Safety of Fermented Foods

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

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





























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http://www.bccdc.ca/resource-gallery/Documents/Educational%20Materials/EH/FPS/Food/Fermented/Fermented%20Foods%20Guideline%20-%203.11%20Kombucha%20and%20Jun.pdf

Additional fermented food guidance can be accessed at:

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

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Contents

Section 3 Fermented foods food safety reviews				
3.11 Kombucha and Jun	5			
Overview	5			
Background	6			
Outbreaks and Recalls	8			
Description of food preparation for kombucha	9			
Description of food preparation for jun	10			
Kombucha food flow chart	11			
Potential issues with jun and kombucha food preparation	12			
Jun and kombucha food safety control points	14			
Potential health issues with kombucha and jun	15			
References	17			
List of Tables				
Table 1 Recalls related to Kombucha and Jun beverages	8			
Table 2 Illness case reports related to Kombucha (and Jun) products	9			
List of Figures				
Figure 1 Fermented foods reviewed in this guideline described by fermentation agent and complexity	4			
Figure 2 Kombucha starter SCOBY	7			
Figure 3 Metabolic pathway for kombucha	7			
Figure 4 Yeast and SCOBY growth in commercial kombucha	13			
List of Boxes				
Box 1 How to use the information in this food safety review	4			
Box 2 Description of fermentative organisms present in kombucha SCOBY	7			
Box 3 Additional resources for alcohol control for operators and inspectors	15			
Box 4 Alcohols in fermented foods	15			

Section 3 | Food safety reviews of fermented foods

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

Each fermented food review includes:

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

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

Figure 1 | Fermented foods described by fermentation agent and complexity

Complexity	Foods Fermenting Agent		Section
high	Sausage	Added LAB ¹ , wild moulds & yeasts	3.13
†	Kefir, Kombucha SCOBY² based: <i>Acetobacter</i> , yeast & mould		3.11-3.12
	Koji, Miso	Aspergillus, wild or added yeast & LAB	3.10
	Tempeh	Rhizopus	3.9
	Natto	Bacillus	3.8
	Yogurt, Plant-based cheese	Added LAB	3.6-3.7
	Dosa, Idli, Fesikh	Wild LAB and Yeast	3.4-3.5
low	Vegetables, Sauerkraut, Kimchi	chi Wild or added LAB 3.	

 $^{^{\}rm 1-} {\rm LAB}\text{-lactic}$ acid bacteria; $^{\rm 2-} {\rm SCOBY}\text{-symbiotic}$ 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.

3.11 | Kombucha and Jun

Author: Lorraine McIntyre and Kelsie Dale

Overview

Description



Jun (photo left) is a fermented beverage made of green tea and honey. Kombucha (photo right) is a fermented beverage made of black tea and sugar. Tea is steeped in boiling water, then sweeteners are added (honey for jun; sugar for kombucha). After the tea is cooled, starter culture is added. Teas ferment for 4-7 days (jun) or 7-10 days or longer (kombucha).

Starter culture

- The culture is referred to as a SCOBY (symbiotic culture of bacteria and yeasts).
- Commercial starter cultures are available.
- The most common practice is to use a portion of a previous batch of mother culture liquid and/or a portion of the SCOBY (i.e. backslopping portion).
- Starter culture microbes include yeasts that convert sugars to alcohol and carbon dioxide, as well as bacterial communities of lactic and acetic acid bacteria (LABs and AABs) that convert alcohol and sugars to acetic acids (vinegar).
- The SCOBY will form a mat (pellicle) on top of the fermentation vessel.

Key features

- Kombucha and Jun are acidic, fizzy beverages, and may contain caffeine if a caffeinated tea was used in production.
- Kombucha and Jun are live, raw products that are served cold from refrigerated sale and storage. If the teas are pasteurized or processed to inactivate yeasts they may be packaged for room temperature storage in shelf-stable containers.
- Fermentation is slowed when product stored in fridge.
- Teas are typically sold as live cultures in bottles, cans or kegs.
- Fermentation usually occurs at ambient temperatures, affecting length of ferment, from 1 to several weeks.
- Vessels should be uncovered (not closed) during fermentation as AABs are aerobic and require oxygen for conversion of alcohol to vinegar.
- Sealing in containers often creates carbonation (fizziness) until carbon dioxide slows fermentation by creating anaerobic conditions.

Hazards of concern

- 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 level is of concern. Federal regulations require 1.1% or less alcohol in non-alcoholic beverages, provincial requirements vary.
- Excess acids during prolonged fermentation, when pH is 2.5 or lower, can cause illness in susceptible individuals. This very low pH and daily consumption of teas have led to metabolic acidosis concerns in vulnerable populations, although rare.
- Pathogens on raw materials (e.g., dried teas, fruit juices) include *E. coli, Salmonella, Listeria* and *Clostridium* spp. are considered hazards. However, these bacterial pathogens and spores are unlikely to survive in the high acid content of kombucha and jun, once a low enough pH (pH of 4.6 or less) is achieved during fermentation.
- Spoilage yeasts and moulds can grow on the surface of the SCOBY culture.
- Excess formation of gas in bottles and cans may cause containers to rupture under added pressure.
- Mould growth may affect performance of SCOBY.

Important control points

- Fermentation vessels should be open to allow aerobic microbial activity. The vessel should be covered with a tightly woven cloth or housed in a room or building with environmental controls adequate to prevent pests (e.g., flies) or dust from entering the vessel.
- This is a two-stage fermentation process. First, alcohol is produced by yeast converting sugars to alcohol under controlled conditions. Second, bacteria convert most of this alcohol into acetic, gluconic, glucuronic and other organic acids. Sugars and flavourings with added sugar added after this second step may cause formation of excess alcohol if fermentation persists.
- Control yeast and added sugar to prevent excess alcohol formation, particularly after packaging, to control anaerobic alcohol formation. Control options include:
 - o Killing yeast by pasteurizing (rarely done), or cold shock using strains vulnerable to cold
 - o Addition of preservatives: 0.1% sodium benzoate and 0.1% potassium sorbate
 - o Diluting kombucha with potable water prior to bottling to reduce alcohol content
 - o Refrigeration
- Labelling should include alcohol content on retail containers.
- Kombucha and jun containing live cultures **must be refrigerated** to prevent excess alcohol and gas formation.
- Fermentation must be long enough to lower alcohol, but not too long to avoid extreme low pH and acid build-up. Recommended pH is between 2.5 and 4.2.

Background

This review is focused on non-alcoholic kombucha and jun beverages. Hard kombucha or jun describes fermented alcoholic teas with halted fermentations that intentionally contain alcohol, and/or added alcohol beyond the scope of this review. Kombucha is a ferment of sweetened, steeped tea with a mixture of culture that includes yeasts, acetic acid bacteria and many other microbes. Generally, kombucha is made with one of the varieties of tea from the plant *Camellia sinensis*, white, green, black, or oolong.¹ Herbal teas are not from the *Camellia* family, and include rooibos teas, mate teas and herbal infusions. Not all herbal teas are suitable for kombucha because oils in some herbal varieties may interfere with the culture and fermentation. To make kombucha, tea is steeped in boiled water, and sugar is added and dissolved into the hot water. Once the mixture is cooled to room temperature, a starter culture is added. The tea is fermented in aerobic conditions for approximately 7 to 10 days or longer at ambient temperatures.

The origins of kombucha trace back to northeast China (Manchuria) in 220 B.C, and later Japan and Russia.² Kombucha fermentations are characterized by a white mat of culture that floats on top of the fermentation. This mat of culture, also called a pellicle and shown in Figure 2, is sometimes referred to as the Manchurian mushroom, the mother, or a Symbiotic Culture of Bacteria and Yeast (SCOBY). To make a new batch of kombucha, the mother culture liquid and/or a portion of the SCOBY is transferred to a fresh batch of sweetened tea and this pellicle will regrow across the top of the fermenting liquid, i.e., backslopping is normal practice for this fermentation.

Figure 2 | Kombucha starter SCOBY

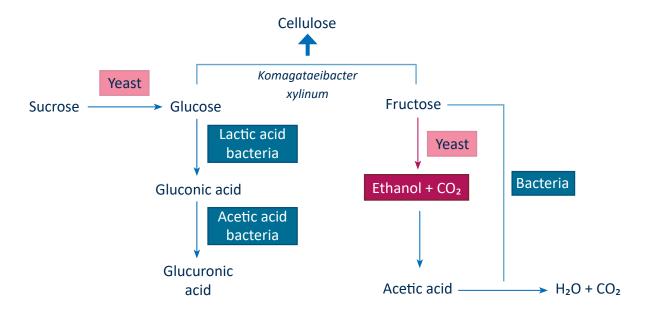


Box 2 describes major bacteria and yeasts during the fermentation process, and Figure 3 (adapted from Villarreal-Soto et al, 2018) describes the metabolic pathway.³

Box 2 | Description of fermentative organisms present in kombucha SCOBY

Many varieties of yeast and bacteria can be present in kombucha. Of interest, varieties can be geographically distinct, changing depending on where in the world the kombucha is fermented. Predominant North American species from commercial kombucha brands are bolded in the following list, and include acetic acid bacteria (*Komogataeibacter*, *Gluconobacter*, *Acetobacter*), lactic acid bacteria (*Lactobacillus*, *Lactococcus*), and yeasts (*Brettanomyces*, *Kloeckera*, *Lachancea*, *Saccharomyces*, *Schizosaccharomyces*, *Starmerella*, *Rhodotorula*, *Torulaspora*, *Zygosaccaromyces*)³⁻⁵.

Figure 3 | Metabolic pathway for kombucha



Jun is often referred to as the sister beverage of kombucha with the primary difference being the ingredients. It is also called "honey kombucha" or "jun kombucha". Kombucha is typically made with black tea and refined sugar, whereas Jun is made with green tea and raw honey. There is debate over whether the Jun starter culture is unique from a kombucha starter culture in terms of specific yeast and bacterial strains. However, the components of a jun and kombucha culture are both a symbiotic mix of bacteria and yeasts.^{6,7}The fermentation process is the same as well. Commercial "pure" jun starter cultures are available.⁶

Outbreaks and Recalls

There were no recalls or foodborne illnesses reported for jun, however, as an emergent food beverage similar to kombucha, it is reasonable that the following issues may be shared.

Kombucha has been recalled in the U.S. and Australia for excess alcohol, extreme pressure, mycrocystin contamination of the tea, and physical issues with glass fragments. No recalls were found for products in Canada, although brands noted in U.S. recalls (not shown in Table 1) are commonly sold in Canada. A fermented ginger beer product, also made in the U.S., was recalled in Canada in 2015 for bursting bottles presumed due to extreme pressure.⁸

Table 1 | Recalls related to Kombucha and Jun beverages

Year(s)	Hazard Category	Hazard Detail	Number Recalls	Country (s)	Product Description
2020, 2010 ^{9,10}	Chemical	Excess alcohol >1.15% ABV (Aus.) >0.5% ABV (U.S.)	1 2+	Australia U.S.	Various flavours kombucha
2019 ^{11,12}	Physical	Extreme pressure	2	Australia	Kombucha
202113	Microbiological	Elevated yeast	1	U.S.	Kombucha
202013	Chemical	Mycrocystin	1	U.S.	Kombucha
2019 ¹³	Other	Process deviation, temperature abuse at wholesale	1 (>50 retailers)	U.S.	Kombucha (and other products)
2018 ¹³	Physical	Glass	1	U.S.	Kombucha

There has been only one outbreak related to kombucha SCOBY being applied to skin¹⁴, but no outbreaks involving 3 or more individuals related to ingestion of kombucha or jun products. However, >10 individual case reports have been reported over the last few decades in the literature, primarily in the U.S. All cases were seen in hospitals or medical clinics and the majority of cases also had underlying medical issues that likely contributed to illness. Individual cases shown in Table 2 are grouped by symptom presentation (reason). Excess acids in kombucha were inferred to contribute to metabolic and lactic acidosis (in 2 cases), hepatitis (or hepatoxicity) and jaundice through injury to the liver (in 2 cases) or to renal function (in 1 case), other gastrointestinal and related symptoms (in 2 cases), imbalance in sodium or niacin levels (in 2 cases) and muscle weakness (in 1 case). Notably, in one case, excess caffeine was measured at 3.8mg/L confirmed with caffeine detection by gas chromatography in the SCOBY mat.¹⁵ In Australia, lead poisoning occurred in two cases following home preparation of kombucha in ceramic containers and tea consumption over a period of six months. It was hypothesized that the lead leached out of the glaze due to the highly acidic tea environment.¹⁶ Food grade containers resistant to leaching properties in high acid environments must be used when making kombucha and jun.

Table 2 | Illness case reports related to Kombucha (and Jun) products

Date	Country	Pathogen/agent causing illness	No. III (no. hospitalized)	Premises	Reason
1995 ^{17,18}	U.S.	NR	2(2) and one death	Home	Metabolic acidosis, respiratory distress
2016, 2014, 2009, 1997 ^{15,19-21}	U.S. and Serbia	NR	4(4)	Home	Renal failure(2), lactic acidosis (n=2), hepatitis (n=1), jaundice (n=1), and hyperthermia (n=1)
1997 ¹⁵	U.S.	NR	2(2)	Home	Respiratory distress, shaking, agitation
2017, 2004, 2007 ²²	U.S., U.S., New Zealand	NR	3(3)	Home	Low sodium (hyponatremia, n=1), muscle weakness (myositis, n=1), niacin imbalance (pellagra, n=1)
1997 ¹⁵	U.S.	Caffeine	1(1)	Home	Nausea, vomiting, headache, neck pain
1998 ¹⁶	Australia	Lead poisoning	2(2)	Home	Brewed in ceramic pot
1996 ¹⁴	Iran	Bacillus anthracis (anthrax)	>20	Community	SCOBY applied externally to skin resulted in anthrax lesions

NR-not relevant. In all cases consumption of kombucha tea linked to illness.

Description of food preparation for kombucha

Preparations of kombucha can be small or scaled up to much larger volumes. Tea leaves are steeped in boiled water 4-5 g/ $L^{2,23}$ for at least five minutes or longer before they are removed by a filtration step. Sugar is added at 50 to 150 g/ $L^{2,23}$ to a concentration of 5 to 15% 23 . This mixture is sometimes referred to as the wort, similar to the brewing industry. When the brew (or wort) has cooled to ambient temperature, previous SCOBY culture is added at 100 to 200 mL/ $L^{2,23}$. The brew should be cooled down from 60°C to 20°C within 2 hrs, and no longer than 6 hrs before the culture SCOBY is added. Liquid SCOBY is usually added, with or without a portion of the pellicle cellulose mat. If a portion of the mat is not added, it will regrow over the top surface area of the container. The addition of backslopped culture acidifies the brew, inhibiting growth of pathogenic microorganisms. The vessel container must allow for aerobic conditions. In small scale and home brewing, food grade 'cheese cloth' (a loosely weaved cotton fabric) covers the vessel to protect from contamination, in large scale operations fermentation tanks may be left open if the room and facility are protected from dust, insects, etc. otherwise the fermentation vessel should be covered with clean porous cloth.

The fermentation usually proceeds for 7 to 21 days (typically 10 days). Longer fermentations increase acidity. The recommended pH of kombucha is below 4.2, to control for spores of *B. cereus* or *C. perfringens* that may be present on tea leaves and not have been destroyed during steeping of the tea, but not less than 2.5.²³ The floating SCOBY pellicle cellulose mat is removed once the fermentation is completed, and kombucha is filtered to remove macroscopic SCOBY culture from the brew. Kombucha may be dispensed into individual glass or aluminum cans, or stored in kegs for bulk sales to food premises. Once bottled, natural carbonation will occur until the formation of carbon dioxide halts the fermentative process. Kombucha is intentionally marketed as a live, probiotic culture, and pasteurization of the tea prior to bottling is not usual practice. However, even after filtration, as the culture is live, there is a possibility for regrowth of SCOBY after bottling. When opening bottles of kombucha, contents may be under pressure from build-up of carbon dioxide, and macroscopic culture can be visible in the beverage.

Variations of the preparation of kombucha include adding a concentrated mixture of steeped tea to filtered water when it is difficult to boil large volumes of water in the vessel; adding a portion of flavoured juice instead of water; adding flavouring agents and juices in a secondary fermentation step, following the first fermentation; and addition of bottling aids, such as carbonation.

Processors have developed several methods to limit over-production of acid and alcohol in finished kombucha. As long as the processor has acid (2.5>pH≤4.2) and alcohol (≤1.1% ABV or lower depending on provincial requirements) under control within their process, then the method is acceptable. All kombucha products containing residual fermentable sugar must be stored refrigerated before sale to the consumer. A survey of kombucha teas in BC found over 30% of samples collected were not compliant in managing alcohol content. Regulations in the province of BC specify non-alcoholic beverages must not contain over 1% alcohol by volume (ABV)²⁴, and over 70% of local BC processors failed to control alcohol levels in kombucha tea.²⁵ There are some shelf-stable kombucha products on the market that are sweetened with sugar alcohols, alternatives or artificial sweetners (non-fermentable sugars).

Description of food preparation for jun

Preparations of jun is nearly identical to that of kombucha. Green tea leaves are steeped in boiled water (2 teaspoons in 2 L) for 3-5 minutes before they are removed by a filtration step. The mixture is cooled to room temperature (20-22°C). Honey is added (250 mL into the 2 L solution) and stirred until completely dissolved. Mixture is then transferred into a ½ gallon container and the starter culture. SCOBY or blackslop is added. To allow for aerobic conditions, the vessel container must be covered with food grade 'cheese cloth' (a loosely weaved cotton fabric) or similar clean porous cloth to protect from contamination while permitting air flow.⁷

Jun ferments faster than kombucha. The primary fermentation usually proceeds for 4 to 7 days at 21-26 °C. Jun also tends to be less acidic with the acidity of the final ferment should be between 2.5-3.5, with maximum pH of jun at 4.2, to control for spores of *B. cereus* or *C. perfringens* that may be present on tea leaves and not have been destroyed during steeping of the tea.²³

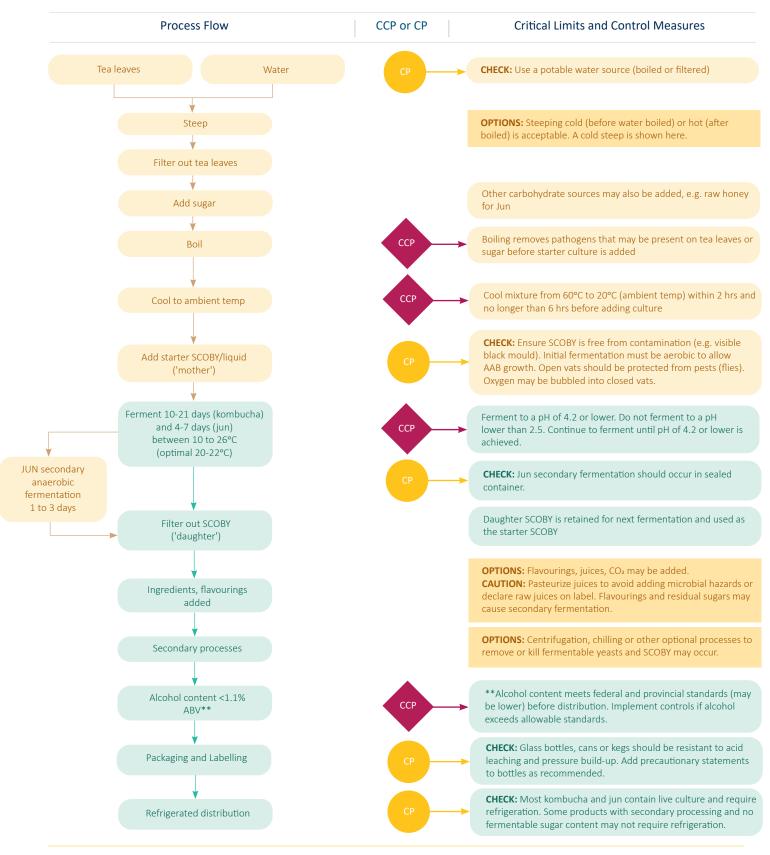
For the second fermentation, the Jun is decanted into airtight bottles. Fruit or juice can be added at this step. This is left to sit for 1 to 3 days before refrigerating. Jun tends to be more carbonated than kombucha so extra caution is needed when bottling and opening. A prolonged second fermentation period when temperatures are warm should be avoided.

Flavouring agents in kombucha and jun. Flavouring agents should be added after initial fermentation, when the pH has dropped below 4.2. If juice is added for flavouring after the fermentation step, a pasteurized, commercially produced juice is recommended. If unpasteurized juice is used it should be processed in accordance with applicable sections of the "Code of Practice for the Production and Distribution of Unpasteurized Apple and Other Fruit Juice/Cider in Canada". Use of unpasteurized juice must be declared on the label in accordance with federal labelling requirements. Other products added to change flavour (herbs, fruit, etc.) must be processed and handled in a manner that prevents contamination of the kombucha or jun, including washing and refrigeration where appropriate. After juice or other flavouring agents have been added, the pH must again be tested to ensure the pH of the finished product is less than 4.2 but above 2.5.²³

The floating SCOBY pellicle cellulose mat is removed once the fermentation is completed, and Jun is filtered to remove macroscopic SCOBY culture from the brew. Jun may be dispensed into individual glass or aluminum cans, or stored in kegs for bulk sales to food premises, and then refrigerated. Even after filtration, as the culture is live, there is a possibility for regrowth of SCOBY after bottling. When opening bottles of jun, contents may be under pressure from build-up of carbon dioxide, and macroscopic culture can be visible in the beverage.

The alcohol content of jun is generally higher than kombucha.⁷ This may be due to the differences in the sugars used to culture the two beverages. Whereas cane sugar contains 50% glucose and 50% fructose, honey only contains 30% glucose, less than 40% fructose, and about 20% of a mix of other sugars. This difference, in combination with the yeasts used in the ferments, produces a higher alcohol content. Processors must have a method in place to limit over-production of acid and alcohol in finished jun. As long as the processor has acid and alcohol levels under control within their process, then the method is acceptable.

Kombucha food flow chart | Process flow and controls



Potential issues with jun and kombucha food preparation

Note: the majority of potential issues are the same, but there are some differences between the two beverages noted in the table below.

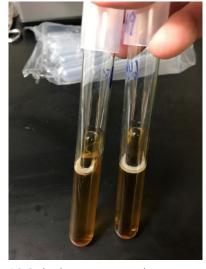
Issue	Description
Ingredients – tea and water	Steeped tea made from any variety of <i>Camellia sinensis</i> (black, green, white or oolong) is acceptable. Herbal teas and infusions may not be acceptable if they contain compounds that interfere with natural fermentative bacteria, if they elaborate toxins or have unknown toxicity. For example, Chaga is a tea derived from a cinder conk fungus that grows on birch trees and is considered a fungal infusion. Because it is a wild 'mushroom', because it has some toxicity, and because it must be treated as a novel food, it is not a suitable substrate for jun or kombucha. ²⁶ It is the processors responsibility to provide evidence that ingredients are acceptable for fermentation.
	Water must be potable and either boiled or filtered before use in the wort.
Ingredients – initial starter culture (SCOBY)	Commercial kombucha and jun starter cultures may be used for initial batches.
Ingredients – SCOBY	Portions of SCOBY from every new batch are stored to inoculate the successive batch. Due to the highly acidic nature of the starter culture, microbial testing of the starter culture is not useful. Sanitary containers and sanitary handling of the SCOBY mother culture are necessary to prevent cross-contamination with undesirable spoilage and pathogenic microbes that may interfere with the successive fermentation. SCOBY pellicle mats may also be retained as inoculum. Liquid culture or mats that change colour, for e.g., grow black or irregular coloured mould or appear abnormal in appearance to the white cream pellicle should be discarded. Additional indications of poor SCOBY pellicle function when finished product fails to each pH 4.2, ²³ and when pellicles sink to bottom of fermentation vessel and do not rise again. Processors should be monitoring fermentation activity to ensure it is normal, if it is not, it may indicate an issue with the SCOBY.
Ingredients – added raw honey (jun), sugar (kombucha), flavouring agents, and yeast strains	Raw honey can contain botulism spores. The acidity (pH<4.6) of the jun and fermentation process should prevent spores from fruiting. Addition of flavouring agents with added sugar following the primary fermentation may provide nutrients for yeast production of alcohol. If the fermentation is halted before conversion to acetic acid, excess alcohol may form after bottling. Before addition of added flavours, processors should evaluate impact on alcohol production. Controlling yeast activity is an option. Processors, for example, can choose strains that are not cold-tolerant, and destroy yeasts by chilling the kombucha or jun before adding secondary flavouring agents. Added juices should be pasteurized, raw juices should be declared on the label.
Fermentation environment	Fermentations should be in aerobic environments to allow the multi-step fermentation towards acetic acid: • Kombucha for 7 to 21 days • Jun for 4 to 7 days The pH of jun and kombucha is recommended to be below 4.2 and not lower than 2.5. Containers should be protected from contamination of pests and soils. Kombucha containers must be uncovered for aerobic fermentation. Jun is different. Primary fermentation occurs in aerobic environment, and secondary fermentation occurs in the airtight containers or bottles (anaerobic environment) and lasts 1 to 3 days at ambient temperatures. Once fermentation is completed, kombucha and jun should be bottled and refrigerated to 4°C or
	lower to stop further fermentation (anaerobic conditions will halt fermentation).
	Raw kombucha and jun with residual sugars need to be refrigerated. They should be labelled with a "keep refrigerated" statement to control for temperature abuse conditions that may cause excess alcohol to form inside the packaged product.

Acid formation	Extended periods of fermentation may result in excess acid production. Kombucha and jun with pH lower than 2.5 is not recommended, although reports of acidosis in kombucha consumers remains rare. All packaging materials must be acid resistant. The very low pH of these beverages will cause leaching in non-food grade containers.
Alcohol formation	Processors must control alcohol levels in kombucha and Jun products to meet provincial and federal limits. In Canada, alcohol levels should be less than 1.1% ABV and it may be lower in other provincial jurisdictions. 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."
Excess pressure in bottle and containers	The build-up of excess carbon dioxide can occur. Opening jun and kombucha bottles can result in fluids leaking out. Some jun and kombucha products can explode if not refrigerated. Jun tends to be more carbonated than kombucha. Precautionary statement labels on bottles are recommended to advise consumers "Do not shake. Contents under pressure."

Figure 4 | Yeast and SCOBY growth in commercial kombucha



4-A. Left tube: no growth; Centre and right tube: gas formation



4-B. Both tubes: no yeast growth or gas formation; SCOBY ring

In Figure 4, commercially purchased kombucha was inoculated into yeast growth media to determine yeast activity. Growth of yeast in the tubes is visualized by gas formation in the inner tube, which pops up, and cloudiness in the growth media. In the other tubes in 4-A, yeast activity was demonstrated, showing gas formation and active SCOBY culture. Some commercial strains demonstrated no yeast growth or SCOBY growth (4-A: left tube), while other commercial strains in 4-B showed yeast had been inactivated, but SCOBY culture was still active as shown by the ring of white in these tubes.

In commercial kombucha, positive yeast activity is an indication of two possible hazards: gas production and higher alcohol content. Gas may form inside containers at non-refrigerated temperatures, exploding cans or bottles. Active yeast may cause alcohol in the presence of added sugars and the right environmental conditions. Operators are expected to control hazards in their products. Raw kombucha and jun containing residual sugars need to be refrigerated. They should be labelled with a "keep refrigerated" statement to control for temperature abuse conditions that may cause excess alcohol to form inside the packaged product. Alcohol content in the finished beverage should be declared and meet all provincial and federal requirements.

Jun and kombucha food safety control points

- To limit microbial contaminants, tea, honey and any additional ingredients must be prepared using potable water in an environment that prevents cross-contamination.
- Containers and bottles used for primary and secondary fermentations should be clean and sterilized.
- Containers must be food grade and non-reactive due to high acid and low pH environment of fermented teas.
- Optimal temperatures for fermentation are between 20°C to 22°C with an upper limit of 26°C and lower limit of 10°C.
- Jun fermentation control points:
 - o Initial fermentation lasts 4 to 7 days, with acidity becoming evident on day 4.
 - o Secondary fermentation occurs in anaerobic environment and lasts 1 to 3 days at ambient temperature (20°C to 26°C) before refrigeration.
 - o Jun is considered finished when total acidity has pH between 2.5 and 4.0.
- Kombucha fermentation control points:
 - o Fermentation last 10 to 21 days
 - \circ Kombucha is considered finished when total acidity reaches 4 to 5 g/L³ and pH<4.2
- Alcohol control options to prevent over-production of alcohol and carbon dioxide during fermentation ('the 4 D's) include:
 - 1. Diluting the batch, or
 - 2. Delay and continue fermenting to convert alcohols to acetic acid, or
 - 3. Divert the batch to alcoholic sales (licensing required), or
 - 4. Discarding the batch.
- Alcohol control options to control yeast (i.e. to prevent over-production of alcohol and carbon dioxide after bottling) include:
 - 1. Pasteurizing the product or destroying the yeast population (cold-shock, if cold susceptible yeast populations are present in the SCOBY), or
 - 2. Using centrifugation or other technology to remove yeasts, or
 - 3. Choosing yeast populations that do not grow at refrigeration temperatures, or
 - 4. Some other method to control yeast.
- Mould contamination of SCOBY cultures, particularly with *Aspergillus* and *Penicillium* could be of concern, affecting the final product and culture performance. SCOBY should be assessed visually, contaminated cultures discarded and healthy culture used in subsequent batches. Discard if fuzzy patches of blue, green, grey, brown or black on SCOBY.
- Acidity must reach a pH of 4.2 or lower for fermentation to be complete and successful. If pH does not drop to 4.2 or below, fermentation has failed and product should be discarded. New, healthy SCOBY culture should be used in subsequent batches.
- Acidity should not reach a pH of lower than 2.5, and this pH should be included as a critical limit in food safety plans.
- Hygiene and sanitation is important throughout production; clean and sanitized utensils and vessels should be used. Vessels should be protected using tightly woven cloth or in rooms with environmental controls adequate to prevent pest and soil contamination of open vessels.
- Once brewed and steeped tea is made it should be cooled from 60°C to ambient temperature (approximately 20°C) within two hours prior to SCOBY addition.
- Kombucha and jun should be labelled appropriately so that consumers are aware of the ingredients, including the amount of alcohol. Raw kombucha or jun containing residual sugars need to be refrigerated. It is further recommended to provide precautionary labels, such as:
 - o May contain alcohol at <0.5% ABV (or state actual alcohol level),
 - o Not a suitable beverage for young children or during pregnancy,
 - o Keep refrigerated, do not shake,
 - o Provide readable Best Before Dates (BBD)

Box 3 | Additional resources for alcohol control for operators and inspectors

Based on findings of a study of alcohol levels in kombucha products that exceeded the provincial threshold of 1% ABV, the BC Centre for Disease Control determined guidance for kombucha processors and inspectors was warranted.

<u>Alcohol testing for kombucha</u>. This guidance lists requirements for alcohol testing and laboratories who use approved alcohol testing methods.

<u>Evaluating alcohol control in kombucha</u>. This guidance provides the regulatory rationale, food safety plan requirements, critical limits, controls, and critical control points for alcohol in kombucha teas. It provides requirements for processors and what inspectors should do to assess for alcohol as a hazard.

Food issue note on kombucha. Summarizes risks and controls for kombucha.

<u>Peer reviewed paper</u> of study and <u>Full Report</u> on BCCDC <u>website</u>.

Potential health issues with kombucha and jun

Because kombucha and jun beverages are sold as non-alcoholic, unintended alcohol content can be a concern for some parts of the general population, especially for children and during pregnancy. 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.²⁷ 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.²⁸

Health Canada acknowledges ethanol in non-alcoholic fermented beverages as a concern in kombucha, kefir, and some soft drinks including ginger beer.²⁹ The Public Health Agency of Canada recommends that alcohol is not consumed during pregnancy, and that youth should delay drinking alcohol. Alcohol toxicity is a concern in young children, and especially those weighing 10 kg or less, who are vulnerable to even low doses of alcohol, should they be present in these beverages. Alcohol toxicity can occur at a dose of 50 to 100 mg/dL, health care practitioners should consider monitoring children in a hospital when exposed to beverages containing alcohol. If a kombucha beverage contained only 2% ABV, 200 mL or 2 dL (an amount that is less than one cup, 250 mL) would exceed the 50 mg/dL amount in a 10kg or lighter weight child.²⁵ Young children lack the enzyme alcohol dehydrogenase and have difficulties in metabolizing ethanol. Acting confused, overheating and fatigue may be symptoms of alcohol poisoning that go unrecognized by care givers, and long term health effects are difficult to predict. In the adult population, there are many people who avoid all alcohol for personal and religious beliefs. There are those who cannot ingest alcohol as it may interfere with prescription medications, have underlying health conditions, or are recovering from alcohol addiction issues. Others may be driving professionally and are unaware that these beverages may contain alcohol. Aside from health concerns, all consumers have a right to know if the products they are consuming contain alcohol.

Operators are recommended to list alcohol as a potential chemical hazard in their food safety plan. Operators are recommended to monitor alcohol levels, test for alcohol in their products, and to implement control measures to control for alcohol. Control options for yeast during and after fermentation (e.g., pasteurize batch, select strains that don't grow under refrigeration, remove by centrifugation or other means), and for batches exceeding alcohol levels following fermentation (e.g., dilute, delay and continue fermenting, divert or discard) are described in this section. Operators are further recommended to add precautionary labels and information about alcohol content in their products.

Raw kefir, kombucha and jun products with fermentable residual sugars need to be refrigerated. Recommended precautionary statements include "keep refrigerated", "do not shake", "not a suitable beverage for young children or during pregnancy" and "may contain alcohol at <0.5% ABV (or state actual alcohol level).

Operators manufacturing fermented beverages must comply with provincial and federal regulations for alcohol content. In Canada, non-alcoholic products are defined as those containing less than 1.1% ABV, and do not require an alcohol declaration label.³⁰ Lower ABV requirements of 1.0% or 0.5% may exist in other provincial jurisdictions.

Table 2 | Summary of health issues with kombucha, jun, and fermented teas

Issue	Description
Alcohol toxicity	Alcohol may form in kombucha, jun and fermented tea products above regulated Canadian levels (>1.1% ABV). Alcohol toxicity is weight and dose dependant, therefore risk is higher in lower weight children, to the fetus during pregnancy, and to the infant during breastfeeding. Beverage processors should ensure alcohol is controlled and are recommended to label products with precautionary statements that include "may contain alcohol" and "not a suitable beverage for young children or during pregnancy".
Acidosis	Prolonged fermentation can create acidic teas with pH levels below 2.5. Although rare, consumption of large volumes of these beverages has led to acidosis in vulnerable people with underlying conditions.
Botulism	Raw honey can contain botulism spores. If acidosis fermentation is incomplete or done incorrectly and the pH is not low enough, there is a potential for botulism spores to grow in the anaerobic environment.
Injury	Jun tends to produce more carbonation than kombucha. If fermentation is not properly controlled, the container lids may come off under extreme pressure or containers may break. This poses a potential risk of injury.
Toxicity from contaminants via leaching when tea fermented in inappropriate containers	Lead poisoning has occurred when inappropriate glazed containers were used to ferment highly acidic teas over time. The high acid environment of ferments involving kombucha, jun and others beverages have caused metal contaminants to leach out into the teas. Ferments must be done in glass or other approved food containers that are not reactive to high acid environements. ¹⁶

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Figure 3: Figure adapted from Villarreal-Soto et al, 2018

Figure 4: L. McIntyre, BC Centre for Disease Control (unpublished data)