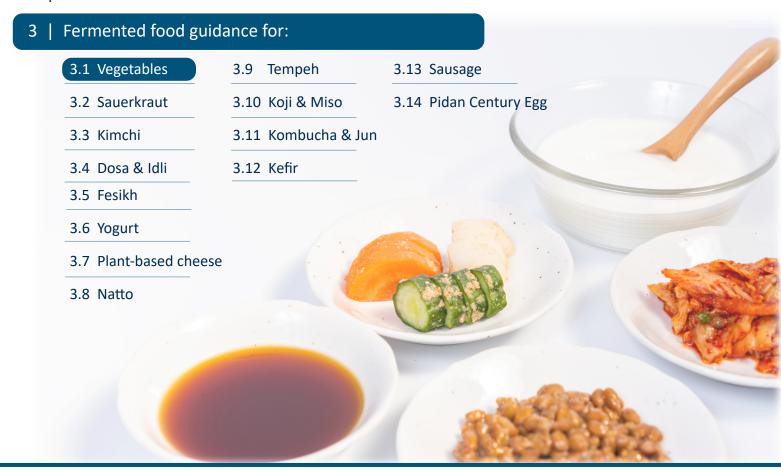
Safety of Fermented Foods

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

- 1 | Introduction to fermented food safety
- 2 | Starter cultures & fermented food standards



























Suggested citation

L. Hudson, B. Coopland, K. Dale and L. McIntyre (authors). Section 3.1 Vegetables. *In* McIntyre, L. (editor) and the Fermented Foods Working Group. (2024). Safety of fermented foods. Assessing risks in fermented food processing practices and advice on how to mitigate them. Environmental Health Services, BC Centre for Disease Control. December 2024. Available from: <a href="http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/Fermented/Fermented Foods Guid-

ance-3.1 Fermented Vegetables.pdf

Additional fermented food guidance can be accessed at:

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

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Acknowledgements

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

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Section 3 | Food safety reviews of fermented foods

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

Each fermented food review includes:

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

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

Figure 1 | Fermented foods described by fermentation agent and complexity

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

 $^{^{\}rm 1-}\text{LAB-lactic}$ acid bacteria; $^{\rm 2-}\text{SCOBY-symbiotic}$ culture of bacteria and yeast

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

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

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

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

3.1 Fermented vegetables

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Overview

Description Vegetables are washed, cut, added to brine, and left to ferment for approximately three weeks or more. • Spontaneous fermentation from wild lactic acid bacteria (LAB) naturally present on ingredients. Starter culture • Backslopping a portion of old culture (introducing a portion of old brine) to new batch is an acceptable common practice. • Commercial LAB may be used to accelerate fermentation. Key features • Fermented vegetables are ready-to-eat, can be served raw, and are held under refrigeration. They may be pasteurized to shelf stable conditions (e.g., to 74°C for 15 min) for room temperature storage. Vegetables are packaged into glass canning jars or equivalent food-grade containers. • Product shelf life is several weeks to several months, depending on packaging and post-fermentation processing. For example, shelf life is extended when products are pasteurized or preservatives are added. Hazards of • Spoilage yeasts and moulds that grow on surface. concern • E. coli from unsanitary conditions, and pathogens on raw materials including Salmonella, Listeria monocytogenes and Clostridium spp., however, vegetative bacteria are unlikely to survive acidic pH development. • C. botulinum spores may germinate in fermented foods when pH rises above 4.6 during spoilage. • Listeria monocytogenes can persist in refrigerated pickled vegetables for months. • Staphylococcus aureus may grow at salt concentrations between 7 to 10%, and to a pH of 4.2, therefore sanitary handling is important during the process and during handling, such as packaging. • Biogenic amine formation. **Important** • Ensure effective fermentation and end pH of ≤4.6 is achieved. Ensure a pH of 4.6 or lower is maintained control points during the intended shelf life to control C. botulinum risk. • Hold completed fermented vegetables for a minimum of two weeks before distribution, particularly short-term fermentations (<1 week), to allow time for acid-tolerant pathogens to die-off. • A pH of lower than 4.4 is recommended for unpasteurized pickled vegetables held under refrigeration for *L. monocytogenes* control. • Use food-grade pickling salt (NaCl) free of caking agents. (continued on page 6)

- Salt (NaCl) concentration and process steps (for example, whether vegetables require a lye wash) vary during fermentation depending on vegetable type. For example:
 - o Cucumbers (pickles) NaCl concentration should be between 3-15%, depending on type of pickle.
 - o Carrots receive a lye wash before they are placed into brine. The lye wash is a sodium hydroxide (NaOH) solution of 1-2%, and the brine is a NaCl concentration of 5%.
- Use validated recipes.
- Wash ingredients to remove soil and contamination, discard damaged, mouldy vegetables. If a sanitizer
 or blanching step is used on vegetables, use of commercial starter culture or backslopped culture is
 recommended.
- This is an anaerobic fermentation, so it is important to ensure air is excluded, keep vegetables fully submerged in the brine.
- During the fermentation, containers should allow gas to vent to avoid containers exploding. Containers should be food-grade and acid-resistant. Avoid ceramic or other pottery with glazes that may leach.
- Ensure appropriate storage conditions based on packaging process (unpasteurized versus pasteurized).

Background

Fermenting vegetables has long been a practice used to preserve food. Fermented vegetables are a staple food product in many cultures and historically were used to ward off scurvy.¹

Vegetables may be preserved by microbial fermentation, direct acidification (acetic acid is used to lower pH of the product to 4.6 or lower), or a combination of these methods.² Generally, fermented vegetables are safe, but improperly fermented foods may pose a food safety risk.³

For clarity, direct acidification of vegetables, where the preservation method is the addition of ingredients such as acetic acid (vinegar), has no fermentation component. **Acidified vegetables** refer to any acid added to preserve nonfermented vegetables that have a natural pH above 4.6.2 **Fermented vegetables** are low acid vegetables that are subjected to activity of acid-producing micro-organisms that release organic acids and reduce pH to 4.6 or lower, regardless of whether any acid is added.² **Pickled vegetables or pickles** can refer to vegetables that are acidified (acid is added) or fermented (acid is created by microbes), so the use of the term 'pickle' can be confusing. Definitions between regulatory agencies also differ. In Canada, the Food and Drugs Regulation (B.11.051) defines a pickle or relish as a "product prepared from vegetables or fruits with salt and vinegar" and may contain other ingredients.⁴ In the United States, a pickle is predominantly made of cucumbers, is prepared by direct addition of vinegar or by fermentation (natural or controlled), and equilibrated to a pH of 4.6 or lower.⁵

Figure 2 | Acidified non-fermented vegetables



Recipes for fermented cucumber dill pickles and quick fresh-pack cucumber dill pickles can be very similar, as both contain added vinegar, as shown in Appendix 1. Health inspectors should review recipes to understand if the process is acidification (low risk) or fermentation (higher risk). In the ingredients list, although the amount of cucumbers and the salt concentration used in both recipes is the same, the amount of vinegar added is very different. The procedure for the recipes is where the differences are more visible. Fermented cucumbers require 3 to 6 weeks to mature when fermented at room temperatures between 20°C to 25°C, while fresh pack cucumber dill pickles are made in one day. A "quick pickle" variation of this is also sometimes referred to as "fridge pickles" and rely on a mixture of vinegar, salt, and sugar rather than fermentation to produce a final product.

Vegetable fermentations are lactic acid bacteria (LAB) based, however, yeasts or other organisms may be involved depending on the salt concentration and other factors. Salt concentration influences the type and extent of microbial activity.² These are described as spontaneous (or wild or natural) fermentations that occur without the use of a starter culture. In some cases, backslopping may be used.⁶

Vegetables most commonly fermented include cucumbers, beets, olives, peppers, radishes, onions, okra, ginger and other vegetables. Fermented vegetables are generally safe. There have been no documented foodborne outbreaks involving sauerkraut (covered in the next guidance section, 3.2) or fermented vegetables that have been made under good manufacturing practices and achieve a pH of 4.6 or lower. However, survival of pathogens in acidified conditions have been demonstrated, and kimchi has been linked to several *E. coli* outbreaks. Fermented table olives have also been linked to botulism outbreaks.

Fermentation is generally described as a 4 step process²:



Spontaneous fermentations are initiated by the addition of salt (NaCl), in the correct quantity in proportion to the ingredients. Cabbage and vegetable ingredients will have a mixture of Gram-positive and Gram-negative bacteria, yeasts and moulds. Primary fermentation begins when the mixture of microbes – LAB, spore-forming bacteria (e.g., *Clostridium botulinum*), yeasts, moulds and Enterobacteriaceae (e.g., *E. coli* or *Salmonella*) are released from the vegetables or fruits. The salt concentration is a critical control point (CCP) to drive the primary fermentation towards specific species of LAB groups. In the secondary fermentation stage, a more homogenous population of LAB continues acidification of the ferment. At this stage the fermentation should have a pH below 4.6. Cucumbers have an initial pH of 5 to 6.4. During fermentation the cucumbers and brine become acidified to a final pH of 3.2 to 3.6. Similarly, sauerkraut will have a final pH endpoint of 3.2 to 3.4.² Fermented products contain live microbes. Depending on the environment, containers, 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 also occur in the post-fermentation period at some point, depending on product process controls.

Box 2 Key items to consider for safety and quality of fermented vegetables.

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, 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 allowing spoilage agents to grow?

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

In Figure 2, lacto-fermented pickles made from the recipe in Appendix 1 are shown. The pickles are in a glass mason jar, weighted down with a glass disc and fitted with a silicone seal that allows gas to escape. The brine becomes cloudy as the fermentation proceeds (week 1), then clears (week 4).

Figure 3 | Fermented cucumbers appearance during fermentation







Week 4 fermented pickles

Outbreaks and Recalls

Very few recalls were found in Canada and elsewhere. Table 1 describes a series of allergen recalls for mixed fermented pickles and fermented carrot juice in 2014 and 2016, respectively. There were recalls in 2011 and 2015 due to spoilage, and an Australian recall in 2019 for lactic acid as a chemical hazard. In 2021, glass as a physical hazard resulted in a recall. No pathogens were identified in this review of fermented vegetable recalls.

Table 1 | Recalls related to pickled, fermented vegetable products (excluding sauerkraut and kimchi) in Canada and elsewhere

Year(s)	Hazard Category	Hazard Detail	Number Recalls	Country (s)	Product Description
2014 ⁷ , 2016 ⁸	Allergen	Mustard, Wheat	3	Canada	Mixed pickles, fermented carrot juice
2019 ⁹	Chemical	Lactic Acid	1	Australia	Hot sauce
2015 ¹⁰ , 2011 ¹¹	Microbiological	Spoilage	2	Canada	Pickles, pickled cubed beets
202112	Physical	Glass	1	Canada	Pickles

Outbreaks and illnesses related to fermented vegetables are shown in Table 2. No outbreaks linked to fermented vegetables have occurred in Canada. Fermented vegetables have been implicated in a number of US foodborne illnesses, but appear to be food handler related illnesses, and not linked to a failure in the fermentation process linked to the food. For example, 15 norovirus illnesses and one hospitalization occurred in 2013 in New Hampshire in patrons served pickles in their sandwiches. Illness and hospitalization from *Salmonella* Newport was linked to the sale of retail store pickles in 2010 in Illinois. Without further details on the process, it is uncertain what may have occurred. Pickles were sold to consumers in plastic bags and it is likely that post-processing contamination of the pickles, arising from improper food handling (e.g., dirty hands) when the pickles were placed into the plastic bags, caused the *Salmonella* infections. In Italy, however, green olives were associated with an outbreak of *C. botulinum*. The olives were soaked in salt water for approximately 35 days, the brine decanted, replaced with fresh water and stored in jars at room temperature. The pH of the olives was measured at 6.5. The recipe used by the restaurant was not standardized, amount of salt or pH through the process used was unknown.

Table 2 | Outbreaks related to fermented vegetables

Country	Date	Pathogen causing illness ¹	No. Ill (no. hospitalized)	Premises where outbreak occurred	Reason
U.S. ¹⁵	2010	<i>Salmonella</i> Newport	6	Deli retail store	Pickles (sold in plastic bag). Food handler or equipment.
U.S. ¹³	2000, 2010, 2013, 2016	Norovirus	19, 32, 15(1), 6	Restaurants	Pickles, sandwiches containing pickles.
US ¹³	1998, 2000, 2013, 2018	Unknown	13, 2, 9, 4	Restaurant	Pickles implicated among other foods.
Italy ¹⁴	2004	C. botulinum	28 (15)	Restaurant	Green olives, pH was 6.5.

Description of food preparation for fermented vegetables

Validated recipes sourced from recognized processing authorities or agencies are recommended, for example, recipes from the National Center for Home Food Preservation.¹⁶

Preparation of vegetables. The first step is the selection of high-quality raw ingredients. Damaged, visibly mouldy vegetables should be discarded. Dirty, poor quality outer layers of vegetables should be removed and discarded to limit potential spoilage. Vegetables are usually rinsed with potable water or washed, then cut or trimmed to desired size. Use of knives, blenders, shredders or other cutting equipment may create a physical hazard from metal fragments. Maintaining equipment in good repair, and observing whether knives, blades or shredders are damaged after vegetables are cut is a control point. Alternatively, a metal detector can be used to ensure no metal fragments are present in the finished product. Other physical hazards include stones and pits (e.g., from olives) or glass fragments from broken containers.

Vegetables may be washed several times, and in some cases, flash pasteurized, for example, blanched by immersion in hot water, or sanitized with a food-grade chemical, for example, 100ppm food-grade chlorine bleach before fermentation.¹⁷ A flash pasteurization step may also be required to remove off-flavours that can develop. For example, when fermenting peppers, blanching at 85°C for 2 min is required to inactivate enzymes that, if present, would result in undesirable tastes.¹⁸ Although washing, pasteurizing, or sanitizing vegetables will reduce overall microbial loads, residual fermentative LAB are still present in high enough numbers to allow spontaneous fermentation to proceed. A backslopping step, i.e., addition of previous brine culture, or addition of commercial LAB culture, is recommended to accelerate progression of the fermentation.

Other vegetables, for example, carrots and olives require de-alkalinization with a dilute solution of lye (sodium hydroxide, NaOH) prior to fermentation. Carrots receive a 1-2% lye wash prior to fermentation in 5% (NaCl) brine.¹⁹ Olives are drupes, which is a fruit containing a stone or pit, other drupes include apricots, plums and peaches.^{20,21} Olives contain oleuropein, a bitter compound that makes the fruit inedible without pre-treatment.²⁰ Green Spanish-style olives (Manzanilla is a common variety) are immersed in a lye wash of NaOH at 2-3.5% w/v for seven to ten hours.²⁰ This treatment, called a debittering process, softens the fruit, removes a waxy layer, and solubilizes other protein elements in the cell walls of the olive. After the lye treatment the olives are washed several times or immersed for six to 24 hours in potable water.²⁰

Vegetables are placed into containers and covered with a brine containing salt between 2 to 10% (or higher), depending on the vegetable. Various other ingredients are added depending on the recipe: usually sugar, vinegar (acetic acid) and other herbs or spices.³ Once the vegetables are prepared the fermentation process should be initiated as soon as possible, and within a maximum of 24 hours. If the initial fermentation is delayed, the cut vegetables should be held under refrigeration. Cutting vegetables increases the surface area available for microbial spoilage and pathogen growth. Containers should be food-grade, in good repair, free of scratches, chips or cracks, and appropriate for acidic conditions. Glass and stone crocks are common and generally well suited.

Metal containers are not suitable due to their reactivity with acid. Plastic can be used but is subject to scratching which can harbour bacteria and may leech potential contaminants. Some pottery glazes are not acid safe and may leach contaminants such as heavy metals into the food if used.^{22,23,24} Oxygen is generally excluded during this anaerobic fermentation. Processes for commercial pickles and pepper include frequent de-scumming of fermentation tank vessels, ultraviolet (UV) light surface treatments and frequent cleaning of tanks.^{23,24}

Surface moulds and oxidative yeasts can grow readily on the surface of fermenting vegetables. Plastic is used to cover the brine, and weights are placed on top of the plastic to prevent pickles from floating above the brine. In Figure 3, mould is clearly visible at the edge of a crock fermentation where plastic has not properly been weighted down over the brine. There should be at least 3 cm or more of liquid brine between the fermented vegetables and the top of the brine. In larger vats, pickles are weighted down with wood over plastic, another acceptable method would be to use a snug-fitting plate in a 20 L (5 gal) plastic bucket. All materials should be cleanable and food-grade. Photos of a successful and failed fermentation are shown in Appendix 2.





Only pickling or canning salts should be used. Iodized salts, sea salts and other speciality salts are not recommended as they may contain heavy metals, anti-caking agents or other agents that interfere with fermentation. Problems linked to non food-grade salts include discolouration of the fermentation brine, and masking of spoilage.³ Recipes calling for the addition of oil should be examined with caution as when oil is added it can create micro-environments of oil bubbles that may allow growth of *C. botulinum*.²⁵ Use of mustard oil is not permitted in Canada.²⁵ In addition to influencing microbial activity, and adding flavour, salt causes rupture of cell membranes during fermentation.² As fluids are released from cells, the percentage of salt decreases in the fermentation.² Some fermentations recommend addition of salt to compensate, for example, weekly additions of a 1% salt brine are added to jalapeño peppers.²³

Table 3 provides examples of salt percentages used for vegetable fermentations. Brine percentages for ethnic recipes may be unpublished and considered proprietary. For example, Spanish Almagro eggplant pickles are made in a brine containing vinegar, oil and many spices that ferments between 4 to 20 days, but the brine percent was not published as this recipe is held as a traditional 'secret'.²⁶

Other ingredients may also be added. Olives are fermented in brines of 10-12%, although lower salt brine levels are also reported when brines are supplemented with organic acids (e.g., lactic acid), and/or preservatives (e.g., sodium benzoate) to control spoilage organisms, lower pH levels more rapidly, and promote growth of fermentative *Lactobacillus* organisms.²⁰ Olives are low in natural sugars and so addition of glucose (0.5%-1.0%) is commonly used to improve fermentations by speeding up acidification thereby decreasing pH.²⁰ Citric and ascorbic acids are added to olives as antioxidants, and organic acids such as lactic, acetic, sorbic and benzoic acids are used to equilibrate the final brine pH and as preservatives in many fermented food products.²

Table 3 | Examples of LAB salt/brine concentrations and processes for vegetable fermentations

Vegetable	Recommended salt %	Additional processes
Cabbage (sauerkraut) ²⁷	1 to 3%, 2.25% optimal	See <u>Section 3.2</u>
Carrots ¹⁹	5%	Pre-treatments include flash pasteurization and a lye wash (NaOH) at 1-2%, removal of lye and acidification with vinegar (acetic acid) to neutralize NaOH absorbed by the carrots. Commercial starter culture is normally added.
Cucumbers (pickles) ^{19,24}	3 to 15%, depending on variety	Fermentation in salt brine for two to three weeks. In the U.S., commercial brines are chlorinated, acidified with acetic acid, buffered with sodium acetate or sodium hydroxide, and inoculated with LAB. Tanks are skimmed daily for debris and insects, and de-scummed regularly. De-salting occurs after fermentation, when fermenting brine salt concentration is too high for human consumption. Final equilibrated pH to 4.6 or lower is required for storage life of product.
Pickled garlic ¹⁹	8%	Heat treatment blanching step required to inactivate enzymes, starter culture is inoculated, fermentation for seven days, preserved by pasteurization.
Olives (green Spanish-style) ^{20,21}	10 to 12%	Lye wash (NaOH) of 2-3.5% to remove oleuropein (bitter component), washing, glucose added to increase acidification, fermented for two to twelve months. Note: black olives are made with the same varietals by exposure to air to darken the fruit.
Mexican peppers ²³	18 to 20%	An additional 1% NaCl added weekly, fermented 4 to 6 weeks, stored in 20% brine with 1% commercially added lactic acid, then rinsed to remove excess salt, and covered with minimum 3% vinegar (acetic acid) before mixing with other vegetables. Tanks are periodically cleaned to remove oxidative yeasts and moulds and treated with ultraviolet (UV light) to remove surface microorganisms.

Vegetables ferment in the brine solution for a desired amount of time depending on the fermentation temperature, the percent of salt in the brine solution, and the penetration time needed for development of lactic acids, other organic acids and sufficient drop in pH.³ The ideal temperature for a cucumber pickle fermentation is 20-22°C for approximately 3 to 4 weeks.¹⁶ However, fermentation can occur at higher or lower temperatures, generally taking less time at higher temperatures and proceeding more slowly at lower temperatures. At temperatures of 15-20°C fermentation periods of 5 to 6 weeks are typical. However, fermentation at temperatures higher than 25°C may result in spoilage. The pH of the finished product should be 4.6 or below and ideally, pH is checked at multiple points during fermentation to track its progress.^{2,3}

In a recent review by Snyder et al., (2020), tracking pH to ensure successful fermentation is occurring is the only critical control of significance, and pH drop is required to establish a 5-log reduction in harmful enterohemorrhagic *E. coli*, the pathogen of significance in vegetable fermentations.³ Snyder recommends a pH drop should be demonstrated by measuring the pH of the brine solution, with time and temperature requirements stated as follows for kimchi (as an example):³

- At room temperature fermentation, test pH of brine every 12 hours and demonstrate pH decrease to 4.6 within 48 hours;
- At refrigerated temperature fermentation, test pH of brine every day and demonstrate pH decrease to 4.6 within 4 days;
- If pH has not dropped to 4.6 within one week consider this a failed fermentation and discard product. (Note: in our guidance for kimchi reviewed in <u>Section 3.3</u>, the critical limit is three days for a room temperature fermentation and one week for a refrigerated fermentation).

Because the length of fermentation required varies for different categories of vegetables, time for pH decrease may also vary. For example, green Spanish olives have four fermentative phases lasting several months.²⁰ In the first 72 hours, LAB will form, in the next 7 days, pH is expected to decrease to a pH of 6.0, over the next few months the pH will decrease to 4.6 or lower. LAB will continue to be present during bulk storage before packaging and bottling. Temperatures for fermentation also vary based on ambient temperature through-out the year, unless they are performed under refrigeration (e.g., refrigerator pickles). For these reasons no standard set of time to a required pH drop is provided based on vegetable category.

Figure 5 | Fermented olives



Our main recommendation is monitoring and recording pH and time as the CCP for a successful vegetable fermentation. Operators making fermented vegetable products should provide expected fermentation times to a pH drop of 4.6 or lower and establish critical limits for their process. It is recommended that log sheets of pH verifications are kept and monitored as this is the main CCP.

To stop fermentation, pasteurize (apply a cook step to) the product. If no step is taken to stop fermentation, and the product has not been verified to be shelf stable (for example, product maintains a pH of 4.6 or lower to end of shelf life), then the fermented vegetables must be stored refrigerated.

Prior to packaging, addition of fresh brine is normal in many fermentations, particularly those with high salt concentrations. In the U.S., maximum amounts of salt for dill, sour dill and relish pickles is 5% (5 grams per 100 mL), for sweet pickles and relishes this is 3%.²⁴ Addition of vinegar, preservatives, stabilizers and other additives are employed to increase shelf life, preserve texture and maintain colour of product, etc. Examples of additives include alum (aluminum potassium sulfate) or lime (calcium hydroxide) to firm fermented pickles.³⁰ Pickles manufacturers, typically recover the pickles from brine, grade them, repack into jars filled with vinegar, sugar, spices and alum, then pasteurize for 15 minutes at 74°C, before storage and distribution.³¹ Hot-packing is recommended, normally brines are heated to 74°C and up to a maximum temperature of 82°C. Lower temperatures are used to prevent softening of the fermented vegetables.

Figure 6 | Fermented vegetables

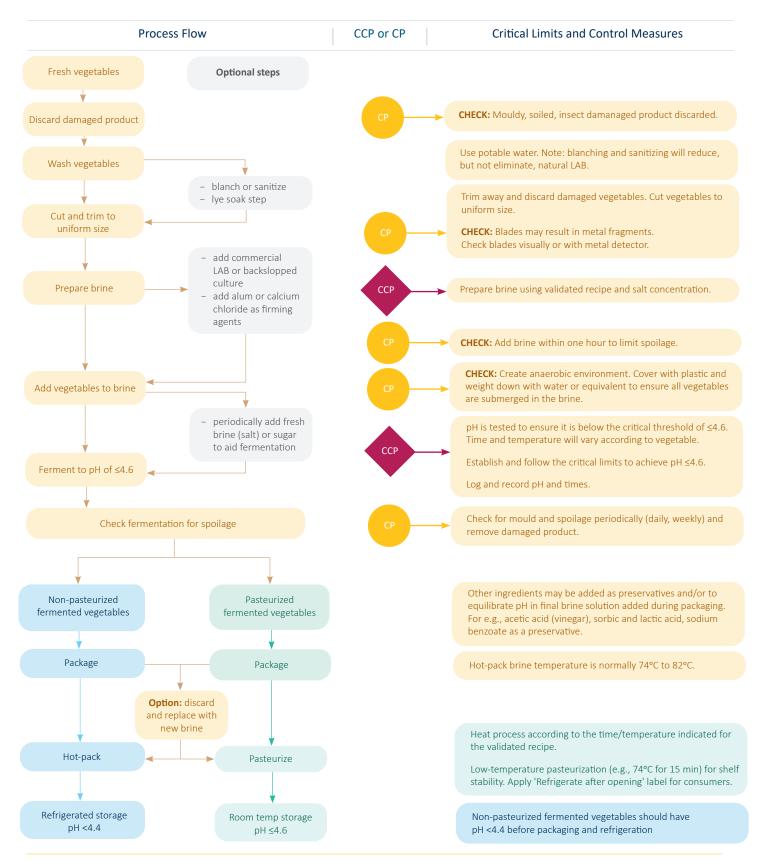


When repacking fermented vegetables from large fermentation vessels into smaller, consumer-sized jars or containers, it is recommended to hot-pack into clean, sterilized jars with hot, fresh, brine. A gas flush to create anaerobic environment in reduced oxygen packaging (ROP) will limit regrowth of microbes in the product if it is not going to be heat-treated. Unpasteurized products, even when hot-packed, will have residual microbes and product should be held refrigerated to avoid regrowth of spoilage moulds and yeasts.

Verification of shelf stability is done by testing products for spoilage and pathogen microorganisms to the end of the intended product shelf life, with testing inclusive of consumer temperature abuse conditions. Because the fermentation contains live microbes, spoilage and regrowth of microbes may increase the pH to levels higher than 4.6, allowing *C. botulinum* spores to grow in the product, creating an unacceptable risk.

The survival of *L. monocytogenes* in fermented refrigerator pickles has been previously demonstrated, showing survival of this pathogen for up to 91 days during refrigerated storage.²⁸ Due to these concerns, authorities recommend that ready-to-eat foods, such as fermented vegetables, maintain a pH of lower than 4.4 (pH<4.4) during their shelf life to prevent growth of *L. monocytogenes*.^{19,29} Acid-tolerant pathogens, such as infectious strains of *E. coli*, have caused illnesses in fermented kimchi. Fermented vegetable products, especially those with short-term fermentation periods of less than one week, are further recommended to be held for another two weeks before distribution and sale, providing additional time for die-off of acid-tolerant pathogens. To ensure microbiological stability and safety, pasteurization of fermented vegetable products prior to repackaging and sale is strongly recommended. If the fermented vegetables are pasteurized, then the product may be stored at room temperature, until opening. Consumer labels for refrigeration after opening are required.

Fermented vegetables food flow chart | Process flow and controls



Potential issues with fermented vegetables food preparation

Issue	Description
Biological hazards	Pathogen hazards in fermented vegetables include <i>C. botulinum, Salmonella, S. aureus</i> and infectious strains of <i>E. coli. L. monocytogenes</i> is also of concern in refrigerated foods. <i>C. botulinum</i> will not grow at pH of 4.6 or below, <i>L. monocytogenes</i> control requires a pH of lower than 4.4. However, <i>E. coli</i> and <i>Salmonella</i> are acid-tolerant and may persist in fermented foods unless held.
Foreign material (metal, glass, stones) physical hazards	Physical hazards such as metal fragments can occur when metal blades and utensils are used to chop raw vegetables and other seasonings. Glass hazards may also occur from broken containers during the fermentation or during repackaging. Stones and pits (e.g., from pitted olives) are also hazards.
Salt concentration is too high or too low	Choice of salt is important, use a pickling or coarse salt that is non-iodized. Sea salts and other speciality salts are not recommended as they may contain anti-caking agents and discolour fermentations or mask spoilage.
	High salt can slow the growth of LAB allowing yeast growth and may result in longer fermentation times. High salt may also drive homogeneous populations of LAB (less diversity in types of bacteria at the beginning of the fermentation) that may not be optimal. Note that other vegetables do require lower or higher amounts of salt, consult validated recipes and processes.
	Low salt , generally less than 0.8% can result in poor LAB growth, and soft-textured vegetables. However, as long as LAB populations recover and organic acids drive down pH, this may not be significant.
Maintaining salt concentration during fermentation	Fermentation breaks down plant cell tissues (cell walls) releasing water into the ferment. This lowers the salt concentration. Some vegetable ferments that require high salt concentrations thoughout the fermentation period may require addition of salt periodically.
Temperature	High temperatures >25°C . LAB grows very fast with rapid acid production resulting in poor flavour. During initial stages, before LAB population increases there is also risk of aerobic yeast and mould growth that impart off-flavours before the increasingly acid environment stops their growth.
	Low temperatures <10°C . Delayed start of fermentation, that can lead to spoilage of product and off-flavours.
Delay between processing and fermentation	Cutting vegetables releases nutrients resulting in rapid microbial growth. Any delay following processing the vegetables can allow aerobic yeasts, moulds, and potential pathogenic microbes to grow.
Delayed or failed fermentation	The critical control point in vegetable fermentation is a pH drop to 4.6 or lower. Delays in fermentation allow spoilage or pathogenic organisms opportunity to grow.
Top of ferment container is not covered	When oxygen is not excluded, aerobic environments at the surface will allow growth of aerobic yeasts and moulds, causing spoilage.
Incomplete fermentation or	Fermentations require enough time to complete, otherwise, acid alcohol formations may be present, arising from activity of cold tolerant yeasts that convert remaining sugars to alcohol.
holding time	It can take up to 7 days and longer for <i>E. coli</i> to die off in an acidified environment. Short-term ferments, for example, products fermented for less than one week should be held for a minimum of two weeks before sale to ensure acid that has developed in the brine has sufficient time to inactivate any <i>E. coli</i> present.
Gas	If fermented vegetables are re-packaged into secondary containers without any pasteurization or heat treatment, the fermentation will continue. Even when brine is replaced, residual LAB on the vegetables will restart the fermentation. Gas build-up may cause leakage from the container and has the potential to explode creating a physical hazard.
Histamine formation	Biogenic amines produced by aerobic microbes may form during initial stages of fermentation before LAB can grow.
Pesticide	Pesticides may be present in raw ingredients. This occurs when pesticides are overused or when produce is harvested too early before pesticide residues dissipate. Washing, salting, and fermentation can remove or reduce pesticides on raw ingredients.

Fermented vegetables food safety control points

Food safety points described in this section are shown in point form below:

- Use validated, evidence and research-based recipes. Recipes should be tested and validated to ensure they support effective fermentation and controls to mitigate spoilage and pathogen growth.
- Ensure proper cleaning and sanitizing protocols are in place for establishment surfaces, containers and equipment.
- Most vegetable fermentations are spontaneous, meaning native, wild, naturally occurring LAB start the fermentation. However, commercial LAB starter may be purchased and added, resulting in safer, more successful fermentation. Backslopping previous culture (liquid) is a normal practice. When backslopping, operators should have a plan to assess quality and safety of the culture. For example, ensure backslopped culture is free of oxidative yeast and mould growth.
- Limit microbial contaminants by choosing best quality vegetables free of moulds, dirt. Prepare vegetables by trimming away soiled areas, wash and rinse in potable water. Blanching or sanitizing vegetables prior to fermentation is acceptable. When this occurs, we recommend adding LAB or backslopping to improve fermentation outcomes.
- Pre-treatment with a lye wash (1-2% NaOH) is normal for some vegetables (e.g., carrots, olives) and this process step is followed by a rinse step in potable water or brining solution to remove excess lye. Acetic, citric acids may be added to counteract NaOH absorbed by the vegetables.
- Cut vegetables to achieve an approximately uniform in size in order to allow for even rate of fermentation and acid penetration.
- Do not prepare (cut) vegetables more than 24 hours before initiating the fermentation to prevent growth of spoilage organisms. If fermentation cannot be initiated after cutting, hold vegetables under refrigeration.
- Fully cover vegetables under brine in order to create anaerobic conditions for optimal LAB growth.
- Ensure formulation contains an appropriate amount of salt, salt range varies from 2 to 20%. Some ferments may require additional salt brine to be added through the course of fermentation as the vegetables break down and release water, diluting the mixture.
- Maintain the optimal fermentation temperature range, room temperature vegetable fermentations are common (20-22°C); some fermentations occur at lower temperatures for longer times.
- Main CCP: Vegetables should be fermented for proper amount of time, based on time required to achieve a pH of 4.6 or less. Operators should determine this and establish critical limits for the process. Logs of pH and ferment times should be recorded.
- Hold all fermented products for a minimum of two weeks before distribution and sale, especially short-term ferments, to allow time for acid-tolerant pathogens to die-off.
- Fermented pasteurized vegetables are accepted as shelf stable and should maintain a pH of 4.6 or lower throughout the intended shelf life of the product. Consumer label should include a 'keep refrigerated after opening' as spoilage yeasts and moulds can grow once opened.
- Fermented non-pasteurized vegetables should be refrigerated as the brining solution contains active culture. These products should maintain a pH lower than 4.4 (pH<4.4) to control for *L. monocytogenes* that may persist in ferments.
- Heat pasteurized fermented vegetables (e.g., hot-water pasteurized or canned, to 74°C for 15 min or equivalent), with a pH of 4.6 or lower (pH≤4.6) are considered shelf-stable.
- When repackaging, first remove product from used brine, hot-pack into fresh, hot, brine (minimum of 74°C), and seal into ROP. A gas flush for unpasteurized products will limit microbial activity, unpasteurized products must be refrigerated and have a pH of 4.4 or lower.
- The operator is responsible for determining shelf life of fermented products. See <u>Section 2</u> of this guidance for shelf life information.

Potential health issues with fermented vegetables

Biogenic amines may form in fermented vegetables, although no reports of illness linked to biogenic amines were found in reports. Information about biogenic amines and how operators are recommended to manage this risk may be found in Box 3 below.

The highest levels of histidine reported for the category of fermented vegetables were 138 mg/kg, above the threshold of 80 mg/kg for causing illness.³² For tyramine, the highest levels reported were 178 mg/kg and within reported levels that cause illness between 100 and 800 mg/kg. For phenylethylamine, the highest levels reported 31.5 mg/kg a level slightly above the threshold of 30 mg/kg for causing illness.³² For other biogenic amines the highest levels reported for tryptamine was 66 mg/kg; putrescine was 549 mg/kg; cadaverine was 316 mg/kg; spermidine was 155 mg/kg; and spermine was 83 mg/kg.32 The highest levels of histidine reported for the category of fermented vegetables were 138 mg/kg, above the threshold of 80 mg/kg for causing illness.³² For tyramine, the highest levels reported were 178 mg/kg and within reported levels that cause illness between 100 and 800 mg/kg. For phenylethylamine, the highest levels reported 31.5 mg/kg a level slightly above the threshold of 30 mg/kg for causing illness.³² For other biogenic amines the highest levels reported for tryptamine was 66 mg/kg; putrescine was 549 mg/kg; cadaverine was 316 mg/kg; spermidine was 155 mg/kg; and spermine was 83 mg/kg.³²

Box 3 | Biogenic amines in fermented foods

Biogenic amines (BAs) can be produced by microbes in fermented foods, such as fermented soybean products, vegetables, cheeses, sausage, and fish. Normal BA intake does not cause illness as intestinal amine oxidases break down and detoxify the BAs.^{33,34} 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.^{33,35,36} The toxic effects of BA may vary between individuals depending on individual sensitivity and on the consumption of alcohol or drugs that are monoaminooxidase inhibitory.^{37,38}

The main BAs are histamine, tyramine, β -phenylethylamine, putrescine, cadaverine and spermidine. Health Canada has set action levels for histamines in anchovies, and fermented fish sauces and pastes at 200 mg/kg and for other fish and fish products at 100 mg/kg. 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.³⁴

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 thethe expected culture activity occurs within correct timeframe; and
- (5) monitoring for expected pH.

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

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Appendix 1 | Recipes and procedures for fermented and acidified cucumbers

The two recipes shown here are adapted from the United States Department of Agriculture (USDA) complete guide to home canning, Guide 6, Preparing and canning fermented foods and pickled vegetables.¹⁶

PICKLED VEGETABLES RECIPES				
	FERMENTED VEGETABLES	ACIDIFIED VEGETABLES		
	Fermented dill pickles	Quick fresh-pack dill pickles		
Ingredients	 Cucumbers – 4 lbs ½ cup salt ¼ cup vinegar (5%) 8 cups water Dill seed, heads or dried – 2TBSP Optional: 2 cloves garlic, 2 dried red peppers, 2 tsp. pickling spices 	 Cucumbers – 4 lbs Soak: 6 TBSP salt in 16 cups water ¼ cup salt 6 cups vinegar (5%) 4 cups water 2 TBSP sugar, 1TBSP pickling spice Add to jars: 1 TBSP mustard, 2TBSP dried dill or fresh dill heads 		
Calculate salt %	113 g NaCl / 1890 mL water (*100) = 6.0%	57 g NaCl / 946 mL water (*100) = 6.0%		
Procedures	Wash cucumbers, cut off blossom end.	Wash cucumbers, cut off blossom end.		
	Layer pickles, dill and garlic into container.	Soak cucumbers in salt and water mixture for 12 hours. Discard water. Pack cucumbers into jars.		
	Dissolve salt, vinegar and water and pour over cucumbers. Ferment for 3 to 4 weeks at 20°C to 25°C or up to 6 weeks at lower temperatures. Weight down pickles and remove surface scum and moulds.	Mix remaining ingredients, boil, and pour over cucumbers. Divide mustard and dill between jars.		
	Store in refrigerator or pack into jars with fresh brine mix and process in a boiling water canner.	Process in a boiling water canner		

Appendix 2 | Fermented cucumber pickles and mould spoilage

The photos in this appendix show a fermentation of cucumbers done in two ways: in a crock and in a glass jar. As the fermentation progresses, mould in the crock pickles that was not successfully managed, illustrates what occurs on the surface of a vegetable fermentation when the product is not properly immersed in the brine.

Week 0



Fermentation ingredients:

- Pickling salt, Household vinegar (5%), pickling spices,
 Russian red garlic, fresh dill weed flowers
- Recipe is shown in Appendix 1
- The cucumbers were washed, trimmed and layered into the crock and the glass jar
- The brine is made and shown in the glass measuring jar on the right

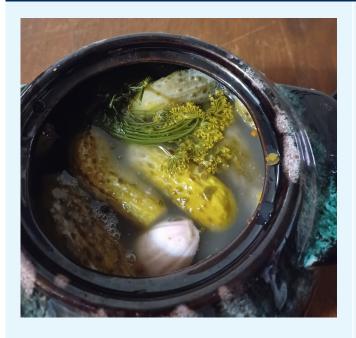






Finished crock and jar:

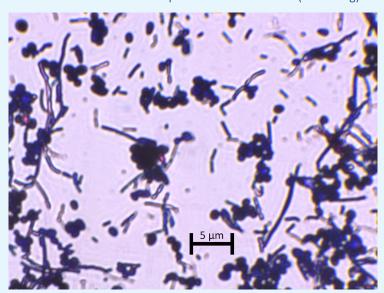
- The brine is added, and the cucumbers are ready for fermentation.
- A piece of plastic was used to cover the crock pickles, and then a round glass weight used to hold down the plastic. Brine that leaked over the plastic also helped to hold it down.
- In jar on the left, another glass weight held down the pickles and the jar was fitted with a silicone venting seal and lid.





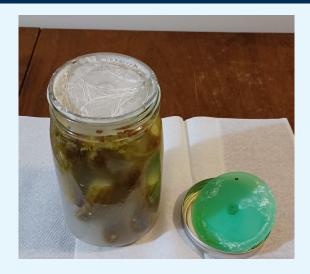
At week 1, the brine made for the fermentation is cloudy showing an active fermentation. The Gram stain photo (below) shows an active microbial culture. There is evidence of large Gram-positive rods, yeasts and the longer strands could be evidence of mould.





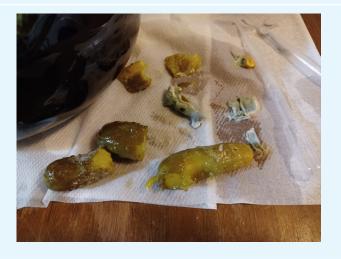


Crock top before (above) and after (below) lid and plastic is removed.

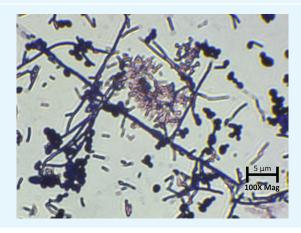


Jar with mould film under lid. The cucumbers are submerged under the glass weight. Brine is cloudy.





By week 2, mould is affecting the quality of the cucumbers in the crock ferment. In the photo on the right, spoilage moulds and yeasts have softened the pickles, so they are inedible. These were discarded.



Gram stain photo from the crock fermenting pickles. Evidence of mould growth.





In the crock, problems are still occurring with pickles floating to the top and becoming slimy and mouldy.

In the jar, bubbles from the LAB fermentation are visible and the cucumbers are held down by the glass insert.



Week 3 moulds on pickle (left). Although the mouldy pickles from week 2 were removed, the method of holding down the submerged vegetables is not working. At the sides of the crock, in the places where the pickles are above the surface of the brine, slimy spoilage agents, moulds and yeasts continue to grow.



More mould is clearly visible on the cucumbers, mainly on the edge of the crock, where cucumbers were not properly held down.

In contrast, the pickles in the jar are satisfactory.

Although the mould growth is still visible on top of the jar where the brine is exposed to oxygen, brine salt in the liquid and an anaerobic environment prevents the cucumbers from spoilage.

In the jar of fermenting pickles, a LAB community is visible.

