A comparison of ethanol content of water kefir products to kombucha products and their compliance to British Columbia's Liquor Control and Licensing Act

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Abstract

Background: Water kefir is an up-and-coming beverage similar to kombucha involving the fermentation of water, sugar, fruits, and cultured microorganisms. The fermentation process develops various metabolites including lactic acid, carbon dioxide, and ethanol. These products need to be controlled to prevent unsafe overproduction, particularly of ethanol, as it can be dangerous to consume alcohol unknowingly. This study examined (i) whether water kefir and kombucha beverages are at-risk of containing elevated levels of alcohol, and (ii) the labelling practices of these products.

Methods: 31 samples of water kefir were collected in various markets in Vancouver, British Columbia to be compared to 107 samples of kombucha previously collected by the British Columbia Centre for Disease Control (BC CDC). The samples were tested using gas chromatography mass spectroscopy (GCMS/D) to determine the concentration of alcohol in each. The data was analyzed using the statistical package NCCS. Two-tailed t-tests assessed differences in alcohol content between the two products, as well as whether kombucha and/or water kefir exceeded the regulatory standard of 1% ABV (alcohol by volume), as set under the Liquor Control and Licensing Act.

Results: Based on the collected data, 53% of kombucha samples and 19% of water kefir samples exceeded 1% ABV for ethanol. There was a statistically significant difference in ethanol concentrations between the water kefir and kombucha samples $p = 0.00002$, power = 100%. More specifically, the kombucha products had a higher alcohol level. Two t-tests compared the kombucha and the water kefir to the standards which resulted in mean kombucha samples being greater than the 1% ABV while mean water kefir samples were less than the 1% ABV regulatory level.

Conclusions: The results indicated that kombucha products had a higher mean alcohol concentration when compared to water kefir samples. However, some samples of water kefir exceeded the 1% ABV level and also lacked an alcohol warning label. Therefore, it is recommended that manufacturers for both kombucha and water kefir products label potential alcohol contents to protect the safety of their consumers – especially vulnerable groups including pregnant women, children, and recovering individuals.

Keywords: water kefir, kombucha, ethanol, fermentation, public health, labelling, food safety

Introduction

Kefir is a fermented beverage, traditionally prepared as a dairy product, that is characterized by its tart and refreshing flavour due to the presence of naturally occurring probiotic bacteria and yeast. This slightly acidic drink can be traced back centuries to the shepherds of the Northern Caucasus mountains in Russia through accidental observations of fresh milk fermenting into carbonated beverages in their leather pouches (Yemoos Nourishing Cultures, 2019). Milk fermentation occurs through the inoculation of kefir grains (resembling small white-yellow cauliflower florets) and kefiran into the milk (Ninane *et al*., 2005). Kefir grains are cultures of symbiotic microorganisms such as *Saccharomyces*, lactic acid bacteria, and acetic acid bacteria. These grains are embedded in a matrix of proteins, lipids, and polysaccharides known as the kefiran (Leite *et al*., 2013). The process involves the kefir grains converting milk sugar into an abundance of lactic acid, acetic acid, ethanol, and CO_2 to ultimately produce the sensory characteristics of the final beverage (Lopitz-Otsoa *et al*., 2006; Wszolek, 2006).

Moving towards the 1800s, kefir grains involving the use of non-milk products was introduced as water kefir, or Tibicos. Research theorises that it originated in Mexico from its existence in the prickly pear cactus plant's sugar-saturated water (Yemoos Nourishing Cultures, 2019). This variation's grain is a mixture of *Lactobacillus*, *Streptococcus*, acetic acid bacteria, *Bifidobacterium*, and many others surrounded by a dextran exopolysaccharide backbone (Moinas *et al*., 1980). Once the crystalline grains are added to a mixture of water, sugar, and fruits, the resulting beverage is cloudy and sparkling with slightly sweet undertones, fruity aroma, and a mild alcoholic taste (Laureys and de Vuyst, 2014).

Due to the limited data and research concerning water kefir, the focus of this project was primarily on this type of beverage and its comparison to a similar fermented product known as kombucha. Both water kefir and kombucha undergo a process where the cultures present create metabolites and, in the case of this study, the production of ethanol was the primary focus. Components related to the starting mixture was studied because of its importance in affecting the end result of the beverage in regard to the chemical composition (i.e. pH, taste, and, alcohol content). This arrangement can be a deciding factor on whether or not suitable conditions are present for the growth of pathogens during the fermentation process thus providing potential preventative or remediation controls. Additionally, as regulatory legislation for this topic is limited, market claims and declarations compared to actual beverage contents would be analyzed to demonstrate ideal standards and guidelines for safe production and consumption.

Literature Review

History of Fermentation

Derived from the Latin word, *fermentare,* meaning to leaven, and from *fervere*, meaning to boil, the process of fermentation dates back to the Neolithic era (circa 10,000 BC), primarily in pre-historic China, when humans first began to farm and create permanent settlements (McGovern *et al*., 2004). However, these fermentations likely occurred by accident and without the settlers' knowledge of the process happening. The first deliberate fermentations involving beer and wine happened around 7,000 BC in Assyria, Caucasia, Mesopotamia, and Sumer until this procedure caught on to other parts of the world, either through their own unintentional discoveries or spreading of the practice. Other popular products resulting from fermentation includes cheese, soy sauce, bread, and milk products (Chojnacka, 2010). In addition to food fermentation, during World War I, fermentation was used to produce solvents, mainly acetone-butanol-ethanol arrangements necessary for explosive production. World War II demonstrated use of the process to produce ethyl alcohol, organic acids, biological warfare, and antibiotics. (Boruff S *et al*., 1947) As modern times progressed, fermentation was continuously used for food production, antibiotic and other medical developments, and waste management (Chojnacka, 2010).

Kombucha & Similarities to Water Kefir

Kombucha is a fermented tea beverage characterized by its refreshing and sparkling cider-like properties. It originated in China during the Tsin Dynasty as the "Tea of Immortality" for its detoxifying and energizing attributes (Dufresne and Farnworth, 2000). Similar to water kefir, kombucha undergoes a fermentation process, however using tea plants (usually black tea) and a combination of symbiotic bacteria and yeasts such as acetic acid bacteria and *Saccharomyces cerevisiae* to form the tea fungus. This mixture produces bioactive metabolites acetic acid, lactic acid, gluconic, glucuronic, and ethanol which contain antimicrobial properties against pathogens (Liu *et al*., 1996). The fermentation process also decreases the pH value of the beverage as the organic acid contents increase.

Starter Mix of Water Kefir Grains

Water kefir grains play an important role as a natural starter culture for the fermentation process based on the mixture of symbiotic microorganisms involved. The basic arrangement of several bacterial and yeast species on the dextran exopolysaccharide matrix present in the grain assist the synthesis of bioactive metabolites. This combination inhibits competitive microbial growth and promotes grain growth (Garrote *et al*., 2010). The microbial mixture works in a symbiotic relationship, so the grain's growth and survival are dependant on the presence of specific yeasts and bacteria (Leite *et al*., 2013). Any variations in the starting mixture in terms of differing concentrations of substrate or added ingredients will also affect the end characteristics of the beverage and thus their quality or risk. For example, a comparison between a starter mixture of only water and sugar led to a product with higher pH, lower alcohol, and lower organic acids while a mixture with added fruits had a higher nutritional variety and lower pH (Laureys and de Vuyst, 2017). The absence of fruits slows down the fermentation process because of the reduced amount of free nutrients available for breaking down. In particular, a study by Laureys and de Vuyst (2017) demonstrated that additions of fruit with higher amounts of glucose were preferred as this sugar was consumed a lot faster than fructose.

Starter Mixture – pH Significance

Other evidence claiming the significance of how certain ingredients raise the risk or require more critical controls was highlighted in kefir-like products made from similar fruit or vegetable juices. According to the *Food Retail and Food Services Code* (2016), foods are considered potentially hazardous if the pH level is greater than 4.6 which provides suitable conditions for pathogen growth. In the kefir-like beverages, the use of melon juice and prickly pear juice which have pH levels of 6.4 and 6.3 respectively, provided favourable environments for microorganisms *Enterobacteriaceae* and *Pseudomonas* to establish themselves even after initial pasteurization and fermentation processes. However, the final pH level of the end products was below 4.6 which is considered low risk while still being contaminated. This was due to the initial high pH allowing favorable conditions for the pathogens to proliferate during the early stages of the fermentation process and outcompeting any metabolites created by lactic acid or acetic acid bacteria in later stages (Corona *et al*., 2016).

Antimicrobial Effects in Water Kefir

When the beginning ingredients in the starter mixture are added in correct concentrations and suitable conditions, the vast majority of microbial inhibition is due to the bioactive metabolites created by the various kefir grain cultures. Since their introduction in each mixture of grain, *Lactobacillus* species such as *L. paracasei, L. hilgardii,* and *L. nagelii* are seen to be a part of the major organisms needed for the fermentation process (Laureys and de Vuyst, 2017). These bacteria produce large amounts of lactic acid through the conversion of one molecule of glucose to two lactic acid molecules. This product assists in pH reduction for microbial growth hinderance and has demonstrated permeabilizing membranes which enhances other antimicrobials through detection of intracellular antigens (Salminen and von Wright, 2004). More specifically, Santos *et al*., (2003) studied the behaviour of these lactic acid bacteria and their ability to inhibit the growth of *E. coli*, *L. monocytogenes, S. typhimurium, S.*

enteritidis, S. flexneri, and *Y. enterocolitica* through denying pathogen entry and attachment to cells. Additionally, a study demonstrated that lactic acid required a supplementary presence of acetic acid in order to create a more effective synergistic effect against *E. coli* and *S. enteritidis* (Garrote et al.,2000; Adams and Hall]

The kefir grains also produce carbon dioxide through the fermentation process which contains antimicrobial activity. The presence of carbon dioxide forms a more anaerobic environment which hinders the growth of many oxygen-requiring microorganisms at higher concentrations. Additionally, carbon dioxide has been studied to suggest its accumulation in the lipid bilayer of cells causes membrane permeability implications (Salminen and von Wright, 2004).

Ethanol Content in Water Kefir and Kombucha

Yeast continues to ferment sugars which produce carbon dioxide and alcohol; therefore, risks of over-producing alcohol becomes an issue to consumers based on recommended serving sizes (BCCDC, 2015). According to the BC Liquor Control and Licensing Act, beverages containing more than 1.00% alcohol by volume need to be defined as a liquor and are subject to the regulations under the Act. However, the ethanol content for both of these products have been observed to be over 1.00% in numerous instances without proper labels. For example, eighteen samples of commercial kombucha were analyzed to contain a range of 1.12 to 2.00% (v/v) of ethanol using headspace gas chromatography (Talebi *et al*., 2017). In two other studies, chemical determinations in kefir-like beverages using a variety of fruits and vegetable juices demonstrated an ethanol content range from 0.09 to 4.96% (v/v) (Corona *et al*., 2015; Randazzo *et al*., 2015).

Regulatory Importance of Ethanol Content

Some individuals may not know that water kefir or kombucha, as well as other similar fermented beverages, contain alcohol. Therefore, proper labelling to define alcohol concentrations on the beverage is vital – especially when the ethanol content is deemed higher than the regulatory standard of 1.00%. There are vulnerable groups that are more susceptible to complications from excessive alcohol consumption such as children and pregnant women. However, the Centers for Disease Control and Prevention stated that "there is no known safe amount of alcohol use during pregnancy". Alcohol-exposed pregnancy increases the risk of disrupting fetal development, spontaneous abortion, and higher rates of psychiatric disorders persisting into adulthood (Babor *et al*., 2016). Children and adolescents undergo significant changes of intellectual, psychologic, and behavioural development by immense hormonal and physical changes (Blakemore and Choudhury, 2006). Early onset of drinking alcohol can have consequences on a child's lasting brain function including cognitive ability, emotional regulation, and social-functioning issues (Babor *et al*., 2016). Additionally, recovering alcoholics are considered a vulnerable group due to the high risk of relapse from unknowingly consuming alcoholic beverages. Long-term consumption of alcohol is associated with higher risks of liver and heart disease, as well as cancer and problems with memory functions (Babor *et al*., 2016).

Research Question

The purpose of this research project was to determine the ethanol content of water kefir products compared to kombucha products and their compliance with regulatory standards for labelling under the Liquor Control and Licensing Act.

Methods and Materials

Sample Collection & Preparation

Due to limited availability in commercial stores, the water kefir products were obtained at any location where all products (from any brand or company) on the shelf were chosen in order to obtain a minimum of 30 samples. Overall, there were a total of 31 water kefir beverages collected from grocery stores from brands including Squamish Water Kefir, LAB Water Kefir, Culture Kefir, Coastal Culture Kefir, and Remedy. The store coolers' temperature was measured with a temperature gun after calibration using Thermowork's (n.d) method of measuring the temperature of ice water. Any water kefir products stored in a self-serve dispenser were collected in a sample bottle about 2/3 of the container. The water kefir samples were placed in a portable cooler and transported to the BCCDC lab for preparation. This process included labelling each centrifuge tube and pipetting approximately 30 mL of water kefir into them before storing in the lab refrigerator until testing began.

Ethanol Testing

Every week, the water kefir samples that were stored at the BCCDC were delivered to the BCIT Natural Health Products Laboratory. The following procedure was validated using Association of Official Agricultural Chemists (AOAC) guidelines. (McIntyre, 2019) The AOAC International organization recognizes and evaluates chemistry, microbiological, and other scientific methods used by regulatory, research, testing, and stakeholders (AOAC, n.d). To measure the ethanol content in the water kefir, a head-space gas chromatography mass spectroscopy method was followed. The unit which the lab technicians in the BCIT Laboratory used is pictured below in Figure 1. First, the samples were diluted and placed in sealed headspace vials containing propanol standard. Next, the vials were heated and agitated until a vapour is injected into the chromatograph and read by the detector. According to the Agilent 5975C Series GC/MSD Agilent Technologies' (2011) manual which includes a method of calibration using the AutoQuant feature for over 2000 compounds, the instrument is certified to confirm accuracy and long-term calibration.

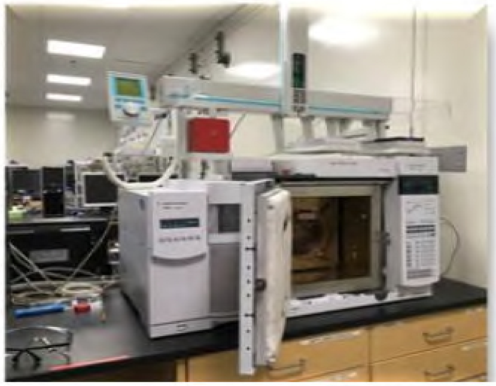

Figure 1. Head-space gas chromatography mass spectrometer at the BCIT Natural Health Products Lab used to measure ethanol levels (McIntyre, 2019).

pH Testing

As an additional test for collecting more information regarding water kefir products, the pH was tested using a pH meter to provide potential reasons for differing ethanol concentrations based on the acidity of each beverage or brand using the Scientific Instruments Model IQ150. For calibration, the meter recognizes 7 buffers for one- or two-point calibration (in this instance, two-point calibration) was implemented with pH4 and pH 7 buffers) for up to 0.01 pH resolution or accuracy of measurements (IQ Scientific Instruments, Inc., 2004). The pH meter was recalibrated every 10 measurements to uphold accuracy. Following the IQ150 manual, cleaning of the probe included rinsing with distilled water before readings and between every individual reading (IQ Scientific Instruments, Inc., 2004).

Inclusion and Exclusion Criteria

For this study's purpose, no dairy kefir products were considered – any kind of water kefir beverage was acceptable. Kombucha products previously chosen and tested by the BCCDC and BCIT Natural Health Products Lab were applicable in this study for comparison to the water kefir products and regulatory standard.

Potential Alpha and Beta Errors

Every test has some potential for an alpha (type I) or beta (type II) error when conducting a statistical analysis. In this study's case, a potential beta error was observed in the results which could indicate that there is no difference when there actually is in reality. Therefore, to reduce the potential type II error, an increased sample size can be implemented. (Chen and Heacock, 2019).

Statistical Analysis

This study analyzed a sample of 31 water kefir beverages in order to determine the ethanol concentration levels and whether they follow labelling requirements for beverages containing 1% or more alcohol by volume under the Liquor Control and Licensing Act (Liquor Control and Licensing Act, 2015). Additionally, the results were compared to a previously studied 107 kombucha ethanol products to determine if there are any significant differences between the two fermented products. The results included continuous numerical data as well as interval (pH and temperature measurements) and ratio data (ethanol concentrations).

Descriptive Statistics

The pH and ethanol concentration measurements were tabulated using Microsoft Excel (2016) as depicted in Table 1 to express the collected 31 datapoints as a mean, range, median, and standard deviation.

Table 1. Tabulated mean, range, median, and standard deviation of the pH and ethanol concentrations (%) in water kefir products

	Mean	Range	Median	Standard Deviation
pH	3.08	2.63 – 3.78	3.11	0.33
Ethanol (%)	0.60	0.12 – 1.87	0.39	0.53

Additionally, percentages were used to determine what proportion of the sample size was at or above the regulatory standard of 1% for ethanol concentrations for both water kefir and kombucha:

- 19% had an alcohol content of greater than or equal to 1% alcohol by volume for water kefir
- 53% had an alcohol content of greater than or equal to 1% alcohol by volume for kombucha

These percentages can also be visualized in the following pie-charts:

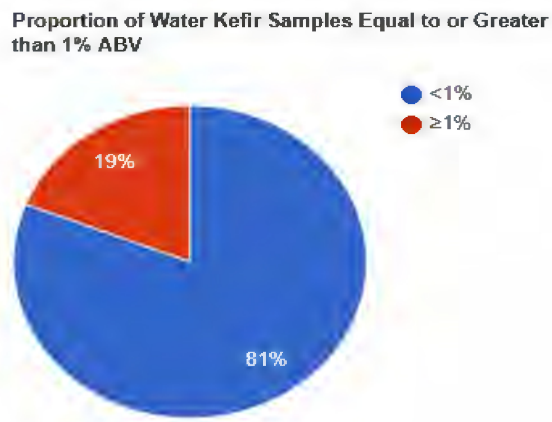

Figure 4. Pie-chart demonstrating the proportion of water kefir samples with an ethanol content equal to or greater than 1%.

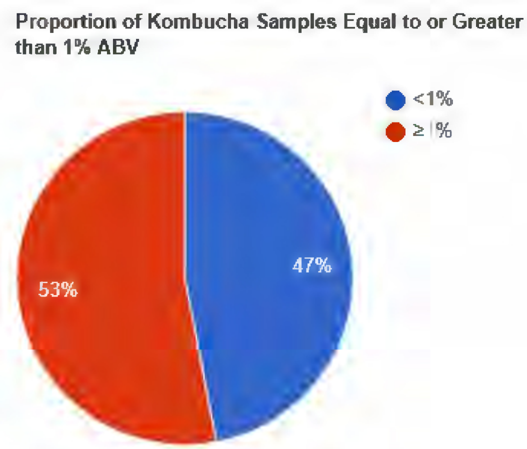

Figure 5. Pie-chart demonstrating the proportion of kombucha samples with an ethanol content equal to or greater than 1%.

To further exemplify the difference between the water kefir and kombucha ethanol concentrations in relation to their compliance with the regulatory standard, a boxplot is illustrated below.

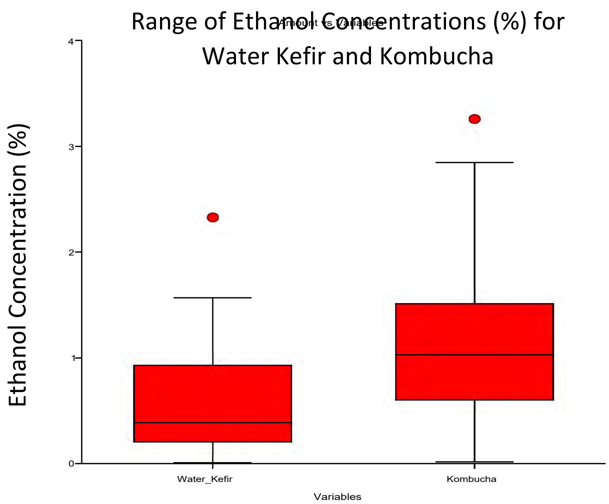

Figure 6. Boxplot for the range of ethanol concentrations (%) for the water kefir and kombucha products.

Solely focusing on the samples that exceeded the 1% ABV, 6 water kefir beverages (as the kombucha samples were kept anonymous, they were not included) are tabulated based on brand, predominant flavour profile, temperature of storage, and pH below.

Inferential Statistics

The statistical package used for analyzing the 31 samples of water kefir and 107 samples of kombucha was the NCSS Statistical Software. Three tests were conducted with three null and alternate hypotheses to determine if there are statistically significant differences between groups – water kefir ethanol content to kombucha ethanol content, water kefir ethanol content to the regulatory standard of 1%, and kombucha ethanol content to the regulatory standard of 1%. The three hypotheses are presented in Table 3, respectively. For the first experiment, these concentrations were compared to one another to determine if there is a statistically significant difference using an Independent Samples T-Test. The next two are comparisons of kombucha and water kefir samples to the regulatory standard of 1% using a two-tailed One-Sample T-Test. Two-tailed tests were used as there are no previous assumptions for the mean ethanol levels.

Sample #	EtOH	Brand	Predominant Flavour	Temperature (deg C)	pH
666	1.57 +/- 0.04	Squamish Water Kefir	Hibiscus	8	2.76
654	1.11 +/- 0.03	Coastal Culture Water Kefir	Lemon	4	3.29
751	1.43 +/- 0.04	Squamish Water Kefir	Hibiscus	1.9	2.72
784	1.17 +/- 0.03	Squamish Water Kefir	Hibiscus	4	3.11
790	1.33 +/- 0.03	LAB Water Kefir	Lemon	4	2.88
791	2.33 +/- 0.05	LAB Water Kefir	Grape	2	3.36

Table 2. Tabulated brand, flavour, storage temperature, and pH for water kefir samples exceeding 1% ethanol concentrations.

Ho: There is no difference between the ethanol concentrations present in the water kefir and kombucha products.	**Ha:** There is a difference between the ethanol concentrations present in the water kefir and kombucha products.
Ho: There is no difference between the mean water kefir ethanol content and the regulated standard ethanol content of 1%.	**Ha:** There is a difference between the mean water kefir ethanol content and the regulated standard ethanol content of 1%.
Ho: There is no difference between the mean kombucha ethanol content and the regulated standard ethanol content of 1%.	**Ho:** There is a difference between the mean kombucha ethanol content and the regulated standard ethanol content of 1%.

Table 3. The null and alternate hypotheses for three experiments.

Interpretation of Results

Using the Mann-Whitney U or Wilcoxon Rank-Sum Test, the two-sided p-value is 0.00002, therefore, the null hypothesis was rejected, and it was concluded that there was a statistically significant difference in ethanol concentrations between the water kefir and kombucha products. More specifically, the kombucha ethanol concentrations are observed to be significantly higher. Additionally, the power of the test was 100% which gives confidence to the results, so there is no or a very unlikely potential beta error.

A Wilcoxon Signed-Rank Test was performed and the resulting p-value for the two-tailed test was 0.00042. Therefore, the null hypothesis was rejected, and it was concluded that the mean water kefir ethanol concentration was statistically significantly different than the regulated standard ethanol concentration of 1%. The power for the test is 98%, so it can be concluded that there is confidence in the results being correct.

For the last experiment, a Wilcoxon Signed-Rank Test was performed and the resulting p-value for the two-tailed test was 0.16615. Therefore, the null hypothesis was not rejected, and it was concluded that there is no statistically significant difference between the mean ethanol concentrations in the kombucha and the standard ethanol concentration for beverage labelling which is at 1.0%. The power is 61%, so there was a potential for a beta error which can be addressed by increasing the sample size.

Discussion

The ethanol concentration results concluded that the kombucha samples had significantly higher alcohol content than the water kefir samples. Moreover, this is evident based on the discrepancy found between the proportions of each sample group being equal to or over the 1% regulatory standard with 19% of the water kefir samples and 53% of kombucha samples measured at those levels. These findings have also been similarly demonstrated in Food Standards Australia's (2019) survey where kombucha samples had a higher percentage of ethanol concentrations of 0.5 to over 1.5% ABV than water-based kefir samples. Over time, through the natural fermentation process as well as during storage, alcohol production will have the chance to rise with increased risks based on shelf life. Both these beverages are affected by temperature, ingredients, and other internal characteristics such as the pH on the amount of alcohol produced which may lead to unintentional levels present. Since each sample was collected from different locations or companies thus causing potential differences in their storage mean times, temperatures, and other brand-specific differences, a reference to Table 2 will be used to determine which water kefir samples were greater than 1% ABV and their corresponding characteristics. Therefore, factors present in kombucha and water kefir that may explain the statistically significant difference in ethanol concentrations can be explored.

Two studies (Ebersole *et al.*, 2017 and Talebi *et al.*, 2017) focusing on a variety of commercial kombucha samples varying in flavours based on the ingredients included have reported a range of ethanol concentrations. However, a limitation to this approach was that the samples were all from different manufacturers which could also include varying fermentation times, handling practices, which could not be controlled for in the end comparison of ethanol levels based purely on ingredients. Beverages that are unflavoured typically had lower alcohol concentrations per alcohol by volume and the flavours incorporating berries were observed to have higher ethanol contents. A possible reason for this could be due to beverages with less ingredients contain lower amounts of nutrients for the SCOBY to breakdown its components and thus lower concentrations of ethanol would result. A similar study by Laureys and Vuyst (2016) observed 3 water kefir samples, all with differing ingredients, but also differing fermentation conditions. The grain with the least amount of sugar and fruits (just two dried figs), but higher fermentation temperature (20 degrees Celsius) was observed to have the lowest ethanol concentration while the other two samples including similar sugar levels, more fruits (figs, apricots, raisins, apple cider vinegar, and lemon) and lower fermentation temperatures (15 and 19 degrees Celsius) had the same higher level.

Based on Table 2 of the 6 samples exceeding the regulatory value of 1% ABV, 3 (50%) were from Squamish Water Kefir's Hibiscus beverages, 2 (33%) were lemon flavoured from LAB Water Kefir and Coastal Culture Water Kefir, and the remaining 1 (17%) was a grape flavour. Since there is not a large number of samples to provide statistical reliability nor validity, only assumptions based on these observations can be made. For one, Squamish Water Kefir can be assumed to have a higher risk of containing greater ethanol concentrations solely for their Hibiscus products thus a proper label is needed, and more vulnerable groups should be prevented from consuming these. Next, lemon flavoured water kefir could be another beverage to avoid. On the other hand, for the brands that had no samples exceeding the 1% alcohol level, consumers may feel safer turning to Remedy or Culture Kefir, though future studies with greater sample sizes are required. Lastly, as ingredients affect the acidity of products, the pH referenced in Table 2 was used as potential reasons for ethanol discrepancies. However, again due to lack of comparison data and variability since all the samples were close in range and were below the 4.6 safety level, there are no significant conclusions to infer from the samples collected.

A discrepancy in just looking at the composition of the beverages was that other factors such as time and temperature of the fermentation process would be overlooked which are just as significant in determining how much alcohol will be produced. A potential reason to the significantly higher concentration of ethanol levels in the kombucha compared to the water kefir is due to kombucha's much longer fermentation time averaging about two weeks while water kefir requires a few days (Happy Gut, ND). As seen in Laureys and De Vuyst's (2014) study of water kefir fermentation over 192 hours, the ethanol concentration increased linearly for the first 72 hours until progressive, slow increases were observed towards the 192-hour mark. Similarly, concentrations of ethanol were observed based on a 7-day and 14-day fermentation time for kombucha products in another study by Gaggia *et al.* (2018). Conclusions from this study included significant increased differences from the longer fermentation period thus providing more evidence that an increased fermentation time does affect the resulting ethanol levels. Since time was not a factor in this study, the temperature was considered. However, since every single temperature was below the 4 degrees Celsius threshold for safe potentially hazardous food holding in coolers (except for one at 8 degrees Celsius), there is no significant conclusion.

Despite these factors affecting kombucha and water kefir products' ethanol concentration production, the concern with overproducing alcohol in beverages remains the same. As seen in this study, the kombucha samples had a statistically significant proportion over the 1% ABV standard while the water kefir, although included some samples over the standard, remained mostly in accordance. Therefore, kombucha products should have a label raising the awareness for potential alcohol in the beverage and a recommendation that water kefir products do the same.

Limitations

Due to the nature of water kefir products, their availability in commercial premises is limited in quantity and variability of brands. Although early research testing for this product is useful and can assist in the extrapolation to future products, a more accurate representation of the mean alcohol content would be obtained if a greater sample size was used. Therefore, for future studies, a greater number available would be preferred to reduce any potential statistical error, namely beta errors. This issue is also seen when comparing the 6 samples over 1% ABV – there is not enough data to support potential claims as to why some are higher. In the future, dedicating more time to the project, rather than just a couple of months, would be worthwhile for a complete representation of the study's findings. Furthermore, as this research study was in collaboration with the BCCDC, frequent communication was needed to keep up to date with any testing results as soon as possible in order to move the analysis stages of the process along. Lastly, the use of the IR temperature gun to measure the temperatures of where each product was stored is not as accurate because the glare of products' packaging could hinder the laser. Therefore, for future testing, a probe thermometer can be applied by placing it in the storage facility, such as a cooler, which will read exactly what the beverages are experiencing without any potential reflections.

Knowledge Translation

Applications of this study focus on the protection of the general public and vulnerable populations through raised awareness regarding the components of water kefir beverages via labelling updates, manufacturer compliance, and general education. As discussed previously, there were instances of the alcohol content in these beverages exceeding the 1% ABV level set by the Liquor Control and Licensing Act. However, many of the products' labels did not include a warning statement of potential traces of alcohol. Consistency in labelling among producers assists in preventing accidental side-effects from consumption of alcohol unknowingly. Legally-speaking, labels indicating these precautions can protect companies if they provide transparency and clarity. For instance, on May 2019 in the United States, a lawsuit concerning the misrepresentation of alcohol and sugar contents in kombucha products included a settlement of up to $3,997,500 to pay claims for those affected (US District Court for the Northern District of California, 2019). The labelling requirements can be monitored by the CFIA to ensure that regulatory levels are being met. Next, manufacturers have a duty to ensure that their products are labelled correctly to meet the requirements of the Act or alter the composition of the beverages to remain compliant. For example, the Squamish Water Kefir company could specifically provide warnings on their Hibiscus products as those samples were consistently over the regulatory standard. Therefore, manufacturers should have access to suitable equipment and testing procedures in order to quantify the alcohol contents. Additionally, they should monitor the fermentation process throughout the production stages in order to mitigate potential risks of over-production of ethanol. Lastly, the overall education of the public (especially the mentioned vulnerable groups) is applicable through online resources, posters, or information sessions available. These initiatives will assist in the goal of prevention thus protecting the health of the public.

Future Research

As explained by limitations experienced in this research study, only a specific focus could be obtained thus leaving a number of potential future projects based on this study or a continuation of it. Some examples include:

- Conducting a research study on water kefir beverages' ethanol contents based on altered environmental factors (temperature, humidity, etc.)
- Studying a new trend of fermented beverage based on the assumptions of this project and determine potential health risks
- Organizing a survey-based study regarding either the general public's or vulnerable group's perceptions or awareness of potential alcohol in fermented beverages such as kombucha or water kefir

Conclusions

Based on the findings of this study, proper labelling and education of the fermented beverages industry, namely water kefir, is required to raise awareness of potential health risks thus protecting the health of the public and vulnerable groups. Water kefir undergoes a fermentation process involving water, sugar, fruits, and a mixture of yeasts and bacteria to create a fizzy and slightly sweet beverage. This process results in a variation of by-products including organic acids, carbon dioxide, and ethanol. Therefore, close regulation of the process can prevent potential over-production of any of the by-products such as alcohol to ensure quality and safety. However, through statistical analysis, 19% of water kefir beverages sampled exceeded the 1% ABV regulatory standard thus requiring proper labelling and designation as an alcoholic beverage. Lack of clarity available to consumers could result in fetal, developmental, and social-wellbeing complications as well as legal cases. Therefore, manufacturer awareness and compliance to the Act is crucial for monitoring their production process as well as distribution to markets. As this product is relatively new to the market, long-term testing of more samples is required for the future in order to increase the accuracy and validity of this study's findings thus allowing for greater extrapolation to other vulnerable groups and similar products.

Acknowledgements

The lead author would like to thank Helen Heacock and Frederick Shaw from BCIT for the ongoing guidance, support, and materials throughout this research study. Additionally, the author would like to acknowledge Lorraine

McIntyre from the BCCDC for providing valuable resources and advice throughout.

Competing Interests

The authors declare that there is no competing interest for this research.

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