonged cooling after the fermentation may lead to quality issues and shorter shelf life. Because fermentation microbes are present in high numbers in the food, this process step is less likely to create new hazards.		CP. Growth of starter culture limits other microbial risks.

Process Step	Guidance	Control	Critical Control Point (CCP)	Check (Control) Point (CP)
Soaking in water	Plant-based cheese	When soaking nuts, soybeans, grains or other substrates in water, hazard controls must be in place to prevent outgrowth of microbes.  Control options are temperature (refrigeration), acidification of the water, or time (no longer than 4 hr).	CCP. No cook or kill step later in process.	
	Natto		CCP. Cook step follows, but water acidification may result in bacteriophage issue.	
	Dosa & Idli			CP. Cook step later in process.
	Koji & Miso			CP. Cook step later in process.
	Tempeh			CP. Cook step later in process.

**Figure 1 | Soaking nut and soybean substrates in water**



Many fermentation steps require soaking of substrates in water for rehydration prior to fermentation being initiated. In the photos shown here, at (left) cashews and almonds are used to make plant-based cheese using LAB. Because there is no cook step later in the process, it's important to control for growth of *Salmonella* and other pathogens. The nuts and soaking water should be acidified or refrigerated or soaking time limited to 4 hours or less. This is a CCP when making plant-based cheese.

The photo (right) show dried soybeans and soybeans soaking in water. Soybeans are used to make many fermented foods, including natto, miso and tempeh. For miso and tempeh, soaking water and soybeans should be acidified or refrigerated or controlled for time to control for *B. cereus*. Even though there is a cook step later in the process, *B. cereus* spores may grow and elaborate toxins that are not destroyed by cooking. This is a CP. However, natto soaking in water may only be refrigerated or controlled for time, even though there is a cook step later in the fermentation process. Water should not be acidified because this may promote growth of bacteriophages that interfere with the starter culture, *B. subtilis*. For natto, soaking is a CCP.

While industry standards vary among and between countries, Canadian guidance was followed where available.<sup>16</sup> The process step of cooling was always assigned as a CCP prior to culture being added, and as a CP after the fermentation was completed. The rationale for assigned cooling as a CCP prior to fermentation was based on the risk of spores not becoming inactivated by heating (cook step). Should spore-forming bacteria germinate, this could result in unacceptable *C. botulinum*, *C. perfringens* or *B. cereus* risk and lead to toxin formation in foods. The cooling process follows Canadian practice and must be achieved within 6 hrs, or from 60°C to 20°C in 2 hr, followed by 20°C to 4°C in 4 hr.<sup>16</sup>

Cooling after the fermentation process step was always assigned as a CP. This was based on presence of active fermentative microbial populations creating unfavorable conditions for spoilage and pathogenic microbes to survive, grow, and cause harm. Unfavorable conditions include acidification, ethanol formation, formation of other inhibitory compounds, depletion of nutrients, and crowding by fermentative organisms occupying substrate. When industry practices recommended stricter guidance in comparison to normally used standard, it was incorporated. For example, milk is a highly perishable food. Industry standards specify starter culture is added within one hour of achieving the inoculation temperature when cooling or reheating milk for yogurt manufacture.

When determining if addition of starter culture should be from spontaneous, backslopped or commercial starter sources, a CCP or CP designation was assigned based on historical illness risk, and issues linked to starters.

## 2.3 Microbiological standards for fermented products

### Overview

This section describes standards and tests recommended for fermented foods, excluding intentional alcohol-based fermentations (e.g., cider, beer), and inclusive of acid-based and alkaline-based fermentations reviewed in section 3 guidance. Types of microbiological tests are described and summarized with respect to application to fermented foods.

The most important tests are pH and water activity. These tests:

- Determine if food safety criteria is met in the finished food product. Does the food meet the food safety objective, for example, fermented sausage should have a pH of 5.3 or lower and  $a_w$  of 0.90 or lower,
- Evaluate the fermentation process, to measure it is proceeding in a satisfactory manner. These parameters should be tested frequently at various points in the process, particularly when the operator is developing the recipe. Examples: to ensure pH is dropping as an indicator of successful lactic acid bacterial fermentation in yogurt, or that water activity is decreased sufficiently to allow room temperature storage of a fermented sausage.

Total bacterial counts, also called aerobic plate or standard plate counts, are **not recommended** to assess cultured, fermented foods because high numbers of bacteria are expected from the starter culture. Total counts for lactic acid bacteria (LAB) may be helpful to establish if a fermentation process is normal. A 'healthy fermentation' may have upwards of 7 log<sub>10</sub> CFU/g of LAB (over 10 million). Examples: enumerate LAB to establish optimal assay activity, baselines for the process during development, and periodic checks to ensure the fermentation is performing as expected within a specific time. Note these culture-based tests take up to 48 hrs for results. This will provide information about a fermentation process after it has occurred but will not provide immediate evidence if there is a problem in the fermentation.

Of course, fermented foods should be free from pathogens: such as *E. coli* O157:H7, *Salmonella* and *L. monocytogenes*. It is expensive to test for pathogens. Indicator tests may be recommended to assess safety of the food (see Box 4 and Table 4 for more information on indicators). Yeast and mould may be tested to assess food quality and spoilage as increasing counts of yeasts and moulds highlight deterioration of food quality. However, yeasts and moulds are not recommended indicators of quality in a food where they are the fermenting agent, for e.g., dosa and idli (yeasts), kombucha (yeasts), tempeh or koji foods (moulds). Sensory analysis, referring to the evaluation of smell, texture and taste of a food, may also be recommended when assessing quality of the food, but is not a reliable indicator of food safety.



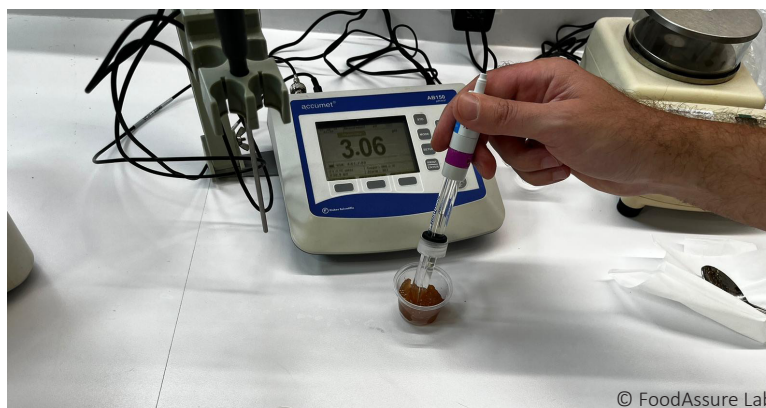
## Chemical tests

**pH** is the term describing the measure of how much acidity (hydrogen ions, H<sup>+</sup>) or alkalinity (hydroxyl anions, OH<sup>-</sup>) are present in food. This is based on a scale of 0 (most acid) to 14 (most alkaline). Acid foods will have a pH below 7, alkaline foods will have a pH above 7, neutral pH is at pH 7.

Vegetables, grains, milk and other ingredients used in fermentations are usually at a neutral pH. During fermentation, LAB will produce lactic and other organic acids lowering the pH. Operators are strongly recommended to test pH in house using a pH meter, and many provincial jurisdictions require a pH meter to be used when manufacturing foods when this is a critical process step. PH strips are not acceptable because they cannot be calibrated, the colour of the food being tested can change or confuse reading the pH results on the strip, and strips have a poor detection limit with variations of  $\pm 1$  pH unit.

Operators are recommended to keep records of pH results. This will provide objective information for what a normal, healthy fermentation process looks like. Table 3 describes when pH and  $a_w$  tests should be used in fermentation processes.

**Figure 2 | pH equipment being used to test acidity of jam**



In these photos the analyst is testing the pH of apricot jam. At top, the sample is placed into a disposable cup. At bottom, the pH result of this jam is 3.06, acidic.

**Table 3 | When pH and  $a_w$  tests should be used**

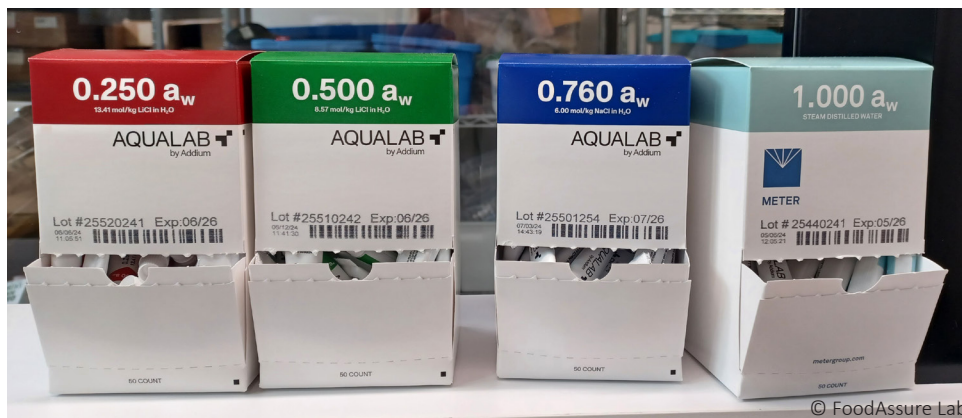
When	Why
Test final pH of the food. The target pH for most LAB fermentations is 4.6 or lower.  Test final $a_w$ of the food. The target $a_w$ for some fermented foods (sausage, pidan egg) is 0.92 or lower; other fermented foods (salt koji) is 0.85 or lower.	To meet food safety requirements.
Test mixture at start of fermentation.	To establish starting pH or $a_w$ .
Test during the fermentation at timed intervals. In most LAB fermentations, test every 4, 8 or 12 hr period over 4 days to ensure the pH is decreasing as expected.	To establish the baseline for a normal fermentation. Periodic re-testing is recommended to validate the process is performing as expected.
Test periodic batches.	To monitor process is normal, as established.
Test when there are changes in ingredients, recipes, starter cultures.	To verify/validate process is still normal, as established.
Test a failed batch.	Test during fermentation at timed intervals as before to identify a failed batch and to troubleshoot why and when it failed. Determine if batch can be saved, re-worked or needs to be discarded.

**Water Activity ( $A_w$  or  $a_w$ )** is the term describing the available water in a food. It is not a measure of % moisture, rather how much water is unbound in the food. When water is bound up it is unavailable for microbes, limiting their growth. For example, foods with high sugar or salt content might appear liquid, but have limited available water content. Similarly, solid foods may look solid, but if there are small pockets of pure water present with little or no salts or sugars dissolved in it, those pockets of water can have high water activity and allow undesirable microbes to grow.  $A_w$  tests are less frequently required in fermented foods than pH, but are recommended for the same reasons as pH.  $A_w$  and pH are parameters used to determine *L. monocytogenes* classification risk suitability for long-term, refrigerated storage.<sup>17</sup> This parameter is a useful measure for these fermented foods: fesikh, sausage, koji, miso and pidan egg.

**Figure 3 | Water activity ( $a_w$ ) testing of sausage samples**



In these photos on page 18 and 19, the water activity ( $a_w$ ) is being tested for 3 sausage samples. Top: sample set-up showing the  $a_w$  meter (left) pH meter (middle) and weight balance (right).



Top left: the meat samples are placed into a small disc and inserted into the machine. Top right: the final result for one sausage sample is  $a_w=0.8831$  at  $22.35^\circ\text{C}$ .

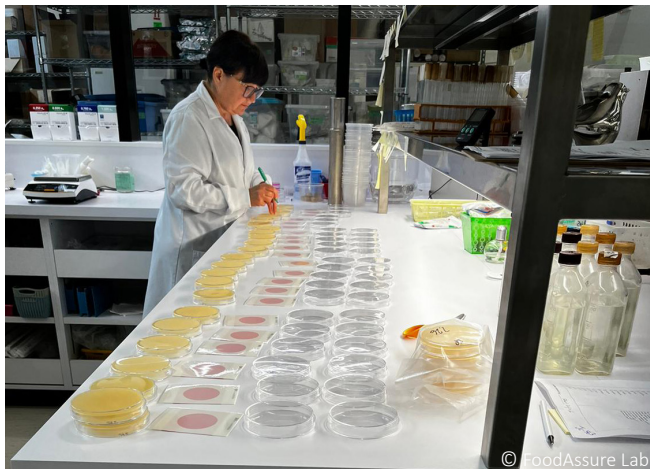
**Salt and nitrite** amounts are not normally tested in finished fermented foods. Instead, the amounts added are recorded into a log sheet. Amounts must be carefully measured when preparing ingredients to establish fermentations and process control. Too little or too much salt cause fermentation failures. Too little nitrite added to meats can allow pathogen growth during the fermentation, but too much nitrite added to meats, when ingested, can cause death. Calculations of percent amounts to add are based on the weight of all ingredients. Calculations of amounts added are CCPs and important information to be documented in log sheets for each process batch to ensure percentages added are correct.

**Humidity** and relative humidity is the measure of moisture or the amount to water vapour in air at a given temperature, important in several types of fermentations. Operators are recommended to obtain humidity meters to monitor rooms where products are fermented or dried, when control of humidity is a requirement. The amount of moisture in the air affects performance of starter cultures, for example, *Rhizopus* moulds in tempeh. If the air is too dry the mould will stop growing in the substrate and begin forming black-coloured spores which are undesirable in the finished product. Control of humidity is a CCP for fermented sausage products. During fermentation and drying steps, when humidity is too low, case hardening may occur on the outside of the sausages, a condition that stops drying of the interior of the product and increases botulism risk.

### Microbiological tests

Fermented foods, as described, are generally consumed as raw and ready-to-eat (RTE), and therefore have high numbers of active culture. For that reason, microbial tests to assess safety and quality will be different from other foods that are consumed as cooked or treated in other ways to make them RTE. Possible indicator and pathogen tests are shown in Figure 4 and Table 4. recommended tests from Health Canada are described in Table 5, and others in Table 6. A summary of recommendations for fermented foods is found in Table 7.

**Figure 4 | Indicator tests set up for food samples**



In this photo, the analyst is setting up Baird Parker plates to test for *S. aureus*, 3M Petrifilm plates to test for fecal coliforms, and nutrient agar plates to test for Aerobic Colony Count.

On RHS: are bottles of liquid nutrient agar that will be poured into the empty plates for the ACC test.

**Table 4 | Indicator and pathogen tests for fermented foods**

Indicator tests	Pathogen tests
<ul style="list-style-type: none"> <li>Lactic acid bacteria counts</li> <li>Yeasts and moulds</li> <li>Enterobacteriaceae</li> <li>Total / fecal coliforms</li> <li><i>E. coli</i></li> <li><i>Listeria</i> spp.</li> </ul>	<ul style="list-style-type: none"> <li><i>Salmonella</i></li> <li>STEC or shigatoxigenic <i>E. coli</i></li> <li><i>L. monocytogenes</i></li> <li><i>Clostridium perfringens</i></li> <li><i>Bacillus cereus</i></li> <li><i>Staphylococcus aureus</i></li> <li><i>Yersinia enterocolitica</i></li> </ul>

**NOTE:** the list of possible indicators and pathogens in the table above does not imply (1) that all fermented foods are required to be tested for these organisms, or (2) that when testing is performed negative test results will assure safety of these products. Testing should be used as verification/validation of the process with all CCPs and CPs. Monitoring pH and water activity as CCPs and implementing corrective actions to deal with any deviations is what will assure food safety. In fact, for fermented foods, when review of documentation for pH and aw demonstrates process control, U.S. authorities do not recommend pathogen testing of end-products.<sup>18</sup> When indicator or pathogen testing are undertaken, for reasons such as fermentation batches are failing, or unsatisfactory sanitation practices are observed during inspections (see Box 4), when establishing process control, it is recommended to take more frequent, smaller samples, rather than one larger, composite sample. Reasons include: large composite samples may dilute out a single positive sample, providing false negative results; contamination, if present, is unlikely to be evenly distributed, and more likely non-homogenous in the food matrix; and smaller, more frequent sampling will pinpoint where and when issues arise.<sup>18</sup>

**Box 4 | Indicator use for food safety and quality**

An indicator test may be used for different purposes:<sup>19</sup>

- Detect likely presence of pathogens in the finished food product
- Assess hygiene and sanitation in the food processing facility
  - Assess the food process
  - Did the CCP work?
- Did post-processing contamination occur?
- Assess spoilage and food quality issues
- Determine shelf life of a food product

(Continued on page 21)

Indicators are present and behave similar to pathogens, share similar characteristics, such as presence in reservoirs in the natural environment (for e.g., *E. coli* in the fecal environment) and growing requirements. They are cheaper and faster to test, should occur in higher numbers and be more resistant to environmental conditions than the pathogen, and should persist in the food or environment. In drinking water, and as a general measure of sanitation, *E. coli* is often used as the surrogate in place of pathogens, *E. coli* O157:H7 or *Salmonella*. The purpose of *E. coli* use as an indicator in fermented foods would be to see if feces from warm blooded mammals was present in the food. The origin of feces might be from contaminated meats, vegetables, or other ingredients, from inadequate handwashing after food employees use the toilet, from rodent's feces getting into the food, or other pests, etc.

Note that *E. coli* would not be a good indicator for the presence of *L. monocytogenes*, yeasts or moulds in foods. *Listeria* are naturally occurring soil microbes. Yeasts and moulds are naturally occurring on the outer surface of many foods, requiring oxygen for optimal growth. Other indicators or tests would be required to detect them, for example, *Listeria* spp. is often used to assess risk of *L. monocytogenes* in food and processing areas.<sup>17</sup>

There is a spectrum of guidance for microbial testing of finished fermented foods that varies from no recommended testing to requirements for validation testing of foods. Health Canada requires validation testing for manufacture of fermented sausages under guideline no. 12, to ensure the process removes verotoxigenic *E. coli* O157:H7 in fermented sausages containing beef.<sup>15</sup> Validation is a scientific proof (usually based on experiment) that shows a process is actually effective.

#### Figure 5 | Analyst reviewing lab requisition for test requests



In this photo, the analyst is reviewing the lab requisition work order for the pathogen and indicator tests requested by the client.

On the bench at left are milk samples, near the side of the bench is an environmental swab sample, and on the cart are food samples being prepared for tests.

The Health Products and Food Branch (HPFB) of Health Canada last published an interpretative summary for standard and guidelines for microbiological safety of foods in April 2008.<sup>20</sup> The guidance specifies microbiological parameters for these fermented foods: cheese made from pasteurized and raw milk, heat treated and raw fermented sausages, and ready-to-eat soybean products (e.g., natto, tempeh, and miso). Tests are suggested within the context of lot testing using 2-class or 3-class attribute lot acceptance sampling plans. Here, we show the maximum amount allowable (M) from these sampling plans. For further information on lot testing, consult the HPFB interpretative summary and associated compendium methods for details.<sup>20</sup>

Recommended tests of HPFB by food category are described in Table 5. The values below do not represent all of the requirements in a 2 or 3-class attribute sampling plan, consult the HPFB guidance and Food and Drug Regulations for complete microbiological criteria.

**Table 5 | Health Canada recommended tests applicable to fermented foods**

Fermented food	Pathogen or indicator test recommended	Maximum amount allowable (M) in CFU/g
Cheese from pasteurized and unpasteurized milk	<i>E. coli</i> <i>Staphylococcus aureus</i>	2.0 X 10 <sup>3</sup> or 3.3 log <sub>10</sub> 10 <sup>4</sup> or 4 log <sub>10</sub>
Fermented sausage – raw and heat-treated (indicators of pathogens)	<i>E. coli</i> <i>Staphylococcus aureus</i>	10 <sup>3</sup> or 3 log <sub>10</sub> 10 <sup>4</sup> or 4 log <sub>10</sub>
	<i>E.coli</i> O157:H7 <i>Salmonella</i> <i>Campylobacter</i> <i>Yersinia enterocolitica</i>	Absent in 25g
Soybean products – ready-to-eat	<i>E. coli</i> <i>S. aureus</i> Psychrotrophic bacteria (aerobic colony count at 15 to 20°C)	10 <sup>3</sup> or 3 log <sub>10</sub> 10 <sup>4</sup> or 4 log <sub>10</sub> 10 <sup>7</sup> or 7 log <sub>10</sub>

Guidance provided by committees, such as the *International Commission on Microbiological Specifications for Foods* (ICMSF) and *National Advisory Committee for Microbiological Criteria in Foods* (NACMCF) varies, as described in Table 6 below.<sup>21,22</sup>

End-product testing for some categories of fermented foods, such as fermented and acidified vegetables (e.g., pickles, sauerkraut, kimchi), was not recommended unless sanitation or process control issues (variances) were observed during inspection.<sup>21,22</sup>

Specific inspection guidance is provided for dairy cultures and meats, along with RTE meals and entrees by NACMCF,<sup>22</sup> with fermented fish products (e.g., fesikh) specified in ICMSF guidance.<sup>21</sup>

**Table 6 | NACMCF and ICMSF recommended tests in fermented foods**

Fermented food	Pathogen or indicator test recommended	Limits identified during inspections
Soft or semi-soft cheese from pasteurized milk (from NACMCF Table J.7)	<i>Listeria</i> spp. in processing environment ----- Coliforms <i>E. coli</i> <i>Listeria</i> spp. <i>Salmonella</i> <i>Staphylococcus aureus</i>	Negative in zones 2, 3 <sup>a</sup> ----- <100/g <10/g Negative in 125g Negative in 375g 100/g, if >10 <sup>4</sup> /g reject lot
Cultured dairy e.g., yogurt (from NACMCF Table J.8)	<i>Listeria</i> spp. in processing environment -----	Negative in zones 2, 3 <sup>a</sup> -----
When fruits added test moulds/yeasts	Coliforms Moulds/yeasts <i>Staphylococcus aureus</i>	10/g 10/g 10 <sup>3</sup> /g or 3 log <sub>10</sub>
Fermented sausage – raw and heat-treated (from NACMCF Table J.28)	Enterobacteriaceae Coliforms <i>E. coli</i> <i>Staphylococcus aureus</i> <i>E. coli</i> O157:H7 <i>Salmonella</i>	100/g or 2 log <sub>10</sub> 100/g or 2 log <sub>10</sub> 10/g 10 <sup>3</sup> /g or log <sub>10</sub> 3 Negative in 125/g
Fermented fish (from ICMSF)	<i>Salmonella</i>	Negative in 10 lot testing of 25/g

<sup>a</sup> – refers to environmental swab testing of surfaces in Zone 2 (close-to-food contact) and Zone 3 (non-food-contact)

Other jurisdictions may have additional requirements, for example, the province of Quebec recommends fermented sausages includes a test for *C. perfringens*.

Based on the guidance provided above and identified recalls and outbreaks affecting fermented foods, this guidance recommends testing finished fermented foods as outlined in Table 7, when the product process is being established, and when inspections indicate an issue with process control or sanitation.<sup>22</sup>

Table 7 also outlines the test parameters to establish process performance and assess shelf life for product quality, discussed in the next section. As previously described, measuring pH during the process is the most important parameter for most fermented foods.

**Figure 6 | Moulds and yeasts**



Moulds and yeasts are shown in these media.

Top row: Specialized agar, for example the Rose Bengal pink plate with a *Penicillium* mould colony has chloramphenicol added to suppress bacterial growth.

Yeasts are visible in the plate to the left of the pink plate (Potato Dextrose Agar, PDA), another *Penicillium* (blue mould). *Aspergillus* (grey mould) is also growing on the agar on the right of the pink plate.

## 2.4 Packaging, Shelf life and Labelling

Information on packaging, shelf life, and labelling are provided here as general guidance for foods reviewed in section 3. Requirements vary widely depending on whether food is raw, cooked or heat treated, is packaged in reduced oxygen environment, is intended to be stored refrigerated or at room temperature, contains antimicrobials or preservatives, and other factors. For example, sauerkraut, once fermented, if

- uncooked, kept in a crock pot (aerobic), may be stored refrigerated for several weeks, or
- uncooked, if vacuum packaged (anaerobic), may be stored refrigerated for weeks to months, or
- cooked, cooled, if cold-packed into glass jars, may be stored refrigerated for many months, or
- cooked, if hot-packed into jars and tins, may be stored at room temperature for years.

The main concerns for fermented foods' shelf life and safety are growth of pathogens and spoilage agents during storage.

**Packaging.** All fermented foods containing live culture should be held refrigerated in some type of packaging. Refrigeration and packaging is required for fermented foods containing live culture to slow down fermentation activity and to limit microbial growth of oxidative moulds, yeasts, and spoilage agents that may change the resting pH of the food to become more neutral, thereby allowing potential pathogens to grow. Once packaged, and there is a barrier between the food and air, a food is considered held in Reduced Oxygen Packaging (ROP). Barrier packaging types vary in the ratios of gas (nitrogen, oxygen and carbon dioxide) but all ROP have less than atmospheric oxygen of ~21%. ROP is inclusive of vacuum packaging where all oxygen is excluded; modified atmosphere packaging where proportions of carbon dioxide and nitrogen gas are flushed into packaging materials, controlled atmospheric packaging where foods are held in rooms with adjusted, lower oxygen levels, and canned, hermetically sealed containers excluding oxygen. Packaging of foods into ROP has greatly increased shelf life of foods by reducing growth of aerobic spoilage microbes, oxidative yeasts and moulds. However, it also increases risk of *C. botulinum* and toxins, a concern for ready-to-eat foods. Achieving appropriate pH in these foods before packaging is the key to preventing proliferation of *C. botulinum* and preventing formation of toxin during storage.

**Table 7 | Fermented foods expected parameters and test recommendations for microbial control, process performance and shelf life**

Food Category	Food	Expected pH range	Expected a <sub>w</sub> range	Starter culture B=backslotted; C=commercial; S=spontaneous	Pathogens/ hazards of concern	Tests recommended in finished foods <sup>a</sup>	Process performance tests		Shelf life of food product for quality & spoilage tests		
							pH, a <sub>w</sub>	LAB counts	Moulds & yeasts	Generic <i>E. coli</i>	Indicator/pathogen
Lacto-fermented vegetables	Kimchi	<4.6		S	Toxigenic <i>E. coli</i> Norovirus	pH generic <i>E. coli</i>	pH	Yes	Yes	Yes	If generic <i>E. coli</i> +, assess VTEC
	Sauerkraut	<4.6		S	Moulds and Yeasts	pH generic <i>E. coli</i> Moulds and yeasts	pH	Yes	Yes	Maybe <sup>e</sup>	If generic <i>E. coli</i> +, assess VTEC
	Vegetables (e.g., cucumber pickles)	<4.6		S	<i>Salmonella</i> <i>C. botulinum</i> Norovirus	pH <i>Enterobacteriaceae</i> Moulds and yeasts	pH	Yes	Yes	Maybe <sup>e</sup>	If generic <i>E. coli</i> +, assess VTEC
Lacto-fermented grains/nuts	Dosa and Idli	<4.6		S	<i>Salmonella</i> <i>E. coli</i>	pH generic <i>E. coli</i> <i>B. cereus</i>	pH	Yes	No	Yes	<i>B. cereus</i>
	Plant-based cheese	<4.6		C-LAB	<i>Salmonella</i> <i>L. monocytogenes</i>	pH <i>Listeria</i> spp. <i>Salmonella</i>	pH	Yes	Yes	Yes	<i>Listeria</i> spp. <i>Salmonella</i>
Lacto-fermented dairy	Yogurt	<4.6		C-LAB	<i>L. monocytogenes</i> <i>Salmonella</i> <i>C. botulinum</i> <sup>d</sup> <i>E. coli</i> O157:H7	pH generic <i>E. coli</i> or <i>Enterobacteriaceae</i> <i>Listeria</i> spp. <i>S. aureus</i> Moulds and yeasts	pH	Yes	Yes	Yes	<i>Listeria</i> spp. <i>Salmonella</i>
Lacto-fermented fish	Fesikh	<6.5	<0.97	S C-LAB preferred	<i>C. botulinum</i> Biogenic amines	pH, a <sub>w</sub>	pH & a <sub>w</sub>	Yes	No	Yes	TVB-N <sup>f</sup>
<i>Bacillus subtilis</i> ferment	Natto			<i>C-Bacillus subtilis</i> natto	PGA- allergic reactions <sup>b</sup>	generic <i>E. coli</i> Moulds and yeasts	No	No	Yes	Maybe <sup>e</sup>	If generic <i>E. coli</i> +, assess VTEC



Food Category	Food	Expected pH range	Expected a <sub>w</sub> range	Starter culture B=backslotted; C=commercial; S=spontaneous	Pathogens/ hazards of concern	Tests recommended in finished foods <sup>a</sup>	Process performance tests		Shelf life of food product for quality & spoilage tests		
							pH, a <sub>w</sub>	LAB counts	Moulds & yeasts	Generic <i>E. coli</i>	Indicator/ pathogen
Mould fermentation	Koji, Miso	~5.0	<0.85 (with salt) >0.85 (no salt)	<i>C-Aspergillus oryzae</i>	No documented illnesses. Recalls for <i>B. cereus</i> , moulds	a <sub>w</sub>	a <sub>w</sub>	No	Yes	No	<i>B. cereus</i>
	Tempeh	~7.0		<i>C-Rhizopus</i> and C-LAB	<i>Salmonella</i> from SC <sup>c</sup> Toxin (coconut) <sup>d</sup>	<i>Salmonella</i>	No	No	Yes, if cooked	No	<i>Salmonella</i>
Mixed starter fermentations	Kefir	<4.6		B, C	Toxigenic <i>E. coli</i> <i>L. monocy- genes</i>	pH Alcohol	pH	No	No	Yes	If generic <i>E. coli</i> +, assess VTEC <i>Listeria</i> spp. <i>Salmonella</i>
	Kombucha	>2.5<4.6		B	Alcohol, lead, caffeine, anthrax	pH Alcohol	pH	No	No	No	<i>Bacillus</i> spp.
	Meats (e.g. salami)	<5.3	<0.90	C-LAB	<i>Salmonella</i> Toxigenic <i>E. coli</i> <i>C. botulinum</i> <i>Trichinella</i> <i>L. monocy- genes</i>	pH, a <sub>w</sub> generic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i>	pH & a <sub>w</sub>	No	No	Yes	If generic <i>E. coli</i> +, assess VTEC <i>Salmonella</i> <i>S. aureus</i> <i>C. perfringens</i> <i>Listeria</i> spp. (if refrigerated)
Alkaline fermentation	Pidan egg	≥9	<0.92	NA	No documented illnesses. Recalls for heavy metals.	pH, a <sub>w</sub>	pH & a <sub>w</sub>	No	No	No	<i>Salmonella</i> <i>B. cereus</i> <i>S. aureus</i>

<sup>a</sup> – Tests are based on evaluation of process controls and sanitation. <sup>b</sup> – PGA=poly-glutamic-acids, <sup>c</sup> – SC=starter culture; <sup>d</sup> – ingredient issue; <sup>e</sup> – If sanitary issue observed, test for generic *E. coli* and manage per kimchi; <sup>f</sup> – TVB-N – total volatile basic nitrogen

In foods where appropriate pH is not achieved, refrigeration and shortened storage time are necessary for food safety to limit *C. botulinum*, other pathogens and spoilage growth. ROP packaging materials may include (but are not limited to) food-grade polypropylene plastic, glass jars, ceramic containers with non-toxic glaze, metal cans or any food-grade vessel or material that excludes atmospheric gas conditions (i.e., air) from the food. Acceptable polymers for packaging and packaging materials may be found on CFIA sites, as listings may not include all acceptable materials, operators are advised to check materials are food-grade before use.<sup>23,24</sup>

**Shelf life.** Shelf life of food is based on intrinsic and extrinsic properties of the food, described in the table, below. It is not possible to determine shelf life of a given food until information is collected that objectively defines the food characteristics (e.g., pH,  $a_w$ , ingredients), including information about process steps and handling of food (e.g., is it raw or cooked?, does it contain live starter culture?, or has it been cooked after fermentation?), packaging, storage temperature, and shelf life testing results for the food at the intended storage conditions. The operator should collect the information, conduct shelf life studies to establish the shelf life of the food, and keep records of results.

**Table 8 | Intrinsic and extrinsic properties to assess food shelf life**

Indicator properties	Extrinsic properties
pH	Packaging atmosphere
$a_w$	Temperature
Redox potential	Light
Competitive microflora	Handling of food (e.g., whole or sliced; cooked or raw etc.)
Antimicrobial ingredients	

The shelf life of a food may be extended when properties present hurdles and limit microbial growth for pathogens and spoilage agents. Hurdles include packaging barriers, thermal treatments, refrigeration and freezing, acidity (pH), water activity ( $a_w$ ), antimicrobials or competitive agents in the foods preventing pathogen and spoilage agent growth, preservatives and other measures. Food safety and extended shelf life can be increased with use of more than one type of food safety hurdle. Shelf life testing differs from microbial challenge testing of foods. These differences are described in Box 5, below.

**Box 5 | Challenge studies and shelf life studies**

Shelf life of foods is the maximum recommended time foods may be stored at a specific temperature, humidity or external factor and still retain acceptable consumer sensory ratings, food quality, and not exhibit spoilage.<sup>25</sup> Shelf life is rarely established based on food safety testing. The end of shelf life is marked by changes in physical attributes (e.g., moisture absorption changing the texture), chemical attributes (e.g., change in pH), or sliminess and odour (e.g., from spoilage growth).<sup>25</sup> Shelf life studies should be inclusive of spoilage agents and indicators, but may or may not include pathogen tests. Any food has to remain safe during and after shelf life since consumer will perceive the shelf life expiration date as exclusively food quality information.

Challenge studies are used to determine whether pathogens inoculated into the food matrix survive in the food. These studies are conducted to evaluate a heat or other lethality step, to assess an antimicrobial ingredient, or to assess survival of pathogens during the food process, storage and distribution to consumer.<sup>25</sup> Generally, challenge studies should show less than a 1-log increase of a pathogen over the intended shelf life.<sup>26</sup> *S. aureus* is assessed by limiting to a 3-log increase, after which enterotoxin development is suspected.<sup>26</sup> When conducting challenge studies, these should be conducted for 25% to 50% beyond the intended shelf life. For example, for a shelf life of one month (30 days), the challenge study period should be 45 days.<sup>26</sup>

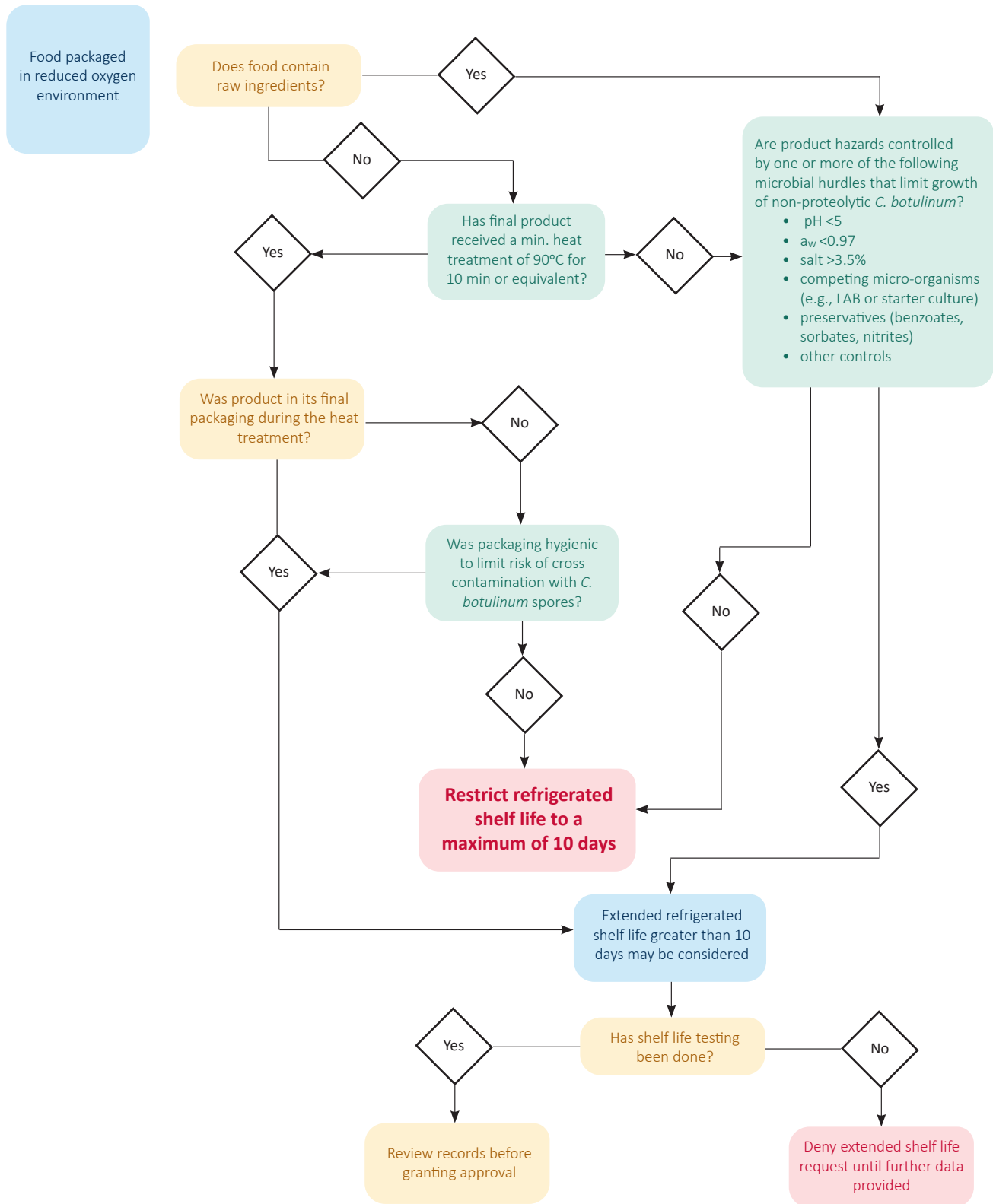
### **Recommendations for storage and packaging of refrigerated fermented foods with live culture**

The four main pathogens of concern in refrigerated foods are non-proteolytic *C. botulinum*, *L. monocytogenes*, cold-tolerant *B. cereus*, and cold-tolerant *Yersinia enterocolitica* (mainly an issue in pork products).<sup>25</sup> Refrigerated shelf life for fermented ROP foods with a risk of *C. botulinum* spores is limited to a maximum of 10 days unless additional food safety hurdles are included, as outlined in Figure 7.<sup>25</sup>

Noted in the figure, the microbial hurdles listed include minimum growth requirements of non-proteolytic *C. botulinum* (pH of 5.0,  $a_w$  of 0.97) therefore any food with pH <5.0 and  $a_w$  <0.97 has intrinsic properties to limit growth of this pathogen.<sup>26</sup> If other properties in the food also limit microbial growth, for example, LAB as a competing microbial culture is present to prevent growth of spoilage agents, or sodium benzoate or nitrite preservatives have been added, then an extended shelf life can be considered. However, there is no general guidance for assigning a shelf life to a food, because this needs to be evaluated by the operator over the intended shelf life and storage conditions for the food. It is the responsibility of the operator to determine shelf life for the products they sell.

For LAB fermented foods, shelf life testing should be inclusive of measuring the pH change, spoilage moulds and yeasts, and assessing consumer sensory characteristics (taste, smell, appearance). LAB-fermented yogurt may have a good shelf life of only a few weeks when made without added stabilizers or preservatives. In contrast, commercial facilities using clean-fill technology during packaging may have a shelf life for yogurt extended to months. Another example, tempeh is a *Rhizopus*-mould fermented food, and once fermentation is complete, operators typically cut the tempeh into blocks, cook (fry or blanch), then package and refrigerate. These processes will inactivate the mould hyphal strands from growing to excess and producing an ammonia-like odour, and stop the mould from sporulating and forming black-coloured spores unsightly to consumers. Vacuum-packaging the product is a protective hurdle that will prevent oxidative spoilage microbes from growing after the cook step. If the tempeh is frozen it will have a long and extended shelf life. If refrigerated after cooking, the tests above should be considered to establish shelf life, generally this product may be held for three or more weeks. However, if tempeh was cut and sold fresh, it may have a shelf life of only a few days before it would be unpalatable to the consumer.

**Figure 7 | Shelf life considerations for refrigerated storage of fermented foods in reduced oxygen packaging**



Evaluating the results of shelf life testing should be based on a combination of objective data collected from laboratory results and subjective consumer sensory data.<sup>27</sup> For example, when is the earliest indication of spoilage and increased mould and yeast growth? Or when do more than 50% of samples have taste, odour or visual issues that consumers would find objectionable?<sup>27</sup> Assessment of increased mould and yeast growth may require some baseline testing of the foods in question.<sup>28</sup> It may be normal to have high levels of aerobic colony counts, LAB counts, moulds and yeasts in finished fermented foods as described in Table 9 for fermented cucumbers.

**Table 9 | Lactic acid bacteria, mould/yeast and Enterobacteriaceae counts in raw and fermented cucumbers**

Total counts in log <sub>10</sub> CFU/g				
Cucumbers	Aerobic	Mould and Yeast	LAB	Enterobacteriaceae
Raw	5.2±0.8	2.8±0.9	3.8±1.2	4.6±1.0
Fermented	7.4±0.2	2.8±0.9	6.9±1.0	Not detected

For example, average microbial counts reported in fresh and fermented vegetables in cucumbers shown in Table 9 are high.<sup>11</sup> Fermented cucumbers typically have total aerobic bacterial counts and LAB counts at nearly 1 billion CFU/g. These numbers, although high, do not indicate spoilage in this fermented food. A single test result for a finished product may not be interpretable for shelf life quality and potential spoilage. From a food safety perspective, absence of *E. coli* is of most importance. From a shelf life perspective, the operator is recommended to assess microbial trends and conduct a combination of microbial and sensory testing to determine shelf life. Operators who lack capacity to test or perform these studies and tests in-house are advised to seek out assistance from accredited laboratories ([scc.ca](http://scc.ca)). When conducting shelf life testing, consider multiple lots (minimum of 3), multiple replicates at each time point tested to account for within lot variations, growth at refrigerated and temperature abuse conditions (one might test at 10°C as this temperature would be expected during food transport, and/or at consumer’s home refrigerator),<sup>25,28</sup> and include process performance tests outlined in Table 7, along with shelf life tests that usually include moulds and yeasts, or generic *E. coli* hygiene indicators.

**Figure 8 | Interior of an accredited food microbiology laboratory**



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**Labelling.** *The Food and Drug Regulations* (FDR, B.01.007(1.1)(b)) and *Safe Foods for Canadians Regulations* (SFCR, 214, 217)<sup>29</sup> specify that prepackaged food with a durable life of 90 days or less and packaged at a place other than the retail premises from which it will be sold must be labelled with.<sup>29,30</sup>

- a durable life date (known as "best before" date), and
- storage instructions (if they differ from normal room temperature).

Voluntary declarations of durable life, storage instructions, or best before date markers are recommended for foods, such as dehydrated fermented sausages with a shelf life of greater than 90 days. They must follow the required manner of declaring (B.01.007(6), FDR).

Operators selling foods to retail may be required to meet federal labelling requirements and are advised to review Government of Canada and Canadian Food Inspection Agency requirements.<sup>31,32</sup> The industry food labelling tool reviews core labelling requirements, claims and statements, and food-specific labelling requirements.<sup>32</sup>

## 2.5 Alcohol and biogenic amine chemical hazards in fermented foods

Chemical hazards that may be unfamiliar to inspectors and operators include unintentional alcohol and biogenic amines. We recommend ensuring operators are aware of these chemical hazards and where required include them in their food safety plan when it is a hazard of concern in the fermented foods. Operators are recommended to monitor and test for unintentional alcohol in kombucha, jun and kefir products, as described in the "Issues with ethanol" section. Biogenic amines may form in fermented vegetables, sauerkraut, kimchi, fesikh, natto, tempeh, miso and sausage, however, there are no recommendations for operators to monitor or test for these hazards in their food process. Should illnesses occur that are linked to fermented foods, we recommend biogenic amines be considered. An evaluation may indicate testing for biogenic amines is appropriate, for example, histamine in a fish flavoured kimchi. Further information on biogenic amines is in the "Issues with Biogenic Amines" section.

### Issues with ethanol

The formation of alcohol during fermentation is a natural process that occurs when yeasts consume sugar. Unintentional alcohol in foods or beverages, also called residual alcohol, may arise when the fermentation is incomplete or may develop from temperature abuse of the beverage when there is live culture and residual sugar in the product.

### ***What fermented food beverages contain unintentional alcohol?***

Fermented foods or beverages that may contain unintentional alcohol include:

- Jun
- Kombucha
- Water or vegan kefir
- Ginger beer

Some manufacturers have controlled live culture and residual sugar in their product allowing these beverages to be stored and sold as shelf stable.

### ***Why is ethanol considered a health hazard?***

Low levels of alcohol that exceed federal or provincial standards are considered a health hazard because they can cause harm. The amount of alcohol that causes toxicity or an intoxication is calculated by the alcohol dose and weight of the individual. Of concern are lower weight toddlers that are susceptible to small volumes of beverages containing low amounts of alcohol, resulting in alcohol poisoning. The table below describes how an alcohol dose is calculated based on weight. The amount of alcohol in a liquid is described as alcohol by volume or ABV, and often provided as a percentage, such as 0.5% ABV.

**Table 10 | Alcoholic doses based on Alcohol by Volume (ABV)**

In this exposure estimate the risk of alcohol intoxication depends on peak blood alcohol level. This table shows peak blood ethanol level following ingestion of 100 ml of liquid at four levels of ABV at three weights. The dose response table is:<sup>33</sup>

Peak blood ethanol level after consuming 100 mL of liquid				
Weight (kg)	0.5 % ABV	1% ABV	2% ABV	2.5% ABV
10	6.7 mg/dL	13.3 mg/dL	26.7 mg/dL	33.3 mg/dL
20	3.3 mg/dL	6.7 mg/dL	13.3 mg/dL	16.7 mg/dL
30	2.2 mg/dL	4.4 mg/dL	8.9 mg/dL	11.1 mg/dL

**Figure 9 | Young boy drinking kombucha**



Ethanol intoxication in infants and young children causes hypoglycemia, lethargy, seizures and, at sufficiently high levels, death. Most cases of serious harm occur when blood ethanol levels over 100 mg/dL are detected during the initial physician evaluation (and blood ethanol ABV test). Children, particularly smaller weight infants and toddlers, have a reduced capacity to eliminate alcohol because their livers do not metabolize ethanol as efficiently as adults.<sup>33</sup> Cases of lethargy, hypoglycemic seizures and fatal hypoglycemia have been documented at blood ethanol levels between 50 and 100 mg/dL. Because of this potential for serious harm, current recommendations are that any child who has ingested sufficient alcohol to produce a blood ethanol level over 50 mg/dL should be observed in hospital.

If a kefir beverage contained only 2% ABV, in 200 mL or 2 dL (an amount that is less than one cup, 250 mL) ethanol would exceed the 50 mg/dL amount in a 10kg or lighter weight child (i.e. from table, 26.7 mg/dL X 2=53.4 mg/dL ethanol). Other populations are also of concern. Physician guidelines recommend that people who are pregnant should avoid all alcohol to protect the fetus. There are also many other individuals in the population who may want to avoid ingesting any alcohol, for example, if they are taking prescription medications, driving, have an alcohol use disorder or choose to avoid all alcohol for personal or religious beliefs. In summary, because kombucha, jun, water kefir and other fermented beverages (e.g., ginger beer) are sold as non-alcoholic, unintended alcohol content can be a concern for some parts of the general population, especially for children and during pregnancy.

***Recommendations for management of unintended alcohol in fermented beverages***

Operators are recommended to include alcohol as a chemical hazard in their food safety plan, control the hazard using a HACCP-based approach, test for alcohol to ensure compliance with federal and provincial alcohol limits, and include precautionary labelling statements for their products, where required, to inform consumers of potential alcohol and handling instructions. Details of these management controls are described below.

**Figure 10 | Bottles of kombucha**



***Managing alcohol as a chemical hazard in a food safety plan.***

Unintentional alcohol above provincial and federal levels should not occur in products during shelf life and through-out consumer use and abuse of the product. Kombucha, jun, kefir and other fermented products that continue to ferment after bottling and distribution may allow alcohol levels to rise. Control of the hazard should be demonstrated by the operator in their process in, for example, a food safety plan by:<sup>34</sup>

1. Testing alcohol in the product at the time of bottling and at the end of the products shelf life to demonstrate compliance with regulations. It is recommended that operators use a combination of in-house testing and verified approved testing methods if they are not available to the operator in-house. Frequency of verification testing should be sufficient to demonstrate stability in the amount of alcohol present with any given process (minimum of three tests per food type for new recipes). Periodic testing is expected to be performed to verify a process (minimum annual verification) or to changes of an existing process.
2. Incorporating alcohol as a CCP into a written food safety plan and explaining how the CCP will be controlled with explanations of the critical limits also in the food safety plan.
3. Having a corrective action plan in place, keeping records of the critical limits and actions taken, and making those records available to inspectors during inspections.
4. Keeping records to show that in-house and verified alcohol testing is being done, and making those records available to inspectors during inspections.

There are critical limit options when alcohol is not controlled. Examples of loss of control include (1) when testing demonstrates alcohol is >1.1% ABV (federal requirements), (2) when a processing error occurs that would result in excess alcohol in the batch, such as addition of extra sugar or flavouring compounds, or (3) fermentation stalls due to cooler temperature. Critical limit options can be summarized as the 4 “D’s” – dilute, delay, divert or discard.<sup>34</sup>

- Diluting the batch;
- Delay and continue fermenting for a longer period so that alcohol is converted to acetic acid;
- Diverting to alcoholic market stream (liquor manufacturing licence would be required); and
- Discarding the batch.

Caution should be exercised when extending fermentation to ensure that the pH of kombucha does not become too acidic and fall below pH of 2.5 (this is a CCP); chemical burns have been reported in the past due to over-fermented kombucha-type products.



If alcohol is a persistent problem the operator should consider reviewing their process and recipe. Recommendations include avoiding added sugars after the fermentation period (as residual sugars provide food for yeast) and controlling yeast populations by:<sup>34</sup>

- Employing technology to remove yeast through filtration or centrifugation, or
- Choosing yeast populations that do not grow at refrigeration temperatures, or
- Pasteurizing the product at time of bottling to ensure yeasts are inactivated and cannot convert added flavouring and residual sugars to alcohol, or
  - Includes extraction and removal of ethanol from the finished product (for example, low-temperature distillation using spinning cone technology)
- Some other method to control yeast.

Inspectors are recommended to review operators' controls by ensuring the hazard is addressed in the food safety plan, production logs and alcohol testing records.<sup>34</sup> During inspections, inspectors may:

- review food safety plans during inspections;
- require evidence that the procedures outlined are adequate and being followed in the form of
  - records for alcohol testing
  - records for control of alcohol as a CCP when critical limits are not met that show actions taken to mitigate the hazard (record of corrective actions).

Monitoring for alcohol should be an ongoing activity with a demonstrated history of compliance to regulations.

#### **Guidance messaging for alcohol in fermented foods**

Consumers have the right to know what products contain alcohol, how much alcohol is present, and if there are any risks to consuming the product. Labelling should include a declaration of the alcohol content, precautionary statements for vulnerable groups (such as during pregnancy and for children), and handling information.<sup>34</sup> For example, labels should include:

- may contain alcohol at <0.5% ABV
- not a suitable beverage for young children or during pregnancy
- keep refrigerated, do not shake
- readable Best Before Date (BBD)

In June 2020 Health Canada created a page to inform the population about unintentional [alcohol in non-alcoholic fermented beverages](#). They ask consumers to keep these beverages refrigerated and to discard beverages past their BBD.

#### ***What are the testing options for alcohol?***

Fermented beverages can contain live culture and other ingredients that may make testing for alcohol using traditional methods more challenging. The Kombucha Brewers Association explains on their site ([Why Traditional Ethanol Testing Methods Prove Inaccurate](#)) that testing using hydrometers (gravity) and refractometers (refractive index) are not reliable because organic acids produced may have the same weight as alcohols, i.e. interfering with hydrometer methods, and suspended particulates in kombucha may interfere with refractometer methods.<sup>35</sup> While these methods may be suitable for in-house testing operations, they are not suitable for regulatory purposes. Operators are recommended to verify compliance using Association of Official Analytical Chemists (AOAC) approved methods. International kombucha expert review panels establish minimum performance requirements for the detection of ethanol in kombucha and similar fermented beverage products. Analytical methods must demonstrate that they can achieve performance requirements through method validation (AOAC validation guidance).

**Figure 11 | Head space gas chromatography mass spectrometry (HS-GCMS) instrument**



The following methods have been validated and shown to meet the performance requirements.<sup>36</sup>

- Head-space gas chromatography (HS-GC) with flame ionization detector (FID) AOAC Method 2016.12
- Head space gas chromatography (HC-GC) with Mass Spectrometry Detection (MS)
- Enzymatic method by r-Biopharm (Enztec Liquid Ethanol Ref. E834), AOAC Method 2017.07
- Headspace solid phase micro-extraction & GC-MS by MilliporeSigma, AOAC Method 2019.04
- Ethanol Assay Kit (K-EtOH) by Megazyme, AOAC Method 2019.08

For contract laboratories, it is recommended that laboratories chosen be accredited and listed on the [Standards Council of Canada approved laboratory website](#).<sup>37</sup> Alcohol testing costs at contract labs using validated methods can range from \$150 to \$300 per test (in 2021).

Information provided above was summarized for fermented beverages that may contain unintentional alcohol (kefir, kombucha, jun) in Section 3 guidance as shown in Appendix 1. Federal and provincial regulations and recommendations pertaining to non-alcoholic beverages are reviewed in Appendix 2.

### **Issue with biogenic amines**

#### ***What are biogenic amines?***

Biogenic amines (BAs) are low-molecular weight bacterial decarboxylation products of amino acids formed during fermentation. Several BAs such as histamine, tyramine, phenylethylamine, putrescine, cadaverine, and spermidine are found in certain foods and are associated with many biological functions.

Health Canada has set action levels for histamines in anchovies, fermented fish sauces and pastes at 200 mg/kg and for other fish and fish products at 100mg/kg.<sup>38</sup> 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 suggested toxicity threshold is identified only for three biogenic amines: 100-200 mg/kg for histamines, 100-800 mg/kg for tyramine and 30 mg/kg for phenylethylamine.<sup>39</sup>

#### ***What fermented foods might be contaminated with elevated levels of biogenic amines?***

Fermented foods that may be contaminated with elevated levels of biogenic amines include<sup>40</sup>:

- Fermented soybean products e.g., soy sauces, natto, miso (Japanese fermented soybean pastes), cheonggukjang, doenjang (Korean fermented soybean pastes), Sufu (Chinese bean curd), douchi (Chinese fermented soybean pastes) and tempeh (an Indonesian fermented soybean paste)
- Fermented dairy products (e.g., cheese, fermented milk)

- Fermented fish (e.g., salted fish, fish sauces, fish pastes, fesikh)
- Fermented meat (e.g., sausages)
- Fermented vegetables (e.g., vegetables, sauerkraut, kimchi)

### ***What are the identified health risks of biogenic amines?***

Normal intakes of BAs are broken down and detoxified by intestinal amine oxidases, however, high concentration of BAs can cause minor allergic reactions and even serious health problems.<sup>41,42</sup> BA-associated adverse health effects mimic allergic reaction and range in potential severity, including nausea, respiratory distress, hot flush, sweating, heart palpitations, headache, bright red rash, burning sensations in the mouth, alterations in blood pressure, diarrhea and hypertensive crises.<sup>43,44</sup> The toxic effects of BA may vary between individuals as it depends on individual sensitivity and on the consumption of alcohol or drugs that are monoamine oxidase inhibitory (MAOI).<sup>45,46</sup> Persons prescribed MAOI drugs (e.g., for anxiety or depression) have increased sensitivity to BAs in fermented foods because the drugs interfere with amine oxidase pathways that detoxify excess BAs.<sup>40,46</sup> Among all the BAs, histamines and tyramines are known to be the most toxic. Histamines are responsible for causing “scombroid fish poisoning” which occurs from high exposure to histamines from the consumption of contaminated fish such as tuna, sardines, anchovies, mackerel, etc.<sup>47</sup> Tyramine intoxication symptoms, also known as the “cheese reaction” or “cheese effect”, occur within the first two hours after consumption and include migraine, gastrointestinal symptoms, tachycardia, an increase in blood glucose, nor-adrenaline ejection and hypertension.<sup>45,47</sup> Dietary exposure to tyramine is very important due to its toxicity and potential interaction with MAOIs that can raise blood pressure. Phenylethylamine, in presence of tyramine has been known to trigger food-induced migraine attacks and increase blood pressure.<sup>48</sup>

### ***What causes or contributes to BA formation?***

BA formation can be influenced by these factors:

- Presence of decarboxylase positive non-starter microbiota in the raw material and environment involved in ripening process.<sup>45</sup>
  - To avoid, use commercial LAB strains that have been selected for their inability to produce high levels of BAs.
- Temperature of the medium (favorable conditions for starter culture growth: 20 to 37°C)
  - Formation of biogenic amines is reduced beneath 5°C or over 40°C.<sup>49</sup>
- Composition, pH, ion strength, water activity, etc. of the raw materials.
- Presence and concentration of additives (sugar, salt, antimicrobial agents, etc.), the microorganisms present.<sup>39,43</sup>
- Poor processing and storage conditions of the food.<sup>39</sup>
- Use of non-hygienic raw material and poor manufacturing practice.

By applying basic good hygienic and food handling practices, formation of BAs can be prevented or reduced. The key methods for BA concentration reduction in food include management of bacterial load (especially decarboxylase-positive microorganisms). Regulation of time, temperature, moisture content, salt concentration, storage conditions and use of high-quality raw materials are also important.

**Figure 12 | High Performance Liquid Chromatography (HPLC) Instrument**



***What are the testing options for biogenic amines?***

The analytical methods used to quantify BAs in foods are mainly based on chromatographic methods, which include thin layer chromatography (TLC), gas chromatography (GC), capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC).<sup>50</sup> Currently HPLC-based methods are considered most reliable for separation and quantification of BAs due to its high resolution, sensitivity, and the advantage of analyzing several BAs simultaneously.<sup>48</sup>

Routine testing for biogenic amines in fermented foods is not recommended. However, when foods are implicated in illness, testing foods with HPLC methods for an appropriate BA is recommended.

Fermented foods with biogenic amines identified as a hazard in Section 3 (vegetables, sauerkraut, kimchi, natto, miso, tempeh and sausage) were provided a summary of this guidance, shown in Appendix 1. BA levels in different categories of fermented foods is shown in Appendix 3.<sup>40</sup>

## References

1. Bramwell P. Food safety plans: three problems to address when analysing microbiological hazards. *Microbiol Aust* [Internet]. 2013 May 13 [cited 2024 Mar 8];34(2):102–5. Available from: <https://www.publish.csiro.au/ma/MA13035>
2. Durso L, Hutkins R. Starter cultures. In: Caballero B, editor. *Encyclopedia of Food Sciences and Nutrition* (Second Edition) [Internet]. Oxford: Academic Press; 2003 [cited 2024 May 1]. p. 5583–93. Available from: <https://www.sciencedirect.com/science/article/pii/B012227055X011469>
3. Capozzi V, Fragasso M, Romaniello R, Berbegal C, Russo P, Spano G. Spontaneous food fermentations and potential risks for human health. *Fermentation* [Internet]. 2017 Dec [cited 2024 May 1];3(4):49. Available from: <https://www.mdpi.com/2311-5637/3/4/49>
4. Laulund S, Wind A, Derkx PMF, Zuliani V. Regulatory and safety requirements for food cultures. *Microorganisms* [Internet]. 2017 Jun [cited 2024 Mar 6];5(2):28. Available from: <https://www.mdpi.com/2076-2607/5/2/28>
5. Bourdichon F, Budde-Niekiet A, Dubois A, Fritz D, Hatte JL, Laulund S, et al. Inventory of microbial food cultures with safety demonstration in fermented food products. *Bull Int Dairy Fed* [Internet]. 2022 Jan 31 [cited 2024 Mar 6];514. Available from: <https://shop.fil-idf.org/products/bulletin-of-the-idf-n-514-2022-inventory-of-microbial-food-cultures-with-safety-demonstration-in-fermented-food-products>
6. Prajapati JB, Nair BM. Chapter 1. The history of fermented foods. In: Farnworth ERT, editor. *Handbook of Fermented Functional Foods* [Internet]. 2nd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2008. p. 1–22. Available from: <https://www.routledge.com/Handbook-of-Fermented-Functional-Foods/Farnworth/p/book/9781420053265>
7. Ranadheera CS, Vidanarachchi JK, Rocha RS, Cruz AG, Ajlouni S. Probiotic delivery through fermentation: dairy vs. non-dairy beverages. *Fermentation* [Internet]. 2017;3(4):67. Available from: <https://www.mdpi.com/2311-5637/3/4/67>
8. Morelli L, Capurso L. FAO/WHO guidelines on probiotics: 10 years later. *J Clin Gastroenterol* [Internet]. 2012 Oct [cited 2023 Sep 11];46:S1. Available from: [https://journals.lww.com/jcge/fulltext/2012/10001/FAO\\_WHO\\_Guidelines\\_on\\_Probiotics\\_10\\_Years\\_Later.2.aspx](https://journals.lww.com/jcge/fulltext/2012/10001/FAO_WHO_Guidelines_on_Probiotics_10_Years_Later.2.aspx)
9. Brown P. Certificates of analysis. *Nutraceuticals World* [Internet]. 2008 [cited 2024 Feb 26];11(10):32–3. Available from: [https://www.bcit.ca/files/appliedresearch/pdf/certificates\\_of\\_analysis\\_nov\\_08\\_nw.pdf](https://www.bcit.ca/files/appliedresearch/pdf/certificates_of_analysis_nov_08_nw.pdf)
10. Government of Canada Canadian Food Inspection Agency. Supplier food safety assurance program [Internet]. 2018 [cited 2024 Feb 26]. Available from: <https://inspection.canada.ca/preventive-controls/sfsap/eng/1523365528734/1528208259725>
11. Pérez-Díaz IM, Breidt F, Buescher RW, Arroyo-López FN, Jiménez-Díaz R, Garrido-Fernández A, et al. Chapter 51. Fermented and acidified Vegetables. In: *Compendium of Methods for the Microbiological Examination of Foods* [Internet]. American Public Health Association; 2013 [cited 2022 Nov 29]. Available from: <https://doi.org/10.2105/MBEF.0222.056>
12. Putnam JH, Chervet ML, Ingham BH. Survival characteristics of *Escherichia coli* O157: H7, *Salmonella enterica*, and *Listeria monocytogenes* in pickling solutions at 5°C. *Food Prot Trends* [Internet]. 2018;38(6):402–9. Available from: <https://www.food-protection.org/files/food-protection-trends/nov-dec-18-putnam.pdf>
13. Harris LJ, Palumbo M, Beuchat LR, Danyluk MD. Outbreaks from tree nuts, peanuts, and sesame seeds: table and references [Internet]. UC Food Safety. 2019. Available from: <https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/Outbreaks%209-3-19.pdf>
14. Peanut and Tree Nut Processors Association, Consumer Brands Association. Industry handbook for safe processing of nuts [Internet]. Third. Alexandria, VA: Peanut and Treenut Processors Association; 2020 [cited 2023 Jul 5]. Available from: [https://cdn.ymaws.com/www.ptnpa.org/resource/resmgr/industry\\_information/2020/Safe\\_Handling\\_of\\_Nuts\\_Dec202.pdf](https://cdn.ymaws.com/www.ptnpa.org/resource/resmgr/industry_information/2020/Safe_Handling_of_Nuts_Dec202.pdf)
15. Health Canada. Interim guidelines for the control of verotoxinogenic *Escherichia coli* including *E. coli* O157:H7 in ready to eat fermented sausages containing beef or a beef product as an ingredient [Internet]. 2000 [cited 2024 Mar 5]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/interim-guidelines-control-verotoxinogenic-escherichia-coli-including-colio157-fermented-sausages-beef-product-ingredient.html>
16. Federal/Provincial/Territorial Food Safety Committee. Food retail and food services code [Internet]. 2020 [cited 2021 Oct 18]. Available from: <https://f.hubspotusercontent20.net/hubfs/7498226/foodservicescode.pdf>
17. Health Canada. Policy on *Listeria monocytogenes* in ready-to-eat foods (2023) [Internet]. 2023 [cited 2023 Oct 30]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/listeria-monocytogenes-ready-eat-foods.html>

18. National Advisory Committee on Microbiological Criteria for Foods. Microbiological testing by industry of ready-to-eat foods under FDA's jurisdiction for pathogens (or appropriate indicator organisms): verification of preventive controls. *J Food Prot* [Internet]. 2022 Nov 1 [cited 2024 Mar 7];85(11):1646–66. Available from: <https://www.sciencedirect.com/science/article/pii/S0362028X22110379>
19. Kornacki JL. Indicator organism assays: chaos, confusion and criteria. *Food Safety Magazine* [Internet]. [cited 2024 Feb 28]; Available from: <https://www.food-safety.com/articles/3850-indicator-organism-assays-chaos-confusion-and-criteria>
20. Health Canada, Health Products and Food Branch, Health Canada. Official methods for the microbiological analysis of foods. Interpretative summary. [Internet]. 2008 [cited 2024 Mar 5]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/official-methods-microbiological-analysis-foods-compendium-analytical-methods.html>
21. International Commission on Microbiological Specifications for Foods. *Microorganisms in foods 8. Use of data for assessing process control and product acceptance* [Internet]. 1st ed. New York, NY: Springer; 2011. 400 p. Available from: <https://doi.org/10.1007/978-1-4419-9374-8>
22. National Advisory Committee on Microbiological Criteria for Foods. Response to questions posed by the Department of Defense regarding microbiological criteria as indicators of process control or insanitary conditions. *J Food Prot* [Internet]. 2018 Jan 1 [cited 2024 Mar 5];81(1):115–41. Available from: <https://www.sciencedirect.com/science/article/pii/S0362028X-22083909?via%3Dihub>
23. Government of Canada, Health Canada. Lists of acceptable polymers for use in food packaging applications [Internet]. 2004 [cited 2024 Mar 25]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/lists-acceptable-polymers-use-food-packaging-applications.html>
24. Government of Canada. Reference listing of accepted construction materials, packaging materials and non-food chemical products database [Internet]. [cited 2024 Mar 25]. Available from: <https://materials-reference-listing-food.hpfb-dgpsa.ca/>
25. Hardin MD. Chapter 2. Food safety factors determining shelf life. In: Taormina PJ, Hardin MD, editors. *Food Safety and Quality-Based Shelf Life of Perishable Foods* [Internet]. Cham: Springer International Publishing; 2021 [cited 2024 Mar 8]. p. 27–40. Available from: [https://doi.org/10.1007/978-3-030-54375-4\\_2](https://doi.org/10.1007/978-3-030-54375-4_2)
26. National Advisory Committee On Microbiological Criteria For Foods. Parameters for determining inoculated pack/challenge study protocols. *J Food Prot* [Internet]. 2010 Jan 1 [cited 2024 Mar 8];73(1):140–203. Available from: <https://www.sciencedirect.com/science/article/pii/S0362028X22115759>
27. New Zealand Ministry for Primary Industries (MPI), New Zealand Government. How to determine the shelf life of food. 2016;43. Available from: <https://www.mpi.govt.nz/dmsdocument/12540-How-to-determine-the-shelf-life-of-food-Guidance-document>
28. Taormina PJ. Purposes and principles of shelf life determination. In: Taormina PJ, Hardin MD, editors. *Food Safety and Quality-Based Shelf Life of Perishable Foods* [Internet]. Cham: Springer International Publishing; 2021 [cited 2024 Mar 8]. p. 1–26. Available from: [https://doi.org/10.1007/978-3-030-54375-4\\_1](https://doi.org/10.1007/978-3-030-54375-4_1)
29. Government of Canada. Safe Food for Canadians Regulations [Internet]. SOR/2018-108 2019. Available from: <https://laws-lois.justice.gc.ca/eng/regulations/SOR-2018-108/index.html>
30. Government of Canada. Food and Drug Regulations (C.R.C., c.870) [Internet]. 2019. Available from: [https://laws-lois.justice.gc.ca/eng/regulations/c.r.c.,\\_c.\\_870/index.html](https://laws-lois.justice.gc.ca/eng/regulations/c.r.c.,_c._870/index.html)
31. Government of Canada CFIA. Food products that require a label [Internet]. 2022 [cited 2024 Mar 12]. Available from: <https://inspection.canada.ca/food-labels/labelling/industry/food-products/eng/1624290469261/1624290680899>
32. Government of Canada CFIA. Food labelling for industry [Internet]. 2015 [cited 2024 Mar 12]. Available from: <https://inspection.canada.ca/food-labels/labelling/industry/eng/1383607266489/1383607344939>
33. McIntyre L, Jang SS. A study of alcohol levels in kombucha products in British Columbia [Internet]. Vancouver BC Canada: BC Centre for Disease Control, Environmental Health Services; 2020 Mar p. 30. Available from: <http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/Kombucha%20report%202020.pdf>
34. McIntyre L. Fact sheet: evaluating kombucha food safety plans for alcohol risk [Internet]. BC Centre for Disease Control Environmental Health Services; 2021 [cited 2024 Apr 30]. Available from: <http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/Evaluating%20Alcohol%20Control%20in%20Kombucha.pdf>

35. Kombucha Brewers International. KBI approved ethanol testing methodology [Internet]. 2020. Available from: <https://kombuchabrewers.org/resources/approved-alcohol-testing-methods/>
36. McIntyre L. Fact sheet: laboratory options for alcohol testing for kombucha [Internet]. BC Centre for Disease Control Environmental Health Services; 2021 [cited 2024 Apr 30]. Available from: <http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/Alcohol%20testing%20for%20kombucha.pdf>
37. Accreditation [Internet]. Standards Council of Canada- Conseil canadien des normes. [cited 2023 Oct 18]. Available from: <https://www.scc.ca/en/search/laboratories>
38. Health Canada. Health Canada's maximum levels for chemical contaminants in foods [Internet]. 2005 [cited 2023 Jan 26]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/maximum-levels-chemical-contaminants-foods.html>
39. Ruiz-Capillas C, Herranz AH, editors. Biogenic amines on food safety [Internet]. MDPI- Multidisciplinary Digital Publishing Institute; 2019 [cited 2023 Jan 26]. Available from: <https://directory.doabooks.org/handle/20.500.12854/42202>
40. Saha Turna N, Chung R, McIntyre L. A review of biogenic amines in fermented foods: occurrence and health effects. *Heliyon* [Internet]. 2024 Jan 30 [cited 2024 Jan 30];10(2):e24501. Available from: <https://www.sciencedirect.com/science/article/pii/S2405844024005322>
41. Doeun D, Davaatseren M, Chung MS. Biogenic amines in foods. *Food Sci Biotechnol* [Internet]. 2017 Dec 13 [cited 2021 Oct 14];26(6):1463–74. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6049710/>
42. Sivamaruthi BS, Kesika P, Chaiyasut C. A narrative review on biogenic amines in fermented fish and meat products. *J Food Sci Technol* [Internet]. 2021 May [cited 2023 Mar 15];58(5):1623–39. Available from: <https://link.springer.com/10.1007/s13197-020-04686-x>
43. Gardini F, Özogul Y, Suzzi G, Tabanelli G, Özogul F. Technological factors affecting biogenic amine content in foods: a review. *Front Microbiol* [Internet]. 2016 Aug 12 [cited 2023 Jan 26];7. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2016.01218/full>
44. Tofalo R, Perpetuini G, Schirone M, Suzzi G. Biogenic amines: toxicology and health effect. In: Caballero B, Finglas PM, Toldrá F, editors. *Encyclopedia of Food and Health* [Internet]. Oxford: Academic Press; 2016 [cited 2023 Jan 26]. p. 424–9. Available from: <https://www.sciencedirect.com/science/article/pii/B9780123849472000714>
45. Barbieri F, Montanari C, Gardini F, Tabanelli G. Biogenic amine production by lactic acid bacteria: a review. *Foods* [Internet]. 2019 Jan [cited 2023 Jan 26];8(1):17. Available from: <https://www.mdpi.com/2304-8158/8/1/17>
46. Santos MHS. Biogenic amines: their importance in foods. *Int J Food Microbiol* [Internet]. 1996 Apr 1 [cited 2023 May 30];29(2):213–31. Available from: <https://www.sciencedirect.com/science/article/pii/0168160595000321>
47. Wójcik W, Łukasiewicz M, Puppel K. Biogenic amines: formation, action and toxicity—a review. *J Sci Food Agric* [Internet]. 2021;101(7):2634–40. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.10928>
48. EFSA. Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J* [Internet]. 2011 [cited 2022 Jul 16];(2011;9(10):2393). Available from: <https://data.europa.eu/doi/10.2903/j.efsa.2011.2393>
49. Ekici K, Omer AK. Biogenic amines formation and their importance in fermented foods. *BIO Web Conf* [Internet]. 2020;17:00232. Available from: [https://www.bio-conferences.org/articles/bioconf/abs/2020/01/bioconf\\_fies2020\\_00232/bioconf\\_fies2020\\_00232.html](https://www.bio-conferences.org/articles/bioconf/abs/2020/01/bioconf_fies2020_00232/bioconf_fies2020_00232.html)
50. Önal A. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chem* [Internet]. 2007;103(4):1475–86. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0308814606006972>
51. Gorgus E, Hittinger M, Schrenk D. Estimates of ethanol exposure in children from food not labeled as alcohol-containing. *J Anal Toxicol* [Internet]. 2016 Sep [cited 2023 Feb 3];40(7):537–42. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5421578/>
52. Blood alcohol content. In: *Wikipedia* [Internet]. 2023 [cited 2023 Feb 3]. Available from: [https://en.wikipedia.org/w/index.php?title=Blood\\_alcohol\\_content&oldid=1136624693](https://en.wikipedia.org/w/index.php?title=Blood_alcohol_content&oldid=1136624693)
53. Health Canada. Ethanol in non-alcoholic fermented beverages [Internet]. 2020 [cited 2023 Feb 3]. Available from: <https://www.canada.ca/en/health-canada/services/publications/food-nutrition/ethanol-non-alcoholic-fermented-beverages.html>
54. Canadian Food Inspection Agency. Labelling requirements for alcoholic beverages [Internet]. 2022 [cited 2023 Feb 3]. Available from: <https://inspection.canada.ca/food-labels/labelling/industry/alcoholic-beverages/eng/1624281662154/1624281662623#c6>

55. Mentis I, Robinson G. CFIA inspections (pers. communication to N. Parto, PHO). 2018.
56. Kombucha Brewers International. Kombucha code of practice, ver. 3 [Internet]. Kombucha Brewers International. 2023 [cited 2023 Sep 11]. Available from: <https://kombuchabrewers.org/kombucha-code-of-practice/>

### **Photo attribution**

Figures 1 to 5: We thank Chris and Anna Piesik and their staff for allowing us to photograph their SCC-accredited FoodAssure Laboratory Ltd. on location in Vancouver, BC. Photos used with permission.

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Figure 10, 11: We thank Dr. Michael Chan and staff for photos of equipment in the Natural Health and Food Products Research Group (NRG) Labs at the British Columbia Institute of Technology's Centre for Applied Research and Innovation in Burnaby, BC. Photos used with permission.



## Appendix 1 | Alcohol and biogenic amine statements provided in food-specific guidance

A summary of the information discussed in this section was provided as information boxes for specific fermented foods reviewed in our guidance. Alcohols were identified as a chemical hazard in kombucha, jun, and water kefir. Biogenic amines were identified as a chemical hazard in vegetables, sauerkraut, kimchi, fesikh, natto, tempeh, miso, and sausage.<sup>40</sup>

### Information summary for alcohols in fermented foods

Fermented foods often contain alcohols in low amounts as a natural by-product of ethanol fermentation, when yeasts convert sugars into ethanol and carbon dioxide. Low levels of alcohols can also occur in non-fermented foods not labelled as containing alcohol such as whole fruits, fruit juices, and bakery products.<sup>51</sup> Small amounts of alcohol consumed in foods and non-alcoholic beverages are normal, as are small amounts of alcohol in the blood. Normal blood alcohol content ranges from 0% (sober) to no more than 0.04%, with higher levels indicating some level of intoxication.<sup>52</sup>

Health Canada acknowledges ethanol in non-alcoholic fermented beverages as a concern in kombucha, kefir, and some soft drinks including ginger beer.<sup>53</sup> The Public Health Agency of Canada recommends that alcohol is not consumed during pregnancy, and that youth should delay drinking alcohol. Alcohol toxicity is a concern in young children, and especially those weighing 10 kg or less, who are vulnerable to even low doses of alcohol, should they be present in these beverages. Alcohol toxicity can occur at a dose of 50 to 100 mg/dL, health care practitioners should consider monitoring children in a hospital when exposed to beverages containing alcohol. If a kombucha beverage contained only 2% ABV, 200 mL or 2 dL (an amount that is less than one cup, 250 mL) would exceed the 50 mg/dL amount in a 10kg or lighter weight child.<sup>33</sup> Young children lack the enzyme alcohol dehydrogenase and have difficulties in metabolizing ethanol. Acting confused, overheating and fatigue may be symptoms of alcohol poisoning that go unrecognized by care givers, and long term health effects are difficult to predict. In the adult population, there are many people who avoid all alcohol for personal and religious beliefs. There are those who cannot ingest alcohol as it may interfere with prescription medications, have underlying health conditions, or are recovering from alcohol addiction issues. Others may be driving professionally and are unaware that these beverages may contain alcohol. Aside from health concerns, all consumers have a right to know if the products they are consuming contain alcohol.

Operators are recommended to list alcohol as a potential chemical hazard in their food safety plan. Operators are recommended to monitor alcohol levels, test for alcohol in their products, and to implement control measures to control for alcohol. Control options for yeast during and after fermentation (e.g., pasteurize batch, select strains that don't grow under refrigeration, remove by centrifugation or other means), and for batches exceeding alcohol levels following fermentation (e.g., dilute, delay and continue fermenting, divert or discard) are described in this section. Operators are further recommended to add precautionary labels and information about alcohol content in their products. Raw kefir, kombucha and jun products with fermentable residual sugars need to be refrigerated. Recommended precautionary statements include "keep refrigerated", "do not shake", "not a suitable beverage for young children or during pregnancy" and "may contain alcohol at <0.5% ABV (or state actual alcohol level).

Operators manufacturing fermented beverages must comply with provincial and federal regulations for alcohol content. In Canada, non-alcoholic products are defined as those containing less than 1.1% ABV, and do not require an alcohol declaration label.<sup>54</sup> Lower ABV requirements of 1.0% or 0.5% may exist in other provincial jurisdictions.

## Information summary for 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.<sup>39,41</sup> 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.<sup>41,43,44</sup> The toxic effects of BA may vary between individuals depending on individual sensitivity and on the consumption of alcohol or drugs that are monoaminoxidase inhibitory.<sup>45,46</sup>

The main BAs are histamine, tyramine,  $\beta$ -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.<sup>38</sup> 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.

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.

## Appendix 2 | Review of Acts and Regulations for alcohol in beverages in Canada

This appendix reviews the requirements for managing alcohol in beverages vary across Canada. A summary of Canadian federal and provincial Acts and Regulations pertaining to alcohol may be found in the table. The table describes the ABV level that defines a beverage as non-alcoholic; legislation source, and labelling recommendations for non-alcoholic beverages that may contain unintentional alcohol. Note: most regulations are worded to state when a beverage is alcoholic. This table is applying that definition to specify levels of ABV that indicate a beverage is non-alcoholic.

**Federal Acts and Regulations.** Alcoholic beverages are regulated in Canada under the [Food and Drugs Act](#) and [Regulations](#), the [Consumer Packaging and Labelling Act](#) and [Regulations](#), and under provincial regulations in each province. Under sections 4 and 7 of the *Food and Drugs Act* and Division 2 of the Food and Drug Regulations, alcoholic beverages are considered a food. A recall process can be initiated when there is reason to believe that a potentially un-safe food has reached the marketplace. The *Food and Drugs Act*, the [Safe Food for Canadians Act](#) and the [Canadian Food Inspection Agency Act](#) provide CFIA staff with authority to conduct consumer complaint investigations and recall follow up activities, including sampling and detaining product at retail, distribution and manufacturing levels, including retail locations.<sup>55</sup> CFIA will conduct complaint investigations of non-alcoholic

beverages, such as kombucha and water kefir, when ABV testing results demonstrate alcohol levels exceed federal regulations ( $\geq 1.1\%$  ABV).

However, there is no definition for alcoholic beverages, or the amount of alcohol which constitutes an alcoholic beverage in the [Food and Drugs Regulation](#), based on a search for the term ‘alcoholic beverage’. Instead, the volume of alcohol in beverages that defines this term is a labelling requirement. This is described as “All alcoholic beverages containing 1.1% or more alcohol by volume must declare the percentage by volume of alcohol contained in the product [B.02.003, FDR]” on the front panel.

**Table 11 | Level of alcohol by volume used to determine if a beverage is non-alcoholic in within provincial jurisdictions in Canada**

Province / Jurisdiction	Federal	BC	AB	SK	MB	ON	QC	NB	NS	PEI
Beverage is <b>non-alcoholic</b> when ABV% is	Less than 1.1% ABV (<1.1%)	1% or less ABV ( $\leq 1\%$ )	1% or less ABV ( $\leq 1\%$ )	0.5% or less ABV ( $\leq 0.5\%$ )	1% or less ABV ( $\leq 1\%$ )	0.5% or less ABV ( $\leq 0.5\%$ ) or 0.4% or less of alcohol by weight.	0.5% ABV or less ( $\leq 0.5\%$ )	0.5% or less ABV ( $\leq 0.5\%$ ) (described as a restricted beverage)	Less than 0.5% ABV (<0.5%)	0.5% ABV or less ( $\leq 0.5\%$ )
Legislation	<a href="#">Food and Drugs Regulation</a>	<a href="#">Liquor Control and Licensing Act</a>	<a href="#">Gaming, Liquor and Cannabis Regulation</a>	<a href="#">The Alcohol and Gaming Regulation Act, 1997</a>	<a href="#">The Liquor, Gaming and Cannabis Control Act</a>	<a href="#">Liquor License and Control Act</a>	s-13 <a href="#">Loi sur la Société des alcools du Québec</a> and others	<a href="#">Restricted Beverages Act</a>	<a href="#">NS Liquor Corporation Regulations</a>	<a href="#">PEI Liquor Control Act</a>
Recommendations for labelling <b>non-alcoholic</b> beverages	None	Yes (in kombucha guidance)	No	No	No		Yes	Yes		

Detailed labelling instructions for alcoholic beverages can be found here <https://inspection.canada.ca/food-labels/labelling/industry/alcoholic-beverages/eng/1624281662154/1624281662623>. Voluntary claims and statements, such as “low alcohol” is acceptable on products with less than 1.1% ABV. The term “dealcoholized” may be used in a process that has reduced alcohol levels to less than 1.1% ABV; “non-alcoholic” or “alcohol-free” statements may be used when alcohol has been reduced to less than 0.05% ABV.

#### Provincial Acts and Regulations

**Alberta (AB):** Gaming, Liquor and Cannabis Regulation [Alberta King's Printer](#): section 86: For the purposes of section 1(1)(q) of the Act, a product that is intended for human consumption in which the percentage of alcohol by volume exceeds 1% is liquor.

**British Columbia (BC):** BC [Liquor Control and Licensing Act](#). The definition of liquor is found in the definitions section of the Act, "liquor" means, subject to the regulations, beer, wine, spirits or other product that is intended for human consumption and that contains more than 1% alcohol by volume;

**Manitoba (MB):** The [Liquor, Gaming and Cannabis Control Act](#)

**New Brunswick (NB):** [NB Restricted Beverages Act](#) In NB, provincial regulations require 0.5 or less in non-alcoholic beverages. Beverages with alcohol levels 0.5 or less ABV are classified as restricted beverages and are not to be sold to anyone under the age of 19 years (difficult for retailers to apply this).

**Nova Scotia (NS):** Nova Scotia Liquor Corporation Regulations made under Section 40 (1) of the [Liquor Control Act Nova Scotia Liquor Corporation Regulations- Liquor Control Act \(Nova Scotia\)](#) describes potable products as exempt when “a beverage containing less than one-half of 1% alcohol by volume at 15.5 degrees Celsius (60 degrees Fahrenheit) of absolute alcohol”.

**Ontario (ON):** ON Liquor Licence and Control Act, 2019 O. Reg. 745/21 [Liquor Licence and Control Act, 2019](#). The actual values in the Act are stated as “0.5 of 1 per cent or less of ABV or 0.4 of 1 per cent or less of alcohol by weight.”

**Prince Edward Island (PEI):** The actual values in the [Liquor Control Act](#) are worded as “liquor” does not include a beverage containing one-half of one per cent or less of absolute alcohol by volume”.

**Quebec (QC):** QC liquor laws are described at this [site](#). The QC definition of an alcoholic beverage is found in the “Loi sur les infractions en matière de boissons alcooliques” at section 2, paragraph 5, <https://www.legisquebec.gouv.qc.ca/fr/document/lc/l-8.1>. Further information is found in Loi sur les permis d'alcool, <https://www.legisquebec.gouv.qc.ca/fr/document/lc/P-9.1>.

**Saskatchewan (SK):** [Alcohol and Gaming Regulation Act, 1997](#). In SK, many of the large "non-alcoholic" beers sold in Safeway, Walmart, etc. are at 0.5% ABV, and not at the federally defined level of “less than 0.5%” per inspection Canada website. Anything greater than 0.5% must be sold via the SK Distribution Centre to a permittee. Using terms "non-alcoholic" or "alcohol-free" follow true nature requirements, meaning the terms "non-alcoholic" or "alcohol-free" may be used to describe a product whose alcohol level has been reduced to a level less than 0.05%, as part of the common name (for example, non-alcoholic beer).

#### **Recommendation statements for non-alcoholic beverages and products**

Federal labelling requirements are directed towards alcoholic beverages only, and specify the amount (percentage) of alcohol allowed for display on the front panel. ***There are no federal recommendation statements for non-alcoholic beverages.*** Claim statements may be made if products meet specific criteria as described previously.

Provincial labelling requirements exist in Quebec; recommendations exist in New Brunswick, BC including the industry association.

- In QC, kombucha products with a label require a statement on the label that bottles should not be shaken since the contents are under pressure. Kombucha and all products that require refrigeration should state on the label “keep refrigerated”.
- In NB, recommendations for restricted beverages include a declaration on the label of the alcohol content, for e.g, if the ABV is 0.25%, consumers should be aware there is a small percentage of alcohol in the product so that they can make an informed decision for their children.

- In BC, recommendations for including consumer warning statements for kombucha were given in a March 2020 report. Specifically, BCCDC recommends that industry processors include labels on their products that state the amount of alcohol they contain (in kombucha); and that labelling of raw kombucha products should also include the statement “keep refrigerated”.<sup>33</sup>
- Guidance on recommendation statements are also given by the Kombucha Brewers Association. Specifically with regards to alcohol, in section 10.3 (May 2023, version 3) example statements include: “Kombucha is a fermented tea that has naturally occurring alcohol.” “Kombucha naturally contains trace amounts of ethanol due to the fermentation process.”, and “Do not consume if you are avoiding alcohol.” are suggested.<sup>56</sup>

**Figure 13 | Customer shopping for wine at liquor store**



**Appendix 3 | Maximum Biogenic Amine Levels in Soybean-based, Fish, Meat, Dairy and Vegetable-based Fermented Foods**

Foods	Maximum levels of BAs (mg/kg) reported in the literature								Method of detection
	His <sup>1</sup>	Tyr <sup>1,2</sup>	Phe <sup>1</sup>	Trp	Put	Cad	Spd	Spm	
<b>SOYBEAN BASED FOODS</b>									
Soy sauce	<b>592</b>	<b>485.9</b>	23.7	31.2	1007.5	550	486	145	HPLC
Natto	<b>457</b>	<b>300.2</b>	<b>51.5</b>	301	43.1	203.3	478.1	80.1	HPLC
Miso	<b>221.0</b>	95.3	<b>42.0</b>	762	34.29	201	35.7	216	HPLC
Cheonggukjang	<b>755.4</b>	<b>2539</b>	<b>562.4</b>	236.4	476.3	268.2	79.0	20.8	HPLC
Doenjang	<b>2795</b>	<b>6616</b>	<b>8704</b>	2808	4292	3236	8804	9730	HPLC
Gochujang	66.6	<b>126.8</b>	24.8	36.6	36.4	18.1	14.4	1.8	ELISA/HPLC
Sufu	<b>1187</b>	<b>1730</b>	<b>341.03</b>	469.9	1244.9	371	32.87	82	HPLC
Chunjang	<b>272.6</b>	<b>131.3</b>	11.8	31.35	28.59	6.6	12.8	2.9	HPLC
Doubanjiang	ND	25.8	<b>185.6</b>	62.43	129.2	0.2	0.2	1.7	HPLC
Douchi	<b>808</b>	<b>529</b>	<b>736.6</b>	440	596	672.3	719	242	HPLC
Tempeh	<b>100</b>	<b>575</b>	ND	15.6	3200	225	105.5	21.9	HPLC
<b>FERMENTED FISH</b>									
Fermented salted fish	<b>579</b>	<b>523</b>	<b>162.4</b>	69.4	241	1205	351	77	IELC/HPLC
Fish sauce	<b>1205</b>	<b>852.6</b>	<b>85.3</b>	410	1257	1429	56.2	19.4	HPLC
Ikan pekasam	<b>195</b>	<b>369.4</b>	<b>37.2</b>	64.9	416.2	211.2	7.4	9.0	HPLC
Fermented fish paste	<b>208.9</b>	<b>1003</b>	<b>152</b>	410	680	502.5	62.07	238.3	HPLC
<b>FERMENTED MEAT</b>									
Fermented sausage	<b>514.5</b>	<b>627</b>	<b>248</b>	790	505.3	790	91.3	119	HPLC
Marinated fermented beef	ND	<b>437.5</b>	NA	NA	139.5	582	ND	ND	IELC
Fermented pork (nham)	45.2	<b>385</b>	NA	NA	531.1	170.9	6.54	26.02	HPLC
Salami	NA	<b>120</b>	<b>51</b>	71	NA	210	NA	NA	HPLC
Pepperoni	48	<b>200</b>	<b>42</b>	390	NA	290	NA	NA	HPLC

Foods	Maximum levels of BAs (mg/kg) reported in the literature								Method of detection
	His <sup>1</sup>	Tyr <sup>1,2</sup>	Phe <sup>1</sup>	Trp	Put	Cad	Spd	Spm	
<b>FERMENTED DAIRY</b>									
Blue cheese	35.7	<b>2010</b>	NA	NA	26.5	16.1	15.9	16	IELC/HPLC
Mesh cheese	NA	<b>905</b>	NA	NA	131	922	ND	7	IELC/HPLC
Feta cheese	84.6	<b>246</b>	4.9	NA	193	82.8	ND	ND	HPLC
Cheddar cheese	29.4	44.5	2.3	NA	4.8	4.5	26.6	8.5	HPLC
Parmesan cheese	28.6	29.9	9.5	NA	75.9	15.6	4.46	7.7	HPLC
Fermented milk	53.9	<b>337.1</b>	NA	NA	22.9	29.1	82.9	NA	HPLC
Kefir	4.0	12.8	ND	ND	12.1	2.2	4.5	ND	HPLC
<b>FERMENTED VEGETABLES</b>									
Fermented vegetables	<b>138.1</b>	<b>177.7</b>	31.5	66.0	549	316.3	154.8	83.2	HPLC, LC-MS/MS
Kimchi	<b>947.3</b>	<b>357.9</b>	23.9	74.8	428	193	550.1	83.2	LC-MS/MS
Sauerkraut	37.0	<b>206.3</b>	0.2	31.6	162.4	79.5	10.98	1.2	LC-MS/MS, HPLC
Fermented fruits	72	86.8	6.3	22.9	286.9	179.2	14.0	9.8	HPLC, LC-MS/MS

His: histamine, Tyr: tyramine, Phe: phenylethylamine, Trp: tryptamine, Put: putrescine, Cad: cadaverine, Spd: spermidine, Spm: spermine.

ND: Not detected.

HPLC: High Performance Liquid Chromatography, IELC: Ion Exchange Liquid Chromatography; ELISA: Enzyme-linked Immunosorbent Assay

<sup>1</sup> – Values exceeding suggested health risk threshold shown in bold for histamine and tyramine  $\geq 100$  mg/kg and phenylethylamine  $\geq 30$  mg/kg;

<sup>2</sup> – Persons taking medications containing monoamine oxidase inhibitors (MAOI) or using alcohol may be susceptible at lower levels.