

# Analyzing Ethanol Accumulation in Different Kombucha Tea Brands During Storage: Relationship Between Storage and Ethanol Production

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## Abstract

### Background

Kombucha tea is a fermented tea beverage that is mainly consumed for its associated-health benefits. These associated-health benefits may range from detoxifying the body to cancer treating. However, there is little to no scientific evidence that suggests that they work on humans. Similarly, kombucha tea is also prone to post-fermentation. This presents possible ethanol production and accumulation within the tea after packaging which can pose a possible health risk to susceptible population if not properly labelled or controlled. This study will investigate if there is any post-ethanol accumulation in commercially produced kombucha tea products under various storage conditions.

### Methods

The ethanol concentration of 3 different kombucha tea brands (i.e.: Pure+, Health-Ade, and RISE) at various storage conditions (i.e.: no storage, refrigeration, and room temperature) were analyzed using GC-FID to determine post-ethanol accumulation. In addition, NCSS software was used to conduct a statistical analysis on the data to determine whether the 3 different kombucha tea brands exceeded the ethanol regulatory limit and whether the ethanol accumulation was dependent on storage temperatures.

### Results

The mean ethanol concentration for Pure+, Health-Ade, and RISE after refrigeration for 3 weeks were 0.722%, 0.696%, and 0.050% relatively which all showed a slight decrease in ethanol compared to their baseline ethanol levels (i.e.: no storage). Similarly, Pure+, Health-Ade, and RISE mean ethanol concentration after room temperature storage were 1.766%, 1.285%, and 0.794% relatively which indicates ethanol accumulation. Statistical analysis showed that there is a significant difference between room temperature storage and the other 2 storage conditions (i.e.: no storage and refrigeration). Also, only Pure+ and Health-Ade under room temperature storage showed a statistically significant mean ethanol concentration above the regulatory limit.

### Conclusion

Results suggests that room temperature storage of Pure+, Health-Ade, and RISE for 3 weeks increased the ethanol levels significantly while refrigerating them will decrease the ethanol levels slightly which can minimize any potential post-fermentation process from happening. Furthermore, only Pure+ and Health-Ade under room temperature storage for 3 weeks were over the 1% ABV regulatory limit. Lastly, the data obtained from this study can be used to develop guidelines and policies in regulating kombucha tea manufacturers and in educating the public and other regulatory agencies on the matter.

**Keywords:** kombucha, fermentation, tea, ethanol, temperature, GC-FID

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## Introduction

Kombucha tea is a non-alcoholic fermented tea beverage consumed mainly for its associated-health benefits. It is made from fermenting sugared tea with a symbiotic culture of bacteria and yeast (SCOBY) at

18°C- 24°C for 14-30 days (Jayabalan *et al.*, 2014).

During the fermentation process of kombucha tea, the yeast mainly hydrolyzes the sucrose into fructose and glucose which is then further converted into ethanol via glycolysis (Jayabalan *et al.*, 2014). Conversely, the

acetic acid bacteria will oxidize glucose and ethanol into acids such as gluconic, glucuronic, or acetic acid (Jayabalan *et al.*, 2014). As a result, kombucha tea may taste slightly sweet and acidic.

As noted above, kombucha tea may contain increased levels of organic acids and ethanol if the fermentation were to continue after packaging the product. The low pH due to high levels of organic acids may present a health risk to susceptible populations as it can cause acidosis which may lead to further implications (BCCDC, 2015). Similarly, the increase in ethanol concentration can lead to non-compliance with current legislations and affect susceptible population like pregnant women (BCCDC, 2015).

With the potential of further ethanol production within kombucha tea, kombucha manufacturers are subject to comply with CFIA's (2019) labelling requirements and standards for products that contain alcohol. Similarly, the BC's *Liquor Control and Licensing Act* defines a liquor as anything that is intended for human consumption with a minimum alcohol content of 1% by volume (Queen's Printer, 2015). Therefore, kombucha manufacturers need to ensure that their practices adhere to current legislations so that any potential health impacts and impairment caused by ethanol consumption to susceptible people are minimized.

Given this information, this literature review will investigate the potential health effects, the ethanol concentration trends due to fermentation, and proposed methodology in analyzing ethanol in kombucha tea.

## **Literature Review**

### **Health Benefits of Kombucha Tea**

Kombucha tea consumption have been advertised as having therapeutic and prophylactic properties. Furthermore, Russian researches and kombucha tea drinkers have testified that kombucha tea possess antioxidant properties and immune enhancing agents (Jayabalan *et al.*, 2014). Ram *et al.* (2000) conducted a study that evaluates the effects of kombucha tea on chromate (VI) - induced oxidative stress in albino rats. In this study, Ram *et al.* (2000) sees that consuming kombucha tea in chromate treated albino rats reversed the immunosuppressive effects caused by the chromate (VI) and increased their antioxidative metabolic processes.

Conversely, Amarasinghe *et al.* (2018) study states that fermentation time and the tea concentration in kombucha tea may affect the antioxidant properties. Amarasinghe *et al.* (2018) found that overall, there is a statistically significant reduction ( $p < 0.05$ ) of antioxidant activity of kombucha tea with increased fermentation time. Similarly, there is also a statistically significant reduction of antioxidant activity when lower tea concentration is used when making kombucha tea (Amarasinhe *et al.*, 2018). Thus, the fermentation time and the amount of tea used to ferment kombucha tea may impact the antioxidant properties of the drink.

### **Kombucha Tea-Related Toxicity**

Despite that kombucha tea have been reported to be an alternative treatment to some ailments, there have been evidence showing toxicity associated with kombucha tea consumption in some individuals. For example, there have been reported cases in adults having anemia

which is due to the lead-gazed ceramic pot that housed kombucha tea (Jayabalan *et al.*, 2014).

Similarly, Srinivasan *et al.* (1997) presents 4 patient cases that were associated with kombucha tea consumption all of which recovered from health complications after ceasing kombucha tea consumption. Thus, these patient cases showed that there may be a probable health risk associated with consuming kombucha tea.

Likewise, there was another incident of a 22-year old Filipino gay male diagnosed with HIV that was admitted into the hospital after consuming kombucha tea (SungHee Kole *et al.*, 2009). He was diagnosed with hyperthermia, lactic acidosis, and acute renal failure without knowing the cause. Through a history examination of the patient, it was indicated that his friend also consumed the same kombucha tea but did not get ill. This may suggest that kombucha tea consumption may also affect immunocompromised individuals.

Even though there are several cases of toxicity from consuming kombucha tea, Vijayaraghavan *et al.* (2000) studied the acute toxicity of kombucha tea in rats where he force fed rats kombucha tea for 90 days.

Vijayaraghavan *et al.* (2000) concluded that there were no toxic effects due to kombucha tea. Thus, further research should be conducted on humans to see if there are possible side effects of consuming too much kombucha tea as currently there are only animal trails and case reports available in the literature.

### **Effects of Alcohol in Susceptible Populations**

As we know, kombucha tea may have traces of alcohol due to the fermentation process. Alcohol is a known

toxic substance to people when consumed excessively, especially susceptible individuals. There is evidence that alcohol consumption during pregnancy, for example, may result in teratogenic effects that can lead to newborns being diagnosed for Fetal Alcohol Spectrum Disorder (FASD) or Fetal Alcohol Syndrome (FAS) (Vall *et al.*, 2015). Therefore, susceptible people should be aware that kombucha tea containing alcohol may cause unpredicted negative consequences such as neurological and biological effects to the body.

Similarly, in Corrales-Gutierrez *et al.* (2019) cross-sectional study, she conducted a survey for pregnant women on their risk perception of the teratogenic effects due to alcohol consumption in pregnancy. She concluded that there was a lower risk perception of fermented alcoholic beverages (wine and beer) versus distilled/spirited ones and that single pregnant women had a lower risk perception compared to pregnant women that were in a relationship (Corrales-Gutierrez *et al.*, 2019). Also, she found that there was a lower risk perception with the less educated compared to the well-educated ones (Corrales-Gutierrez *et al.*, 2019). Thus, this shows us that the risks of consuming alcoholic beverages are still unclear.

Given the evidence on the alcoholic effects on health and the cross-sectional survey above, kombucha tea may present a concern as it is a fermented beverage that may contain alcohol. Furthermore, most susceptible people who drink kombucha tea may not be aware of its potential health effects as seen in Corrales-Gutierrez *et al.* (2019) study where not all pregnant women have the same perceived risks of alcoholic beverages. Therefore, manufacturers and regulatory bodies like BCCDC or CFIA should inform consumers that kombucha tea may

contain traces of alcohol and that susceptible populations should be cautious in drinking it.

### Standard Method of Ethanol Detection and Relevant Studies of Ethanol Accumulation in Kombucha Tea

As the amount of kombucha tea manufacturers steadily rises, an urgent need in developing a standardized method in testing ethanol content in kombucha tea is required. As a result, the Association of Official Agricultural Chemists (AOAC) International have established a Standard Method Performance Requirements (SMPRs) for testing ethanol in kombucha (AOAC International, 2016). The SMPRs for ethanol testing on kombucha outlines that the method needs to meet the following minimum performance requirements (AOAC International, 2016):

**Table 1: Method Performance Requirements**

Analytical range (%ABV <sup>a</sup> )	0.1 to 2.0
Limit of quantitation (LOQ) (%ABV)	≤0.05
Accuracy (% of mean spiked recovery over the range of the assay)	97 to 102
Repeatability (RSD <sub>r</sub> ), %	≤4
Reproducibility (RSD <sub>R</sub> ), %	≤6
<sup>a</sup> ABV= Alcohol by volume.	

In Ebersole *et al.* (2017) study, he tested his methodology of ethanol determination in kombucha tea using GC-FID to see if it met AOAC's specified method performance requirements. Ebersole *et al.* (2017) determined that analysis through GC-FID met the requirements of the method performance requirements set out by AOAC.

On the contrary, Pinu *et al.* (2017) studied a rapid quantification method using GC-MS to determine major volatile metabolites in fermented food and beverages to

investigate if her proposed method were quicker and more reliable than traditional ones. She determined that the ethanol quantitation using GC-MS to analyze balsamic vinegar, sourdough, whisky, wine, and beer had a Limit Of Quantitation (LOQ) of 2ppm (0.0002%) which is more sensitive than Ebersole *et al.* (2017) GC-FID (Pinu *et al.*, 2017).

Currently, there are few literatures on ethanol accumulation in commercial kombucha tea products. For instance, Talebi *et al.* (2017) analyzed the effects of storage under 4°C and room temperature (22°C) in kombucha tea for 60 days using GC-FID. He determined that the refrigerated samples had a lower ethanol content compared to the samples stored at room temperature (Talebi *et al.*, 2017). Similarly, Fung *et al.* (2019) conducted a study of ethanol accumulation between two different brands of kombucha tea which are stored for a week under room temperature. Fung *et al.* (2019) concluded that there was an increase in ethanol content in one brand while the other brand had a decrease in ethanol content after a week of storage.

### Scope

The purpose of this research is to determine if there is any ethanol accumulation in 3 different kombucha tea brands during storage at refrigeration and room temperatures for 3 weeks and whether they are over 1% alcohol by volume which will then be classified as a liquor. Furthermore, this will help provide additional scientific evidence for health authorities on the ethanol accumulation of various local kombucha tea products due to mishandling. Thus, health authorities can use it to educate and inform small local kombucha manufacturers to have procedures in place to monitor and control these variations in ethanol content. As a

result, it will limit any potential negative effects on susceptible populations such as pregnant women drinking kombucha and/or exceeding recommended serving sizes due to discrepancies in labelling.

## **Materials and Methodology**

### **Sample Collection**

A total of 54 bottles of 3 different kombucha tea brands were purchased from Whole Foods Market in Vancouver, BC. Each brand consisted of 27 bottles each.

### **Kombucha Brands**

The 3 different kombucha tea brands that were used in this research are Health-Ade, Pure+, and RISE.

### **Apparatus**

The analysis of ethanol concentration within each kombucha tea brands was done using GC-FID. The instrument model used was a Hewlett Packard (HP) 6890 Series Gas Chromatograph (GC)-FID coupled with a HP 7683 Series Injector.

### **Reagents**

The reagents used to create the working stocks and calibration standards are 95% ethanol ACS reagent, 99.5% N-propanol ACS reagent, and ultrapure deionized water.

### **Standard and Sample Preparation**

Standard and sample preparations were done in BCIT's chemistry laboratory at SW3, Room 4680. First, a set of ethanol calibration standards were made to 0.00% (blank), 0.10%, 0.20%, 0.50%, 1.00%, 2.00%, and 3.00%. Furthermore, a final 10% internal standard were added to each calibration standard. The calibration standards were then used to calibrate the instrument.

Once the calibration standards were made, 27 kombucha bottles, 9 bottles each brand, were prepared immediately for analysis of the ethanol concentration to acquire the baseline level for each brand before storage. On the other hand, 27 new bottles, 9 bottles each brand, were incubated under room temperature (22°C) for 3 weeks prior to sample preparation for analysis of the mean ethanol concentration. Similarly, the 27 bottles used to analyze the baseline ethanol concentration were re-capped and incubated under refrigeration temperature (4°C) for 3 weeks prior to analysis.

Samples were prepared by adding internal standard to the kombucha tea followed by diluting it with ultra-pure water. This was done with all the kombucha bottles across the 3 brands. Similarly, a set of sample spikes were also prepared for the baseline ethanol concentration sample set to ensure accuracy and reliability of the analysis. Furthermore, the baseline ethanol concentration sample set were spiked with ethanol.

### **Sample Analysis**

Sample analysis was done in the BCIT's chemistry laboratory at SW3, Room 4635 and in accordance to AOAC International's (2016) standardized minimum method requirements of analyzing ethanol in kombucha tea.

The instrumental analysis conditions were followed as per the instructions given by Ebersole *et al.* (2017) except for the injection volume which was modified to 100µL as opposed to 200µL.

After the conditions were set, instrumental analysis using GC-FID began. First, the instrument was calibrated using the calibration standards. Then, the prepared samples and/or spiked samples were analyzed

using single injection via headspace. The peak areas were then measured to determine the ethanol concentration in each sample. Furthermore, within each kombucha brand, a mean ethanol concentration was calculated between the 9 bottles in each brand and storage condition (i.e.: no storage, refrigeration, or room temperature).

## Results

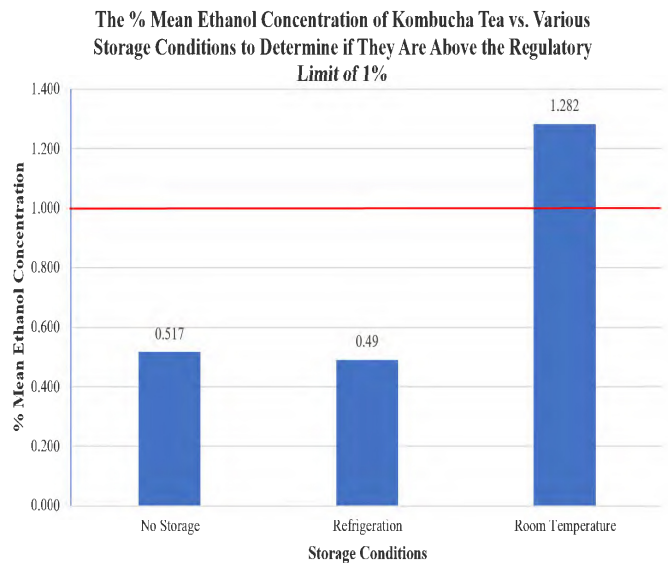
### Description of Data

In this experiment, numerical data was collected on a continuous and ratio scale (Heacock, 2019b).

Furthermore, the data was measured in % ethanol by volume within each sample and then averaged to obtain a mean ethanol concentration within each brand at various storage conditions (i.e.: no storage, refrigeration, and room temperature). Thereafter, the dataset within each brand at various storage conditions were then categorized as to whether they met the regulatory limit of having less than 1% ethanol concentration by volume.

### Descriptive Statistics

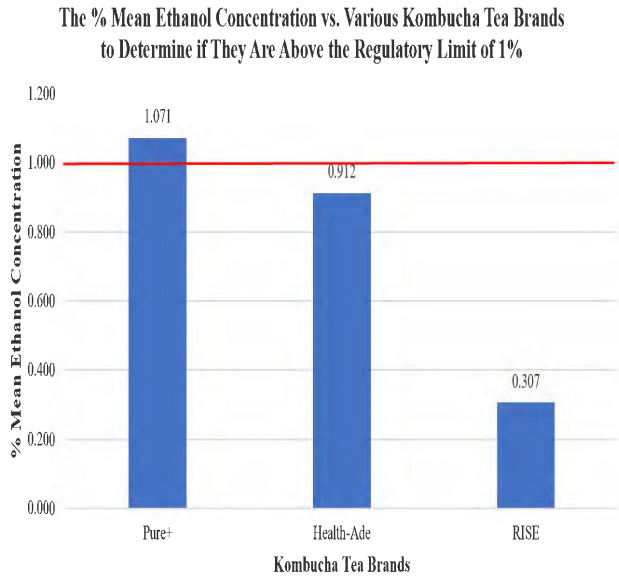
The figure below shows the mean ethanol concentrations across various storage conditions to determine whether there is a difference in ethanol concentrations due to storage and whether they exceed the 1% regulatory limit.



**Figure 1: The % Mean Ethanol Concentration of Kombucha Tea vs. Various Storage Conditions to Determine if They Are Above the Regulatory Limit of 1%.**

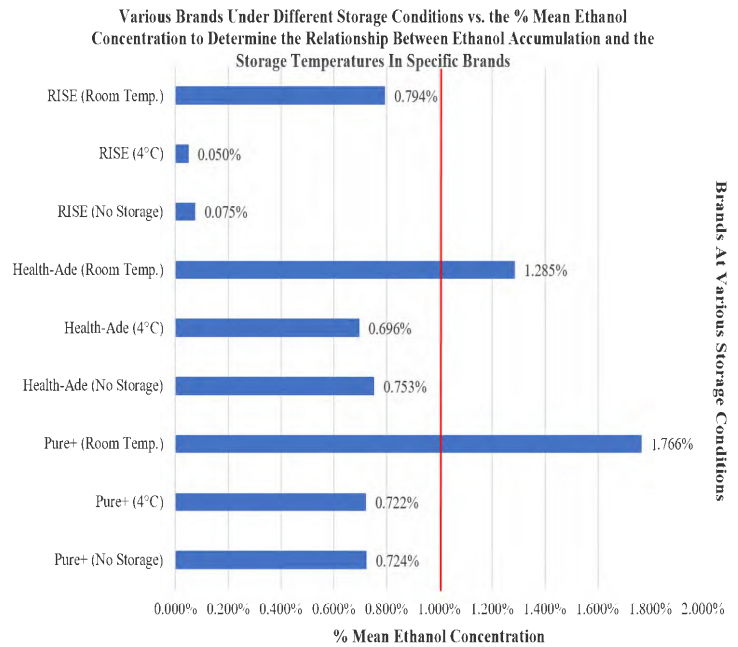
In Figure 1, it was observed that across all kombucha tea brands, in general, the no storage and refrigeration conditions were well below the regulatory limit of 1% ethanol by volume as defined by the *Liquor Control and Licensing Act*. However, the refrigeration condition showed to have a slight drop in the mean ethanol concentration from 0.517% to 0.490% after 3 weeks of storage. Also, the kombucha tea stored under room temperature for 3 weeks showed to have an increase in the mean ethanol concentration of 1.282% which is over the regulatory limit.

Similarly, below shows the % mean ethanol concentrations across various kombucha tea brands and whether they adhere to the 1% regulatory limit.



**Figure 2: The % Mean Ethanol Concentration vs. Various Kombucha Tea Brands to Determine if They Are Above the Regulatory Limit of 1%.**

In Figure 2, only Pure+ had a mean ethanol concentration of 1.071% which is over the regulatory limit. On the other hand, Health-Ade and RISE were shown to be below the ethanol regulatory limit. Nonetheless, Health-Ade and RISE mean ethanol concentrations were at 0.912% and 0.307% respectively. Finally, Figure 3 shows a summary of the descriptive statistics of the % ethanol concentrations of various brands under varying storage conditions.



**Figure 3: Various Brands Under Different Storage Conditions vs. the % Mean Ethanol Concentration to Determine the Relationship Between Ethanol Accumulation and the Storage Temperatures in Specific Brands.**

The above figure shows that, in general, room temperature storage for 3 weeks increased the mean ethanol concentration in all kombucha tea brands. Furthermore, the mean ethanol concentrations of Pure+, Health-Ade, and RISE after room temperature storage were 1.766%, 1.285%, and 0.794% respectively. However, only Pure+ and Health-Ade were over the regulatory limit. On the other hand, all kombucha tea brands showed a slight decrease in the mean ethanol concentration when refrigerated for 3 weeks.

### Inferential Statistics

The statistical software that was used for analyzing the datasets was NCSS (2019). Furthermore, the inferential statistics used were one-sample t-test, one-way ANOVA, and Scheffe's Multiple-Comparison Test (Heacock, 2019c, d). Below is a table showing a summary of the inferential statistics results:

**Table 2: Summary of Inferential Statistics**

<b>H<sub>0</sub> and H<sub>A</sub></b>	<b>Test(s) used</b>	<b>Result</b>	<b>Conclusion (If relevant, Type I/II errors and Scheffe's Multiple-Comparison Test results)</b>
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of Pure+ without storage is less than or equal to 1% by volume.  <b>H<sub>A</sub>:</b> The mean ethanol concentration of Pure+ without storage is greater than 1% by volume.</p>	One-sample t-test	p-value = 1.000 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>0</sub> and conclude that without storage there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of Health-Ade without storage is less than or equal to 1% by volume.  <b>H<sub>A</sub>:</b> The mean ethanol concentration of Health-Ade without storage is greater than 1% by volume.</p>	One-sample t-test	p-value = 1.000 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>0</sub> and conclude that without storage there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of RISE without storage is less than or equal to 1% by volume.  <b>H<sub>A</sub>:</b> The mean ethanol concentration of RISE without storage is greater than 1% by volume.</p>	One-sample t-test	p-value = 1.000 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>0</sub> and conclude that without storage there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of Pure+ at refrigeration is less than or equal to 1% by volume.  <b>H<sub>A</sub>:</b> The mean ethanol concentration of Pure+ at refrigeration is greater than 1% by volume.</p>	One-sample t-test	p-value = 1.000 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>0</sub> and conclude that under refrigeration there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of Health-Ade at refrigeration is less than or equal to 1% by volume.  <b>H<sub>A</sub>:</b> The mean ethanol concentration of Health-Ade at refrigeration is greater than 1% by volume.</p>	One-sample t-test	p-value = 1.000 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>0</sub> and conclude that under refrigeration there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of RISE at refrigeration is less than or equal to 1% by volume.  <b>H<sub>A</sub>:</b> The mean ethanol concentration of RISE at refrigeration is greater than 1% by volume.</p>	One-sample t-test	p-value = 0.996 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>0</sub> and conclude that under refrigeration there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<p><b>H<sub>0</sub>:</b> The mean ethanol concentration of Pure+ at</p>	One-sample t-test	p-value = 0.000	Reject H <sub>0</sub> and conclude that under room temperature there is a statistically significant mean ethanol concentration above



room temperature is less than or equal to 1% by volume. <b>H<sub>A</sub></b> : The mean ethanol concentration of Pure+ at room temperature is greater than 1% by volume.		Power ( $\alpha = 0.01$ ) = 100.0%	the regulatory limit. Power = 100.0%, therefore there is truly a difference between Pure+ stored at room temperature and the regulatory limit.
<b>H<sub>O</sub></b> : The mean ethanol concentration of Health-Ade at room temperature is less than or equal to 1% by volume. <b>H<sub>A</sub></b> : The mean ethanol concentration of Health-Ade at room temperature is greater than 1% by volume.	One-sample t-test	p-value = 0.000 Power ( $\alpha = 0.01$ ) = 100.0%	Reject H <sub>O</sub> and conclude that under room temperature there is a statistically significant mean ethanol concentration above the regulatory limit. Power = 100.0%, therefore there is truly a difference between Health-Ade stored at room temperature and the regulatory limit.
<b>H<sub>O</sub></b> : The mean ethanol concentration of RISE at room temperature is less than or equal to 1% by volume. <b>H<sub>A</sub></b> : The mean ethanol concentration of RISE at room temperature is greater than 1% by volume.	One-sample t-test	p-value = 1.000 Power ( $\alpha = 0.01$ ) = 0.000%	Fail to reject H <sub>O</sub> and conclude that under room temperature there is not a statistically significant mean ethanol concentration above the regulatory limit. Type II error exist because Power = 0.000%. To minimize Type II error, increase sample size (Heacock, 2019a).
<b>H<sub>O</sub></b> : There is no difference in the mean ethanol concentrations between no storage, refrigeration, and room temperature. <b>H<sub>A</sub></b> : There is a difference in the mean ethanol concentrations between no storage, refrigeration, and room temperature.	One-way ANOVA, Scheffe's Multiple-Comparison Test	p-value = 0.000 Power ( $\alpha = 0.05$ ) = 100.0%	Reject H <sub>O</sub> and conclude that there is a statistically significant difference in the mean ethanol concentrations between different storage conditions. Scheffe's Multiple-Comparison Test shows that only room temperature storage is statistically different from no storage and refrigeration. Power = 100.0%, therefore there is truly a difference between storage conditions.

## Discussion

The descriptive data shows that the 3 kombucha tea brands at baseline ethanol content are below the regulatory limit of 1% alcohol by volume (ABV). These findings were in contradictory to both Talebi *et al.* (2017) and Fung *et al.* (2019) studies where they found that all or most of their commercial kombucha tea products at baseline were well above the 1% ABV regulatory limit. These discrepancies may be due to the public, manufacturers, and regulatory agencies being more aware of the issue as of present, thus, having more stringent policies in place to prevent the percent alcohol

from going above the limit. Likewise, this discrepancy may also be a result of different manufacturing practices which led to varying difference in ethanol concentrations between brands.

Similarly, the data suggests that there is a strong association between ethanol accumulation in kombucha tea and room temperature storage. However, only Pure+ and Health-Ade were above the 1% regulatory limit after the 3 weeks incubation period. Comparably, Talebi *et al.* (2017) findings shows a similar upward trend in ethanol accumulation of kombucha tea under room temperature storage. On the other hand, in Fung

*et al.* (2019) study one of his kombucha tea brands showed a decrease in ethanol content when stored under room temperature for a week. He further mentions that this decrease in trend may be due to the evaporation when stored in gas-tight vials and that the brand, Kevita, is a manufacturer that pasteurize their kombucha products prior to shipment. Nonetheless, this may explain the reason for the discrepancies of the results.

On the contrary, the descriptive and inferential data also suggests that storage under refrigeration has no statistically significant difference in ethanol content compared to the 3 kombucha tea brands' baseline level. However, based on the descriptive data, all 3 kombucha tea brands showed a slight decrease in ethanol content after being stored under refrigeration for 3 weeks.

These results refute Talebi *et al.* (2017) findings where he observed that there is an increase in ethanol content in various kombucha tea brands in both unopened and opened bottles under refrigeration for 60 days. The most probable explanation for this discrepancy may be due to the type SCOBY used between brands that enable better efficiency and further conversion of ethanol into acetic acid.

Overall, the results suggest that storing kombucha tea under room temperature for 3 weeks increased the ethanol content drastically. Both the Pure+ and Health-Ade after 3 weeks incubation under room temperature exceeded the 1% ABV regulatory limit while RISE did not. Similarly, out of the 3 kombucha tea brands analyzed, RISE is the safest as it is the only one that conforms to the 1% limit after 3 weeks of room temperature storage. Conversely, storing kombucha tea under refrigeration will slow down and potentially decrease the amount of ethanol within the product itself, making it comply with current regulations.

## **Knowledge Translation**

The findings from this research can be translated into a variety of ways. For instance, the results from this study can provide scientific evidence to health authorities and other agencies in educating and advising the public on the possible ethanol accumulation in kombucha tea due to improper storage (i.e.: room temperature). For example, BCCDC may update their current Food Issue Note regarding the safety of kombucha tea and how to properly store them to avoid possible health effects due to further fermentation.

Comparably, the findings in this study can also influence Environmental Health Officers' routine inspection of small kombucha tea manufacturers. For instance, Environmental Health Officers will be more proactive in looking for potential sources of temperature abuse when inspecting these small kombucha tea manufacturers because the results from this study strongly associate ethanol accumulation with room temperature storage. In addition, these findings may lead to possible policies being developed from health authorities to ensure that ethanol accumulation is minimized. For example, health authorities may require all kombucha tea manufacturers to have a pasteurization step prior to packaging.

Lastly, these findings may translate into CFIA having to modify their labelling requirements for kombucha tea as there are increasingly more evidence suggesting that they exceed the regulatory limit of being considered as a non-alcoholic beverage. Further on that note, these findings will then open possible committee meetings between CFIA and the Liquor Control Board on whether which regulatory agency is responsible for inspecting these kombucha tea manufacturers as

evidence can prove that kombucha tea products can be categorized as a liquor.

## **Limitations**

Some limitations that were presented in this research was mainly due to time and economic constraints. For instance, the research was time restricted where only one analysis was conducted at one time point, after 3 weeks, as opposed to analyzing the ethanol accumulation at multiple time points within the 3 weeks time frame. This limitation prevented the use of a correlation and regression curve to establish a relationship between ethanol accumulation at different storage temperatures in relation to time. Similarly, the time availability of the Chemistry Professor, Hsin, that helped run the instrumental analysis inhibited the number of samples that were run and interpreted. Therefore, the samples were only run once without any duplicates or triplicates.

An economic constraint within the experimental design was not being able to purchase enough kombucha tea bottles to ensure a sufficient sample size so that Type II error is minimized during the statistical analysis. In addition, the economic constraints did not also permit another set of 27 bottles of kombucha tea to be purchased to analyze the effect of ethanol accumulation due to refrigeration. Thus, the methodology was compromised by reusing the baseline sample set for the refrigeration condition which ultimately affected the soundness of the experiment.

To improve the experimental design of this study, internal and external validity should be considered. Furthermore, internal validity can be improved in a variety of ways. For instance, triplicates should be used to increase the repeatability/reliability of the

experimental design. Also, purchasing sealed sample set for the refrigeration condition as opposed to repurposing the baseline sample set will increase the soundness where the study can then directly assess the relationship between various storage temperatures and ethanol accumulation. Similarly, analyzing the ethanol accumulation at various time points within different storage temperatures will allow for a correlation and regression analysis. Thus, it will permit an accurate conclusion about the cause of the ethanol accumulation in relation to time within different storage conditions and at which point in time has the highest ethanol accumulation.

Finally, a recommendation to improve the generalizability of the data to all commercially produced kombucha tea, the external validity should be extended to incorporate kombucha tea brands that are pasteurized, have added probiotics or preservatives, and/or had a post-treatment process done prior to packaging.

## **Future Research**

Some potential areas of research that are presented based on this study are as follows:

- Analyze the ethanol accumulation under refrigeration and room temperature conditions at various time intervals to determine the relationship between ethanol accumulation and time. Thus, performing a regression and correlation test of the results.
- Re-test the reliability of the results to see if the ethanol content increase, decrease, or remain constant under refrigeration as there was a discrepancy in the results between this research and in Talebi *et al.* (2017) study.

- Analyze commercially produced kombucha tea brands that are either pasteurized, have added probiotics and preservatives, or have undergone a post-treatment step to see if a process or additive has any effect on ethanol accumulation after packaging.
- Conduct a survey on the public's knowledge and risk perception of kombucha tea potentially having alcohol levels over the regulatory limit of 1%.

## **Conclusion**

Overall, the results suggest that room temperature storage of the 3 kombucha tea brands for 3 weeks increased the ethanol levels significantly. Both the Pure+ and Health-Ade after 3 weeks of room temperature incubation have ethanol levels over the 1% ABV regulatory limit while RISE was the only brand that was below it. Thus, this may present possible health risk for susceptible population if they were to drink kombucha tea from Pure+ and Health-Ade that is stored under room temperature for prolong periods of time. On the other hand, all the kombucha tea brands stored under refrigeration showed a slight decrease in ethanol levels relative to the baseline. Furthermore, the data suggests that storing kombucha tea under refrigeration will minimize ethanol accumulation due to fermentation.

Finally, the information obtained from this study may be used to develop guidelines and policies in regulating

kombucha tea manufacturers so that ethanol accumulation is minimized during the production step. Thus, it will limit the public and/or susceptible population in exceeding recommended serving sizes due to discrepancies in labelling. Similarly, these results may initiate possible CFIA labelling requirement changes towards kombucha tea products and open committee meetings between CFIA and the Liquor Control Board to discuss possible roles and responsibilities. Lastly, the findings from this study can also be used by health professionals and other agencies as a tool to educate the public that kombucha tea may undergo further ethanol fermentation due to improper storage and their associated health affects to susceptible population.

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## **Competing Interest**

The authors declare that they have no competing interests while conducting this study.

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