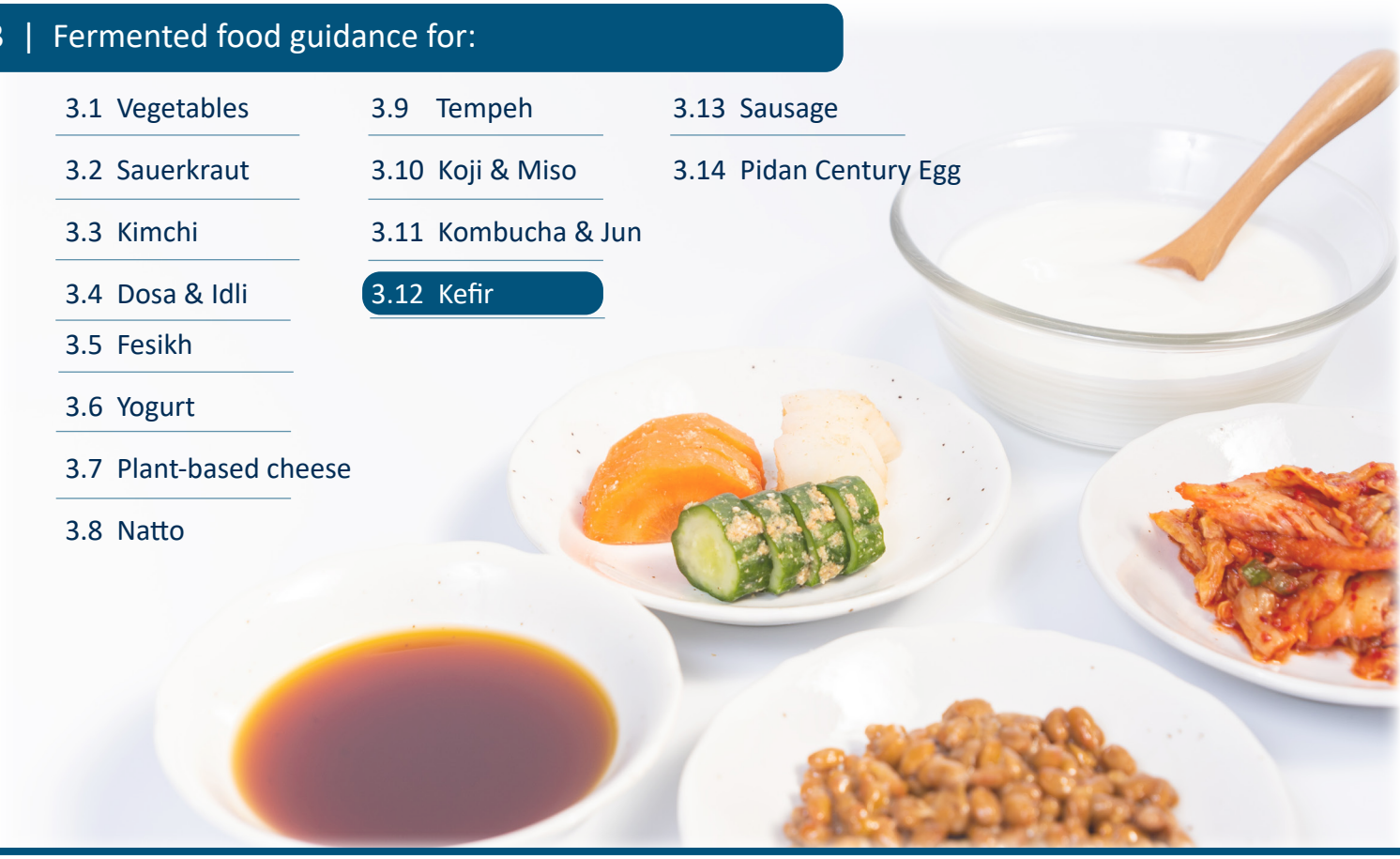
December 2024

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 3 | Food safety reviews of fermented foods

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

Each fermented food review includes:

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

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

Figure 1 | Fermented foods described by fermentation agent and complexity

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

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

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

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

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

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

3.12 | Kefir

Authors: Naghmeh Parto, Celeste Loewe | Public Health Ontario

Overview

Description	 Photo showing slightly foamy milk kefir (left) and milk kefir grains (in spoon, and right) Kefir is a fermented product, made from cow's, goat's, buffalo's or sheep's milk. It can also be produced from a plant-based beverage, or water supplemented with sweetened water or fruit juices (water kefir). Kefir is characterized by its fizziness, acidic taste and sometimes, may contain alcohol.
Starter culture	• The culture is referred to as Kefir grains. • Milk kefir grains range in size from 0.3 to 4 cm in length and look similar to small clumps of cauliflower florets, white to yellow in colouration. • Water kefir grains are smaller than milk kefir grains and look like tiny, irregular-shaped translucent, crystal granules. • Kefir grains contain a mixture of lactic-acid bacteria (LAB), acetic-acid bacteria (AAB) and yeasts that are immobilized on a polysaccharide and protein matrix known as kefiran. • Kefir grains are recovered from finished beverages by filtering (straining) them out. Grains are re-used in subsequent batches of kefir (a type of backslopping) and this process is acceptable. New kefir grains (frozen or dehydrated) will need to be reactivated before use. • Alternatively, a commercially sourced, powdered kefir culture can also be used in place of kefir grains.
Key features	• Kefir is a fermented drink characterized by its fizziness, acidic taste, and may contain low levels of alcohol. • Kefir has a pH of less than 4.6. • Kefir is not a shelf-stable product and should be refrigerated. • The recommended shelf life for milk kefir is 3-12 days; commercial milk kefir may be stable for up to 21 days.
Hazards of concern	Chemical Hazards: • Alcohol. During fermentation, alcohol is produced that can be higher than acceptable limits. Alcohol is a danger to children, during pregnancy, and to other susceptible populations seeking to avoid all alcohol. • Formation of alcohol above regulated levels is of concern. Federal regulations require 1.1% or less alcohol in non-alcoholic beverages, provincial requirements vary. *(Continued on page 6)*

Hazards of concern cont'd	• Water kefir is well documented to contain elevated alcohol, although residual alcohol levels have been found rarely in milk kefir. Biological Hazards: • *E. coli* is linked to illness reports in milk kefir. • *L. monocytogenes* has been detected in recalled milk kefir in Canada. • *Salmonella* may be a concern with addition of dried fruits or nuts used in manufacture of plant-based kefir. • The low pH (less than 4.6) and presence of bacteriocins inhibits growth of pathogens. However, if kefir or added ingredients, such as fruit juice sweeteners are contaminated with pathogens, these may survive in kefir. Because milk kefir has a short fermentation time of <24 hrs, *E. coli* and other acid-resistant pathogens may survive.
Important control points	• Only use pasteurized milk and juice for fermentation media. • Kefir grains recovered from a previous batch or sourced as commercial dehydrated culture must be activated before inoculating into milk or juice. This may require several transfers of kefir grains to fresh substrate (milk or juice) before the grains are fully activated. • Ensure the pH of final product is below 4.6 but not lower than a pH of 2.5. o Critical limit for milk kefir: maximum time to achieve a pH drop to <4.6 is within 36 hours. o Critical limit for water kefir: because starting pH is already <4.6, operators must define process based on grain mass growth, soluble sugar reductions (BRIX), or other process method. • Check the alcohol content of the final product. Label the product if it contains alcohol. • Refrigerate kefir. • Use a verified recipe (see Box 2).

Background

Kefir is a fermented beverage. Milk kefir is made from cow, goat, sheep or plant-based beverages (commonly referred to as vegan milk). Water kefir is made with sweetened water or fruit juices.[1] Kefir is characterized by its fizziness, acidic taste and sometimes alcohol content.[2-5] Milk kefir origins can be traced back to 2000 years B.C. to the Caucasus mountains area (Armenia, Georgia, Azerbaijan and Russia),[6] while the origin of water kefir is unclear.[1]

Kefir is the Turkish word for “good feeling” and kefir has been associated with health benefits, such as improved digestion.[7] It also contains tryptophan, an essential amino acid, that has relaxing effects on the nervous system.[1] Kefir is usually sold as a refrigerated ready-to-drink beverage in both plain and flavoured varieties.[8] Traditionally, dairy milk is inoculated with milk kefir grains to make milk kefir, which is well-known for its carbonated quality, acidic tart taste, and high viscosity. Milk kefir can be made from a variety of animal milk products (e.g. goat, sheep, and buffalo milk) and plant-based beverage (e.g. soy beverage, rice beverage, walnut beverage and coconut milk).[2,3,7]

Kefir fermentation is referred to as a combined fermentation because it contains a mixture of bacteria and yeast in the starter culture. The fermentation period for milk kefir is generally 18 to 24 hours. The starter culture used in the production of kefir are referred to as “kefir grains”. Milk kefir grains range in size from 0.3 to 4 cm in length and look similar to small clumps of cauliflower florets with white to yellow colouration. The grains contain a mixture of lactic-acid bacteria (LAB), acetic-acid bacteria (AAB) and yeasts that are immobilized on a polysaccharide and protein matrix. In milk kefir, the grain exopolysaccharide is known as kefiran.[6] The community of bacteria and yeasts in milk kefir grains converts the milk sugar into other components such as lactic acid, acetic acid, carbon dioxide, ethanol and aromatic compounds during the fermentation process.

The community of bacteria and yeasts in milk kefir grains may differ depending on the geographical origin of the kefir grain but commonly include:

- Bacterial species: *Lactobacillus kefiranofaciens*, *Lacticaseibacillus paracasei* (*basonym Lactobacillus paracasei*), *Lactiplantibacillus plantarum* (*basonym Lactobacillus plantarum*), *Lactobacillus acidophilus*, and *Lactobacillus delbrueckii* subsp. *Bulgaricus.*
- Yeast species: *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Candida kefyr*, and *Kluyveromyces marxianus sp. Marxianus*.[8]

To produce water kefir, a mixture of water, sugar and fruit or fruit juice is inoculated with water kefir grains and allowed to ferment from one to 8 days between temperatures of 21°C to 30°C, typically fermentation is 2 to 4 days at room temperature.[8-11] Many have described traditional water kefir as a cloudy sparkling beverage with a fruity aroma, mild alcoholic taste, and slight sweet acidic note. Water kefir grains are smaller than milk kefir grain and have a jelly-like appearance (see Figure 2).[12] They look similar to tiny, irregular-shaped translucent, crystal granules, and the grains are 5 to 20 mm in diameter.[10] Similar to milk kefir grains, water kefir grains contain a mixture of LAB, AAB, yeasts, and may contain anaerobic bacteria, bifidobacterium, surrounded by a polysaccharide matrix known as dextran exopolysaccharides (EPS or α-glucans).[10] Water kefir beverages can also be made from other sugary liquids (e.g. fruit juices, vegetable juices, coconut water, ginger beer).[9,13-16]

This review is focused on both milk kefir and water kefir.

Activating Kefir Grains

From previous fermentations. Kefir grains should be properly preserved to retain their microbial activity so they can be reused to make the next batch of kefir. Microbial activity can be maintained in milk kefir grains recovered from fermented milk when they are kept wet. When re-using milk kefir grains, store in fresh milk for up to 7 days, or in water for up to 3 days at refrigeration temperatures for optimal activity. Similarly, water kefir grains can also be stored for short periods when stored in sugar solutions. Acidified water and low temperatures will stress water kefir grain activity, requiring longer periods to reactivate.[17] As described in one online recipe, brown sugar was used to store kefir grains trained on plant-based coconut milk.[12] After this period, kefir grains will lose their activity, and will require activating again. This means the grains should be placed into fresh substrate (milk or juice) and fermented one or more times. For longer term storage (12-18 months) kefir grains can be frozen (at -20°C), air dried and refrigerated or freeze-dried.[2,3,18-21]

From purchased commercial culture. Alternatively, commercially purchased kefir culture can also be used in place of kefir grains. Kefir culture is usually in the form of a dry powder that will contain fewer microorganisms than kefir grains.[12] If using kefir culture follow manufacturer directions for the weight/volume of culture per media used. Kefir grains received in air-dried, freeze-dried or frozen forms do not have freshly cultured microorganisms in their exponential growth phase, so the grains are considered inactive. Inactive kefir grains must be "activated" before use. To activate dehydrated milk kefir grains, stir the grains into pre-cooled, pasteurized milk following manufacturers' specifications (for example, one pack containing between 2 to 5g of dehydrated kefir grain to 250mL of milk or more, volumes vary between manufacturers') in a clean container covered with a cheese cloth or coffee filter and secured by a rubber band and held at room temperature (20-25°) for up to 24 hours. When the texture becomes gel like, separate the grains from the milk by a strainer and discard the liquid. The basic technique is to recover the kefir grains, discard the old milk, add fresh milk, increasing the volumes of milk each time. The process is repeated until the grains can ferment a full batch, generally one litre or more.[12] When the kefir grains float to the surface of milk or when the milk clots, the activation process is completed.[22] If the grains are frozen or freeze-dried, the activation process can take longer (up to 1 month) and require kefir grain transfer to fresh milk daily or at minimum transferred into fresh milk three times weekly.

Figure 2 | Water kefir grains

Similarly, inactivated water kefir grains can be activated by placing the grains in 10-15% sugar solution. Pasteurize the sugar solution first, for example, boil water and sugar and cool to inoculation temperature, from 60°C to 21-24°C within 2 hours before addition of the water kefir grains. Following manufacturers' specifications, add, for example, one pack of grains to 3-4 cup of sugary water cover and hold at 20-30°C for 3-4 days, until the grains look plump and translucent.[1] When the water kefir grain mass increases to a desirable amount (for example from 100 to 600g in weight), they can be used for fermentation inoculation.[13,14,23,24]

Using activated milk or water kefir grains is a critical control point (CCP). If the grains are not fully activated, the fermentation will be compromised, for example, in milk kefir, the pH may not drop within the allocated time. Because grains are a mixture of bacteria and yeast, microbial indicator tests are not helpful to assess the quality of the kefir grains as a starter culture. It is recommended to assess the activity of the culture using one or more of these methods:

1. by measuring an adequate pH drop to pH 4.6 or less occurs when the activated grains are added to the media (milk, plant-based beverage or sweetened water);
2. by monitoring grains for healthy colour, shape, odour and activity;
3. by measuring time taken for grains mass to multiply;
4. by measuring another process parameter, for example, soluble sugar and solids content (BRIX) or titratable acidity.

If kefir grains grow slowly, if the grains develop unusual colour, odour, or if grains lose activity, grain function is compromised, and they should be discarded. In one study assessing milk kefir growth, grain growth was best at 20°C in comparison to growth at 25°C or 30°C. Temperature was not a factor in water kefir grain growth. For milk and water kefir grains, there was no change in appearance, odour or texture of healthy grains, transferred weekly over a four week period (note: use of brown sugar adds tan colour to water kefir grains).[25] Healthy kefir grains (milk, left and water, right) are shown in Figure 3 from this study.[25] Other research has found optimized growth of water kefir grains occurs with lower inoculations of wet grain mass, because acidic conditions, which would occur when more starter is added, will result in smaller, brittle kefir grains.[26] In another study, >60% of kefir grain cultures tested were found to be contaminated with fecal bacteria (*E. coli*), and other pathogenic strains of bacteria.[27] Good sanitary handling practices are required during all stages of kefir production.

Figure 3 | Milk and water kefir grains

Suggested critical limits and CCPs for milk and water kefir activation and fermentation performance are shown in the table below, however, operators are advised to assess and document their fermentation process performance, kefir grain growth and activity. Activities include measuring size of grain growth, verifying pH drop and evaluating sensory characteristics described.

Table 1 | Kefir grain activation and fermentation critical limits and CCPs

	Activation of kefir grains	Fermentation performance
Milk kefir (traditional method)	Critical limits for milk kefir grain activation assessment are based on ability of kefir culture to ferment the substrate within a normal fermentation time period as demonstrated by a pH drop to 4.6 or lower	CCP: Verify pH of less than 4.6 is achieved. Critical limit: culture will ferment one litre of milk at room temperature within 24 hrs at ambient temperature. If pH does not drop to 4.6 or less within 36 hours, the batch has failed.
Water kefir	Critical limits for water kefir grain activation assessment are based on ability of kefir culture to ferment the substrate within a normal fermentation time period as demonstrated by a pH drop to 4.6 or lower (if the starting pH of the water kefir is >4.6), OR by monitoring grains are active, healthy and grain mass multiplies or by some other process measure.	CCP: Verify pH of less than 4.6 is achieved. Critical limit: culture will ferment within a validated time frame using a process measure at ambient temperature (see Box 3). CCP: Alcohol is within regulatory limit.

Although reduction of pH to 4.6 and lower is a CCP for milk and water kefir, starting pH of backslopped activated kefir grains is approximately 3.5, depending on ingredients added.

Outbreaks and Recalls

One Canadian recall for *Listeria monocytogenes* was linked to kefir (1%, 2.5%) and probiotic kefir (2.5%, 3.25%) and other fermented cheese products from one processing facility. One reported illness was associated with this recall but no details regarding the illness, including what product(s) from the facility were consumed that led to the illness, was available.[28] A search of the European commission rapid alert system[29] for food and feed identified two recalls. Notable in one recall, high alcohol content was detected in ginger water kefir. Other recalls were linked to failure to declare allergens (in Belgium), a physical hazard from a container defect in the cap (France) and *E. coli* in milk kefir (Australia). Table 2 below summarizes the findings.

Table 2 | Recalls related to fermented sausage and meat products in Canada and elsewhere

Date	Hazard Category	Hazard Detail	Number Recalls	Country	Product Description
2011[28]	Biological	*Listeria monocytogenes*	1	Canada	Dairy kefir of 1% & 2.5% and probiotic kefir 2.5% & 3.25%
2019[30]	Biological	*E. coli*	1	Australia	Milk kefir
2022[31]	Physical	Cap defect	1	France	Cap on container result in projectile risk
2023[32]	Allergen	Milk	1	Belgium	Milk not mentioned on label for pomegranate, banana or pistachio flavoured kefir
2023[29]	Chemical	High alcohol content	1	Netherlands	Water kefir (ginger curcuma)
2024[29]	Other	Labelling error	1	France	Milk kefir

A search of Center for Disease Control and Prevention (CDC) outbreak database (NORS Dashboard) did not identify any illnesses or outbreaks associated with this product.[33] However, one *E. coli* outbreak was identified from a search of published foodborne illness outbreaks associated with consumption of kefir in the Public Health Agency of Canada's Publicly Available International Foodborne Outbreak Database from 2000 to May 01, 2024. Additional google searches also identified two more records of illnesses associated with kefir consumption. Results are summarized in Table 3 below:

Table 3 | Illness and outbreaks associated with consumption of kefir

Date	Country	Pathogen	No. Ill (hospitalized)	Premises where outbreak occurred	Reason
2007[34]	Russia	Not available	203 (97)	Kindergartens	Diary kefir from a local dairy factory. It is suspected that cross-contamination from ill employees may be the cause of the outbreak.
2005[35]	Ukraine	"Dysentery bacteria", no specific pathogen was identified in the report	330 (37)	Kindergartens	Dairy kefir was implicated, no root causes were identified
2012[36]	Russia	*Escherichia coli*	37 (28)	School	A number of dairy products including kefir was implicated in this outbreak. No root causes identified.

Description of food preparation for animal milk kefir

Animal milk kefir made at home or by small scale producers occurs by inoculating animal milk with milk kefir grains. This is referred to as the "traditional method" and will be our focus here. Milk kefir made commercially, by large scale producers use milk kefir grains to initiate a series fermentation process, called the "Russian method", or use pure starter culture with defined kefir microflora strains.[2,4,18,36-39] Kefir fermentation must always use pasteurized and not raw milk as pathogenic bacteria may occur in kefir produced with raw milk.[40,41] This is a critical control point (CCP).

In the traditional method, pasteurized milk is warmed to 20-25°C before inoculation with activated milk kefir grains. Heat cold, pasteurized milk to inoculation temperature (i.e., to 20 to 25°C) within one hour, and within no more than two hours (this is a CCP). The inoculation weight to volume (w/v) ratio of milk kefir grains to milk ranges from 2% to 10%, with the average being 5%. Higher rates of grain inoculum increase lactic acid levels and provide faster decreases in pH.[21] This mixture is then incubated at 20-25°C for a period of 18 to 24 hours to allow fermentation to occur. Fermentation can occur in any acid resistant clean and vented or unsealed container to allow fermenting gases to escape. Cover the container loosely with a clean cloth to prevent cross-contamination such as pest intrusion.

Milk has a starting pH between 6.5 and 6.9. Fermentation is completed when the pH of milk kefir is verified to be at pH of 4.6 or less. After 18 to 24 hours of fermentation milk kefir pH is generally between 4.0-4.6, pH as low as 3.6 has been reported in cow's milk kefir made from 10% (w/v) kefir grains fermented for 36 hours. In this guidance we have defined the critical limit for pH drop as 36 hours, if milk kefir has not achieved a pH of <4.6 within that time, the batch is considered failed. Operators should monitor pH every 6 hours to ensure the CCP of pH <4.6 is achieved.

The next step is to filter the kefir milk through a sterile sieve to recover the grains. Sieves can be made from various materials, for example, metal, food-grade plastic, cloth, as long as they are sterilized before each use. For example, immerse sieves in boiling water for a minute to sterilize before use. Single use cloth sieves supplied in sterile pouches should be used only once and discarded. Hygienic food handling practices during the sieving process are important to prevent cross-contamination.

The recovered milk kefir grains can be reused immediately in new fermentation or at a later time if preserved in appropriate conditions as described earlier in the activated kefir grains section. Ensure proper hygiene practices and prevention of cross-contamination during this step.

Following filtration, the milk kefir should be cooled down within 4 hours and refrigerated at 4°C. At this point some recipes consider the kefir as complete and ready for consumption, other recipes require additional time for kefir maturation prior to consumption.

During fermentation, excess alcohol production is a concern primarily from yeast activity.[38] The type of animal milk used to make kefir may also lead to higher alcohol content. Milk from different animals have different chemical compositions and characteristics, for example, differing in protein, cholesterol, calcium and enzyme contents.[38] For example, buffalo milk has higher calcium/protein/tocopherol level, lower cholesterol content, and increased peroxidase activity compared to cow's milk. In this study, Gul et al., found as cold storage time increases, the ethanol content in buffalo milk kefir increases at a faster rate compared to cow's milk kefir, and that reusing kefir grains produced kefir with higher alcohol content over use of starter culture.[38] Levels of alcohol in kefir reached 7.0% ABV (buffalo milk kefir) and 4.2% ABV (cow milk kefir) made with kefir grains stored for 21 days. In another study with cow milk kefir, increases in alcohol content were observed with longer storage times (21 days) and higher temperatures (20°C room temperature storage over lower temperatures of 15°C) during fermentation and storage. However, the highest alcohol content in cow milk kefir in this study was 0.49% at 21 days at 20°C, compared to 0.31% at -10°C at 21 days.[42] Reports of elevated alcohol in milk kefir, as described above, are rare. Alcohol levels exceeding 1.1%ABV occur when fermentation times exceed 48 hours.[43] When long fermentation, prolonged storage times and temperature abuse is suspected, to verify control for alcohol, we recommend testing for alcohol content in milk kefir.

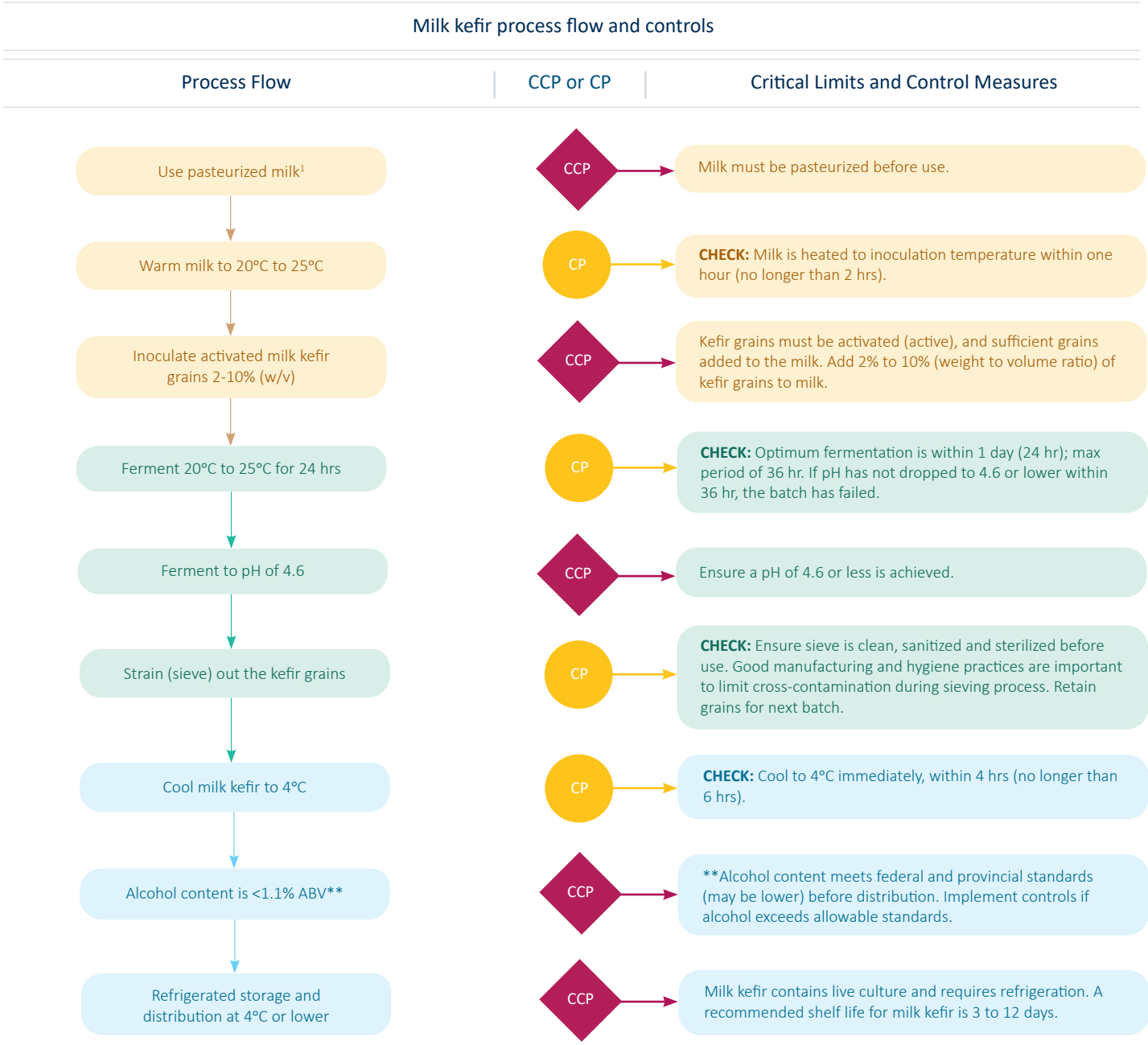
Store kefir at 4°C or lower. The recommended shelf life for traditional style milk kefir made with kefir grains under hygienic, good manufacturing practices is between 3-12 days. Commercial kefir milk production allows for storage for up to 3 weeks. Cold storage at 4°C for a longer period may be possible based on limited research showing the ability of milk kefir to maintain stable pH and organic acid content.[38,44-46] Operators are responsible for testing to establish a shelf life for their products, and to demonstrate that pH and alcohol levels are stable. Records of testing should be available for review by the inspector. More information about shelf life testing and alcohol issues can be found in Section 2 of fermented food guidance.

Box 2 | What is a validated recipe?

A validated recipe should come from a trusted authority. It is a recipe that has been evaluated, tested and then published by a trusted authority. The recipe should demonstrate good manufacturing practices, process steps that are reviewed and validated for safety, for example, process steps that limit and control microbial, physical, and chemical hazards, and provide clear guidance. In general, trusted authorities would include government sources, for example, United States university food extension sources, recognized organizations, and sometimes (not always) may include recipes and guidance from peer-reviewed literature or published books. A trusted source would not include, for example, online blog sites for recipes.

Another option is for the operator to validate their own recipes for use in their premises. This would involve documenting the recipe, starting with a list of the ingredients, quantities, and sources. The recipe should have an accompanying food safety plan that includes critical control points (CCPs), control points (CPs), critical limits, what needs to be monitored during the process (for e.g., pH, aw, temperature, time) and records of log sheets to document important information, such as pH changes over time, etc. The operator should retain this information for food safety health inspectors' review. When a recipe changes, when an ingredient changes, or when a new product is introduced, the operator should repeat the steps needed to validate or revalidate the recipe.

Animal milk kefir food flow chart | Process flow and controls



[1] - Operators with valid dairy licenses who are pasteurizing raw milk are advised to review process flow steps in the yogurt section.

Potential issues with animal milk kefir preparation

The main food safety concerns with animal milk kefir production include unintentional production of alcohol, and survival of pathogenic microorganisms introduced in ingredients or from cross-contamination.

Issue	Description
Alcohol production	Alcohol above federal regulated limit (1.1% ABV) may occur when: • There is temperature abuse during storage when milk kefir is stored above 4°C. • There is prolonged cold-storage of milk kefir before sale, for example, when shelf life exceeds two weeks. • When milk kefir is made with buffalo milk.[38] • Fermentation times greater than 48 hrs may lead to alcohol contents of >1.1% ABV.[43]
Biological contamination	Pasteurized milk must be used. While kefir fermentation inhibits growth of foodborne pathogens, the short fermentation time may not eliminate existing harmful bacteria, if present or introduced during the process. Hygiene and facility sanitation are important to prevent introduction of contaminants into the process. Kefir grains contain different mixes of microbial species depending on geography, animal source, etc. leading to differences in their ability to inhibit pathogens.[37,47-49]
Milk kefir grains activity	The ability of the bacteria and yeasts contained in milk kefir grains to produce lactic acid, acetic acid, bacteriocins, carbon dioxide (CO_2), hydrogen peroxide (H_2O_2), and ethanol during the fermentation process is essential to its intrinsic inhibitory power of food-borne pathogens.[18,50] Milk kefir grains must be preserved appropriately to retain their microbial activity if they are to be reused for milk kefir production at a later time. Milk kefir grains just recovered from fermented milk can be kept in fresh milk for up to 7 days or water for 3 days (at refrigeration temperature) when preserved wet. For longer term storage, milk kefir grains that are air-dried or freeze-dried and kept refrigerated or frozen can retain microbial activity for 12-18 months.[2,3,18-20] The microorganisms in air-dried, freeze-dried or frozen milk kefir grains are not in their exponential growth phase, so the milk kefir grains are considered inactive. Inactive kefir grains must be "activated" before use.[12,22]
Fermentation time and temperature	Milk kefir is commonly fermented for 18 to 24 hours at 20-25°C. Studies have suggested that longer fermentation time (e.g. 48 to 72 hours) may further reduce the pathogen load in the end product kefir. However, certain pathogens such as acid-resistant bacteria *E. coli* O157:H7 have been reported to survive prolonged fermentation time.[48,51,52]
pH and acidity	The pH of animal milk kefir is affected by various factors including size of kefir grains inoculation, length of fermentation process, and storage time. The milk kefir pH after 18-24 hours of fermentation is generally between 4.0-4.6, but pH as low as 3.6 has been reported in cow's milk kefir made from 10% (w/v) kefir grains fermented for 36 hours. It must be noted that the inhibitory effect on pathogens observed in kefir does not come only from the low pH per se but rather the array of fermentation end-products such as lactic acid, bioactive peptides, exopolysaccharides, antibiotics and bacteriocins.[6,39,47,51,53] Extended periods of fermentation may result in excess acid production. Kefirs with pH lower than 2.5 are not recommended.
Food handling hygiene practices	Proper hygiene practices must be practised during food production, handling and storage of kefir to reduce the likelihood of inadvertently introducing pathogenic microorganisms into kefir. Studies have shown that pathogens in kefir may survive up to 21 days in cold storage.[51,53]
Storage containers	Kefir should be stored in acid resistant food-grade containers.
Storage temperature	Once kefir production is deemed complete, it must be refrigerated at 4°C to maintain the shelf life. Increased alcohol concentration has been attributed to longer storage time and higher temperatures (15-20°C).[38,42]
Shelf life	The recommended shelf life for properly made and stored kefir using milk kefir grains is between 3 days to 3 weeks. Cold storage at 4°C for a longer period may be possible based on a limited number of research showing the ability of milk kefir to maintain stable pH and organic acid contents, but more studies are needed to confirm.[38,44-46]

Animal milk kefir food safety control points

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

- Use pasteurized milk. Ensure milk is heated to the inoculation temperature (20–25°C) within one hour and no more than two hours. This is a CCP.
- Use activated milk kefir grains. This is a CCP.
- Prevent cross-contamination, clean, sanitize food surface areas and equipment.
- Sanitize sieves before each use. Single use sieves should be used only once and discarded.
- Ensure fermented kefir has achieved a pH of 4.6 or less within 24 hours and within no longer than 36 hours. pH should not be lower than 2.5. This is a CCP.
- Cool kefir to 4°C immediately, or within 4 hours.
- Store kefir refrigerated at 4°C or less. This is a CCP. The shelf life of kefir is between 3 days to 3 weeks (21 days).
- Check for alcohol content of final product. If product contains alcohol, ensure it is not above regulatory limit. Label the product. This is a CCP.

Plant-based kefir beverages

Milk kefir grains have also been used to ferment plant-based milk substitutes such as soy beverage, rice beverage, walnut beverage and coconut milk to make plant-based kefir. Compared to animal milk kefir, there is very limited research on plant-based kefir. Plant-based kefir is prepared the same way as animal milk kefir with the pasteurization of plant-based milk substitute and other ingredients (e.g., sugars, glucose, fruits) prior to initiation of fermentation. This is a critical control step to eliminate pathogens present in the ingredients. Fermentation of plant-based and water-based kefir will proceed even though there is no fat (from milk) in the starting ingredients. Dried, freeze-dried, or frozen milk kefir grains must be activated before use in fermentation. Proper hygiene practices must be practised during the production, handling and storage of kefir, and during activation, separation (from plant-based fermented beverage) and storage of kefir grains. After fermentation, plant-based kefir must be stored at 4°C.

The optimal fermentation temperature and duration may vary with different plant-based beverage substitutes because of the different compositions (for e.g., different sugar, carbohydrate and protein types). From the few studies available on plant-based kefir, longer fermentation times are required for smaller kefir grains inoculation size (in terms of weight-to-volume) and when lower fermentation temperatures are used. Plant-based beverage substitutes such as soy beverage require the addition of sugar (sucrose) to encourage optimal growth of LAB and yeast during fermentation.[12,44,54]

There is very limited research on the best recipe for plant-based kefir:

- For soy beverage with added sugar, fermentation generally occurs for 16 to 24 hours at a temperature range of 20-25°C. When the kefir pH reaches 4.5-4.6, the fermentation is generally considered complete.[44,54]
- For walnut beverage kefir, one study suggests adding 8 g of sucrose per 100 mL of walnut beverage before fermenting with 3% (w/v) kefir grains at 30°C for 12 hr for optimal kefir product with a pH of 4.2.[55]
- For coconut kefir, fermentation is generally done without extra sugar addition for 12 to 24 hours at 20-28°C. When the coconut beverage has become gel-like the fermentation is completed. During fermentation the kefir grains can sink to the bottom of the jars; slight shaking of the jars a few times can enhance the fermentation. Final pH of the product is below 4.6.[12,56,57] After kefir grains are removed, the coconut kefir can also be stored at 20-28°C for another 6 to 12 hours for a second fermentation. This optional step enhances the taste and flavour of kefir. Water kefir grains can also be used for making coconut kefir.[12]
- For red rice beverage kefir, one study found 9% (v/v) kefir grains made with *L. bulgaricus* and *C. kefir*, and incubated at 43°C for 6 hours produced kefir with acceptable nutritional content, although alcohol was at 1.3% ABV.[58]

Description of food preparation for water kefir

Research on water kefir is limited in comparison to milk kefir. Although water kefir can be obtained commercially, and water kefir can be purchased from retail stores from a variety of companies, a reliable industrialized process for water kefir production has not been created. Unlike milk kefir, a defined strain of starter culture has not been developed for water kefir.[10,15]

A recent review (2024) of commercial kefir production suggested 5 to 10% (w/v) water kefir grains (15g water kefir grains per 100mL of water kefir) in 5 to 10% sucrose, incubated between 20 to 25°C for 24 to 72 hr, followed by storage at 4°C.[59] Water source was an important control point mentioned. As trace minerals are required by the kefir grains, use of reverse osmosis, carbon filtered, and distilled water was not recommended, as was use of tap water containing chlorine or fluorides, and equipment made from aluminum, iron or other metals as these may interfere with kefir grain microbial activity.[59,60] Of interest, figs are high in calcium, and reports that both figs and hard water (containing more calcium and magnesium ions) promotes water kefir grain growth in comparison to other fruits lower in calcium and soft water.[59,60] Barriers to industrialization of water kefir include unstable fermentation, and low recovery of water kefir grains, a requirement for the next kefir fermentation cycle.[59]

Traditional water kefir is prepared by first pasteurizing a staring mixture of water, sugar, and fruits. Fruits may be whole, cut, rehydrated from dried forms or juiced. Sugars are normally added (5 to 10%) to supplement the low amount of naturally occurring sugars in the fruits added. Dried figs are the most common fruit added, also dried raisins, plums, apricots, dates[14] and juices from fruits and vegetables, for example, apple, strawberries, tomatoes, melons, onions, carrots, fennel, grape, cocoa, plum etc., are also reported.[61] Sugar sources include table sugar (sucrose), brown or organic brown sugar, honey, molasses, including high-test molasses made from cane syrup, honey and grape molasses solutions, glucose and fructose.[10,59,61] Regardless of ingredients added, the mixture should be pasteurized at a time and temperature sufficient to obtain 5 log reduction of pathogens. A minimum heat treatment of 71.1°C for 36 seconds is required for acid-tolerant *L. monocytogenes*, and will also inactivate *Salmonella* and *E. coli*.[62] The mixture is cooled to room temperature before it is inoculated with water kefir grains. Cooling to inoculation temperature should occur within 2 hrs, and a critical limit of 6 hrs (per Canadian food code for cooling).[63] Use activated water kefir grains.

The mixture is fermented at 21-25°C for 48-96 hrs, under anaerobic conditions. Water kefir grains that have been recovered from a previous batch should have a baseline pH of approximately 3.5.[60] When added juices and sugars also have low pH, measuring the time for pH reduction to below 4.6 for safety reasons may not be useful, as the starting mixture is already at or below a pH of 4.6. However, pH reduction can still be a useful measurement of the fermentation activity, operators may also want to monitor other components such as water kefir wet grain mass or density (see Box 3 below).[60] Typically water kefir fermentations are conducted in sealed containers that are vented to allow gases to escape.[14] The fermentation can take up to 8 days, a pH of 4.6 or lower should be verified, regardless within 2 to 4 days (48-96 hrs).[10,15] Fermentation can also be performed aerobically. In the presence of oxygen, AAB proliferate resulting in the production of high acetic acid that may not be aesthetically desirable, although alcohol content will be lower.[14,26,61]

Box 3 | Quality evaluation of the water kefir process

Operators should follow the kefir activation process described earlier. If kefir grains are commercially purchased, operators should follow the activation process described in the Product Specification Sheet.

Operators wanting to establish consistent performance for water kefir production should be establishing baseline process performance for this fermentation. Metrics that could be measured:

- Grain mass growth
- Soluble solids (soluble sugar) or BRIX
- Titratable acidity, alongside pH
- Product quality attributes, such as acidity, flavour, carbonation

Operators should measure process performance characteristics to establish baseline by taking measures every 6 hours (for example) to ensure grain mass growth is successful, that BRIX content is decreasing, and acidity is acceptable.

(Continued on page 16)

Although these are not food safety measurements, a consistent process will ensure other food safety requirements, such as alcohol content below regulatory levels, and pH of <4.6 but not lower than pH 2.5, are consistently achievable.

For example, when optimized water kefir grain mass growth should increase by 75% over a 24 hour period. Optimization may require improving calcium concentrations, buffering capacity, lower grain mass inoculum to reduce acidic stress, and substituting sucrose for glucose.[14,16,17,26,60]

After the fermentation process is completed, remaining fruit solids are separated out using good manufacturing hygienic practices and the mixture is filtered through a sterile sieve to recover the water kefir grains. Grains can be reused immediately in a new fermentation or at a later time if preserved in appropriate conditions.[15] Secondary fermentation steps that add additional fruit or fruit juices, for example, apple, pineapple, lime, lemon, mango, cherries, or strawberries occur after grain removal and should be performed in the refrigerator for at least 24 hrs.[61] Water kefir beverages are stored at 4°C before consumption.[9,13-16,64] A few studies have reported a final pH of 3.3-3.6 for water kefir fermented at 21°C for 72 hours from a starting mixture of water, sugar and fruits (figs, apricots, or raisins).[13,14,16] Another study reported a final pH of 3.6-5.0 for kefir-like beverages made from vegetable juices fermented with 4% water kefir grains at 25°C for 48 hours.[23] Fermentation time may be shortened by a higher incubation temperature (e.g. 20-24 hrs at 25-30°C) based on limited research.[15,24]

The ingredients included in the starting mixture will affect the chemical compositions of the starting mixture, which in turn will affect the characteristics of the final water kefir beverages produced (i.e. pH, taste, aroma, alcohol content). Sugar is the primary substrate used by microorganisms in water kefir grains as an energy source for metabolic activity. Other nutrients (for e.g., amino acids, vitamins, and minerals) required for growth are provided by fruits. In one study, a water kefir beverage made from a starting mixture of only water and sugar was found to have very different characteristics (i.e. higher pH, lower alcohol, lower organic acid) compared to that made from water, sugar and fruits.[14] Low nutrient concentration, when no fruits are added to the starting mixture may cause the fermentation process to slow and a higher kefir pH. High sugar concentration in the starting mixture can also encourage pathogenic bacteria to compete with kefir grains microorganisms.[9,14] One study recommended use of up to 10% w/v sucrose addition of fresh or dried fruit or extracts for water kefir.[10] The significance of choosing the appropriate ingredients in the beginning is highlighted in two studies looking at pathogen growth in kefir beverages made from vegetable and fruit juices. *Enterobacteriaceae* and *Pseudomonas* were found in the beverages made from melon juice and prickly pear juice despite initial pasteurization and a final pH <4.5 (4.4 and 4.1 respectively). Both melon juice and prickly pear juice have a near-neutral pH (6.4 and 6.3 respectively), providing a suitable environment for pathogens to grow early on during the fermentation process before the fermentation products like lactic acids and acetic acids accumulate to sufficient level to inhibit further pathogenic growth.[23,64]

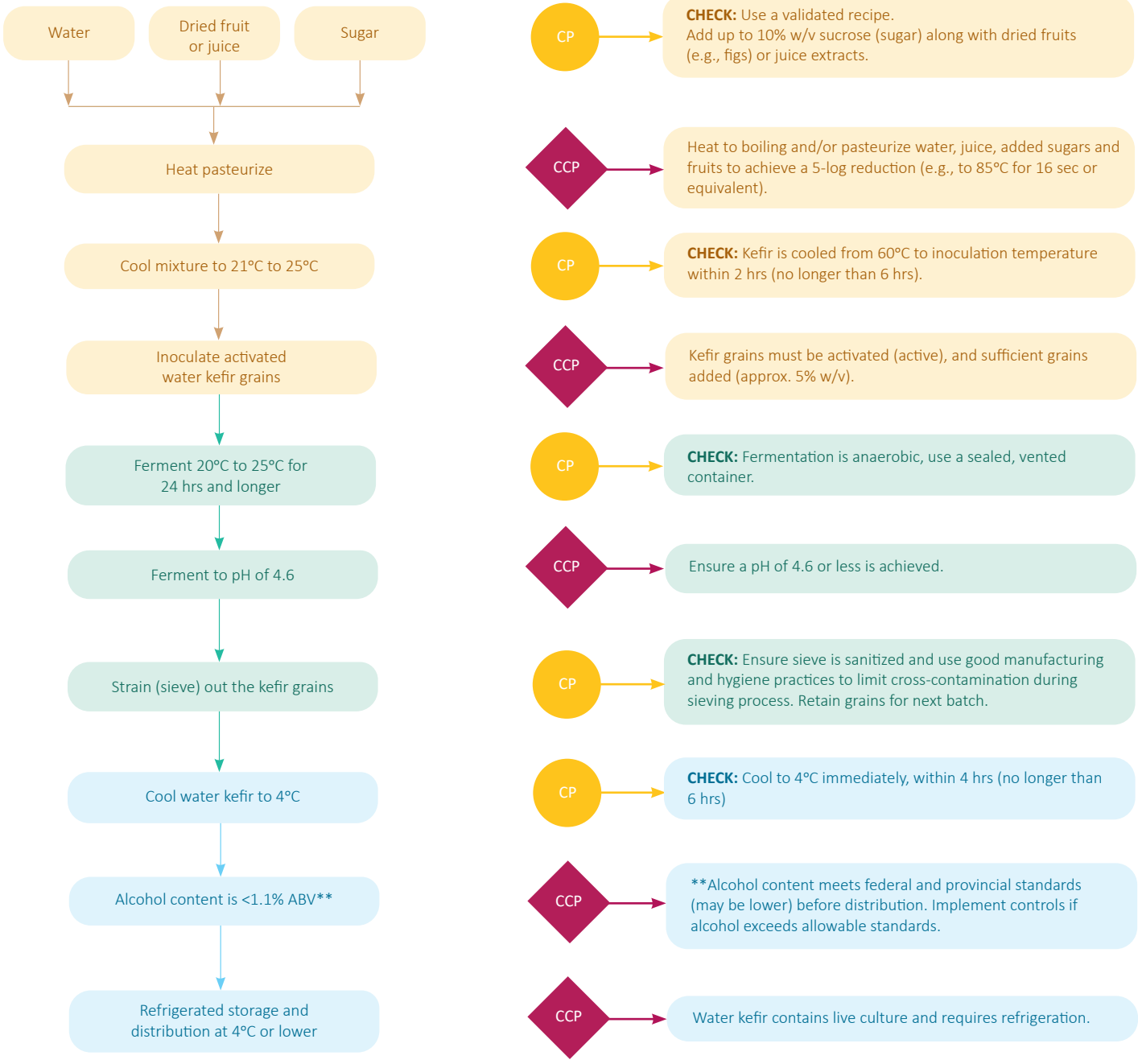
Novel kefir beverages fermented with water kefir grains are continuously being innovated, and include:

- "Tapache" or pineapple-based kefir. This popular beverage in Latin America countries is made from pineapple juice, brown sugar and cinnamon, and is fermented with water kefir grains;[15]
- "Kefir d'uva" or grape-based kefir. This is a popular beverage in southern Italy;[15]
- Ginger beer. This is a popular beverage in Eastern Africa and rural Greece, made from ginger fermented with water kefir grains;[15]
- Pumpkin-based kefir. A recipe of 22.28% (w/v) pumpkin puree and 9.07% (w/v) brown sugar in water fermented by 5% (w/v) water kefir grains at 32°C for 24 hours is suggested by one study.[24]

Alcohol can be elevated in water kefir. Of concern, water kefir made from vegetable or fruit juices may contain high levels of alcohol (≥1%). Following are examples of alcohol content in fruit and vegetable water kefirs. Kiwifruit water kefir: 1.0%; tomato water kefir: 1.5%; prickly pear water kefir: 2.3%; strawberry water kefir: 2.4%; melon water kefir: 2.6%; apple water kefir: 2.7%; carrot water kefir: 3.0%; grape water kefir: 4.4%; quince water kefir: 4.5% and 5% alcohol for pomegranate water kefir.[23,64] It is recommended to use a verified recipe (see Box 2) and check alcohol content of the final product meets federal and provincial requirements at bottling and through to the end of the shelf life of the product. Alcohol content should be declared on the label and include precautionary statements that the beverage may not be suitable for children or women during pregnancy.

Water kefir food flow chart | Process flow and controls

Water kefir process flow and controls



Potential issues with water kefir

Similar to milk kefir, unintentional alcohol production and survival of pathogenic microorganisms introduced in ingredients or from cross-contamination are the main food safety concerns with water kefir production.

Issue	Description
Alcohol content	Kefir beverages made from vegetable or fruit juices may contain high alcohol content.[23,64] Check for alcohol content in the final product is <1.1% ABV. To ensure compliance with federal or provincial regulations, the alcohol content through to the end of the shelf life should be measured and ideally the shelf life test should predict the evolution of the alcohol content so as not to exceed the permitted limit. Actual end-of-life measurements should be taken for each product. Precautionary statement labels on bottles are recommended to advise consumers "May contain alcohol. Not a suitable beverage for young children or during pregnancy." Higher volumes of water kefir grains can consume sucrose rapidly in the first 24 hours of fermentation, leading to higher alcohol contents.[61] However, secondary aerobic fermentations, enabling acetic acid bacteria conversion of alcohol to vinegar, or substitution of sucrose with glucose and fructose sugars can lower alcohol formations.[61]
Fermentation time and temperature	Traditional water kefir is commonly fermented for 48-96 hours at room temperatures between 21°C to 25°C. In fact, one study suggests that most microorganism metabolic activity occurred within the first 72 hours of fermentation at 21°C.[13] Fermentation time may be shortened by a higher incubation temperature (e.g. 20-24hrs at 25 to 30°C) based on limited research.[15,24]
Food handling and hygiene practices	Proper hygiene practices must be followed during food production, handling, and storage of kefir to reduce the likelihood of inadvertently introducing pathogenic microorganisms into kefir. For example, sieves must be sterilized after each use.
Kefir grains activity	Use activated kefir grains that have been preserved appropriately to retain their microbial activity. The ability of the bacteria and yeasts contained in kefir grains to produce various fermentation products such as lactic acid, acetic acid and CO_2, is essential to its intrinsic inhibitory power of food-borne pathogens.[9,13,18,50]
Insufficient pasteurization	Use pasteurized ingredients. The starting mixture should be heat-treated to eliminate pathogens (e.g., *E. coli* O157:H7, *Salmonella*, *Listeria*) before inoculation with water kefir grains. For example, heat treatment of 71.1°C for one minute or longer.[62] Water kefir grains from different origin contains a different mix of microbial species and will inhibit different pathogens to different extent.
Insufficient sugars (nutrients)	Low nutrient concentration (i.e., absence of fruits or added sugars) in the starting mixture can slow fermentation, resulting in lower metabolic activity of kefir grain culture, higher kefir pH and create potential for pathogenic growth.
pH and acidity	The pH of water kefir beverages is affected by various factors including types of starting mixture, size of kefir grains inoculation, length of fermentation process, and storage time. A few studies reported a final pH of 3.3-3.6 for water kefir fermented at 21°C for 72 hours from a mixture of water, sugar and fruits (fig, apricot, or raisins).[13,14,16] If the pH of the starting mixture is too high, pathogens may still be able to grow early on during the fermentation process before the fermentation products like lactic acids and acetic acids accumulate to sufficient level to inhibit further pathogenic growth, such as in the case of kefir-like beverages made from melon juice and prickly pear juice.[23,64] Reduce pH by adding citric acid (lemon slices) or equivalent to lower pH. Use a verified recipe. Extended periods of fermentation may result in excess acid production. Fermenting to a pH of 4.6 or lower is a CCP, however, acidity should not be lower than pH 2.5. Note: extended periods of fermentation may result in excess acid production. Kefirs with pH lower than 2.5 are not recommended.
Storage containers – excess pressure	Kefir should be stored in acid resistant food-grade containers. Excess pressure can build-up from carbon dioxide, causing fluids to leak out. Precautionary statements on bottles are recommended to advise consumers "Do not shake." and "Contents under pressure."
Storage temperature	Once kefir production is deemed complete, it must be refrigerated at 4°C. This is a CCP.
Shelf life	No information on the shelf life of kefir made with water kefir grains can be found.

Water kefir food safety control points

- Use a verified recipe.
- Pasteurize the starting mixture to achieve a 5-log reduction, for example, 71.1°C for 60 sec or equivalent.[62] This is a CCP.
- Use activated grains. This is a CCP.
- Cool the mixture from 60°C to 20°C within 2 hrs, and no longer than 6 hrs before water kefir grains are inoculated.
- Prevent cross contamination, clean, sanitize food surface areas and equipment.
- Sanitize sieves before each use. Single use sieves should be used only once and discarded.
- Ensure the pH of final product is 4.6 or less, but not lower than pH of 2.5. This is a CCP.
- Store kefir refrigerated at 4°C or less. This is a CCP.
- Alcohol content of kefir-like beverages made from vegetable or fruit juices may not be minimal, for example, 5% ethanol in pomegranate-juice-based kefir beverages. Check the alcohol content of final product. This is a CCP.
- Alcohol control option to prevent over-production of alcohol and carbon dioxide during fermentation (the 4 D's) include:
    - Diluting the batch, or
    - Delay and continue fermenting to convert alcohol to acetic acid (ferment in open container, aerobically to promote acetic acid bacteria), or
    - Divert the batch to alcoholic sales (licensing required), or
    - Discarding the batch.

Further information on alcohol control may be found in Section 2 – Starter cultures and fermented food standards.

Alcohol: a potential health issue with kefir

Because dairy and water kefir are sold as non-alcoholic, unintended alcohol content can be a concern for some population sub-groups, especially for children and pregnant individuals. Further information about concerns of alcohols in fermented foods is discussed below.

Box 4 | 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.[65] 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.[66]

Health Canada acknowledges ethanol in non-alcoholic fermented beverages as a concern in kombucha, kefir, and some soft drinks including ginger beer.[67] 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 kefir 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.[68] 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.

(Continued on page 20)

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). Operators are further recommended to add precautionary labels and information about alcohol content in their products.

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Figure 2: https://en.wikipedia.org/wiki/Tibicos#/media/File:Wasserkefir-Kristalle.jpg

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