Kombucha: An Exploration of Microbial Composition, Brewing Conditions, and the Effect of Fermentation Time on the Rate of Alcohol Production

Erin-Leigh Gallop
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Abstract

Kombucha is a fermented tea created with the help of a symbiotic culture of yeast and bacteria. The presence of bacteria and yeast in the broth of kombucha, and not solely in the top-floating pellicle layer, permit fermentation and alcohol production to continue after the "mother" SCOBY has been removed and after bottling has occurred for mass consumption. In the normal metabolic path of kombucha fermentation, the yeast breaks down the disaccharide sucrose into the monosaccharides glucose and fructose. However, the microbial composition of kombucha varies. This study included the microbial identification of a species of kombucha currently being marketed in the United States. Through isolation, gram staining, bacterial DNA extraction, PCR, and Agarose Gel Electrophoresis, the bacteria used in this study was identified as lactobacillus. Using traditional brewing preparation methods for kombucha, ideal brewing conditions were identified; samples of kombucha incubated in 26°C decreased in color during the initial 14-day fermentation and what was initially a slightly brown-colored liquid became more opaque and slightly yellow in color. Lastly, the effect of temperature on alcohol production was observed. When brewed in ideal conditions, 0.0% – 0.5% alcohol was produced within 6 weeks of bottling.
Thesis Signature

MONTCLAIR STATE UNIVERSITY

Kombucha: An Exploration of Microbial Composition, Brewing Conditions, and the Effect of Fermentation Time on the Rate of Alcohol Production

by

Erin-Leigh Gallop

A Master’s Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Masters of Science

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KOMBUCHA: AN EXPLORATION OF MICROBIAL COMPOSITION, BREWING CONDITIONS, AND THE EFFECT OF FERMENTATION TIME ON THE RATE OF ALCOHOL PRODUCTION

A THESIS

Submitted in partial fulfillment of the requirements
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ERIN-LEIGH GALLOP

Montclair State University
Montclair, NJ
2011
# TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... 1

THESIS SIGNATURE ............................................................................................................. 2

TABLE OF CONTENTS ........................................................................................................ 4

ACKNOWLEDGEMENTS ....................................................................................................... 5

LIST OF FIGURES ................................................................................................................ 7

INTRODUCTION ................................................................................................................... 8

HISTORY .................................................................................................................................. 8

RATIONALE ........................................................................................................................... 9

STUDY AIMS ....................................................................................................................... 12

OBJECTIVES ....................................................................................................................... 12

HYPOTHESIS ....................................................................................................................... 12

MICROBIOLOGICAL COMPOSITION ................................................................................ 13

MATERIALS ............................................................................................................................ 13

METHODS ................................................................................................................................ 14

ISOLATION ................................................................................................................................ 14

GRAM STAIN .......................................................................................................................... 14

BACTERIAL DNA EXTRACTION .......................................................................................... 15

POLYMERASE CHAIN REACTION ....................................................................................... 16

AGAROSE GEL ELECTROPHORESIS ..................................................................................... 17

NUTRITION & FOOD SCIENCE ......................................................................................... 18

MATERIALS ............................................................................................................................ 18

METHODS ................................................................................................................................ 18

RESULTS .................................................................................................................................. 20

OBSERVATIONS .................................................................................................................... 22

DATA COLLECTION ............................................................................................................... 23

FINDINGS ................................................................................................................................ 26

IMPLICATIONS IN MARKETING & CONSUMER HEALTH .................................................. 27

REFERENCES .......................................................................................................................... 29
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List of Figures

Figure 1. Discontinuous streak from kombucha sample K1 isolated in culture.

Figure 2. Isolated bacteria from kombucha sample K1 in culture.

Figure 3. Bacteria isolated from sample K1 of Kombucha, gram-positive bacilli. (1000X).

Figure 4. Yeast Isolated from Sample k1 of Kombucha (1000X).

Figure 5. PCR amplification.

Figure 6. New SCOBY produced in sample K1 at 56 days, measuring 2mm thick.
Introduction

History

Kombucha is a fermented tea created with the help of a symbiotic culture of yeast and bacteria (Bauer-Petrovska et. al, 2008). It is known by many names; Kombucha has been called the Manchurian Mushroom or Tea Fungus, and was formally traded in the Orient under the name Mo-Gu (Hartmann et al., 2000). Kombucha finds its roots in Chinese, Japanese, Manchurian, Russian and Tibetan cultures and can be traced as far back as 220BC in China (Hartmann, et al., 2000), where local monks often receive credit for its discovery and initial cultivation (Jarrell et. al, 2000).

Kombucha has been consumed throughout Asia for thousands of years, with the habit spreading as new trade routes opened. Regular consumption became widespread in Europe through World War II, and declined when the necessary brewing ingredients became scarce (Hartmann et al., 2000). Recently, the consumption of kombucha has gained popularity in American society. The sweet-sour beverage has caught the attention of American consumers for reasons stemming from its many claimed health benefits. Kombucha is purported to have a positive effect on many conditions including improvement of T-Cell count in AIDS patients, relief of arthritic symptoms, the rejuvenation of hair in balding persons, and even the cure of cancer (Hartmann et al., 2000). Unfortunately, despite the volume and range of these claims, little research has been conducted to substantiate them. However, Hartman et al. (2000) conducted a 3 year study on mice which did potentially support claims of weight loss, increased longevity,
and suggests that kombucha may have a positive effect on digestion and nutrient absorption.

Rationale

Kombucha has received criticisms for its potential health risks. Reports of hepatotoxicity and necrotizing pancreatitis, have earned kombucha a split reputation, being either praised as a miracle cure-all or chastised as hazardous medicinal tea (Hartmann et al., 2000). Recently, kombucha has been flagged for its potential alcohol content. In June of 2010, kombucha was removed from shelves of Whole Foods Market, a current major grocery chain in America, due to potentially elevated levels of alcohol, which were believed to be in excess of the legal limit for “non-alcoholic” beverages. According to the FDA (2010), beverages that are marketed to consumers to as “non-alcoholic” may contain traces of alcohol, less than 0.5 percent alcohol by volume, provided that the alcohol is the result of the use of flavoring extracts or is a product of natural fermentation.

During the fermentation process, sugar is converted into alcohol and carbon dioxide by sugar-loving yeast (Mo et. al, 2008). The bacteria do not possess the necessary hydrolases and kinases to make use of the disaccharide sucrose. In the normal metabolic path of kombucha fermentation, the yeast breaks down the sucrose into the monosaccharides glucose and fructose (Cvetkovic et al., 2008). The glucose and fructose are then fermented into ethanol, which is later used by the bacteria to produce acid. As a result, kombucha contains trace amounts of alcohol, has an acidic taste, and possesses a naturally occurring effervescence. It is hypothesized that alcohol production in kombucha
is strongly influenced by type of tea (green tea, black tea, etc.), amount of sugar, incubation temperature and fermentation time.

Much of what is known about kombucha from research has been gained by studying batches of kombucha, which have been allowed to ferment for a period of 7 days (Mo et al., 2008). Many home brewers follow a recipe that recommends a 7-10 day fermentation time (Laurel Farms, 2010). However popular American brands, such as GT’s Synergy, allow their kombucha to ferment for 30 days. This discrepancy in initial fermentation time may present problems with generalizing what is learned about kombucha in the lab to what is actually being consumed by American consumers. Prolonged fermentation is not recommended due to the accumulation of organic acids that may reach potentially harmful levels for direct human consumption (Chu & Chen, 2006). The scientific community must establish a standard fermentation time to be used both in the mass-production of kombucha, and for purposes of research. Standardization of fermentation time in kombucha preparation may provide consumers with the tools to make educated choices regarding their storage and consumption behaviors of this beverage.

Kombucha has been traditionally prepared in the home. However, in recent years, kombucha has become commercially available at grocery stores, health food stores, and delis, among other retail venues. Commercially produced Kombucha has the misfortune of travelling across long distances to reach most consumers. One major producer in the United States, for example, is headquartered in California. The shipping method of choice remains truck, as it is with most major food products in this country. As kombucha is an unpasteurized, probiotic beverage, such transportation conditions may encourage the fermentation process to continue long after bottling. This stimulates
increased activity in the bacteria and yeast leading to the increased production of alcohol and carbon dioxide, which can result in a beverage that is above the legal alcohol limit, before the product has even reached it's final destination.
Study Aims

Objectives

This study may establish a standard fermentation time for kombucha and establish a shelf-life for kombucha. This study may also provide food scientists with a standard means of kombucha brewing preparation for future research.

Hypothesis

It is hypothesized that the microbiological composition of kombucha contains the probiotic species lactobacillus.

Temperature maybe a determining factor in the rate of alcohol production in kombucha. It is hypothesized that Kombucha samples stored at room temperature or higher will have a higher alcohol percentage than kombucha stored in cold, refrigerated temperatures and that kombucha stored in a refrigerator will maintain an alcohol percentage of < 0.5% for at least 6 weeks. Additionally, it is hypothesized that kombucha allowed to ferment at room temperature will yield a healthy and desired broth as interpreted by sensory qualities, while kombucha fermented in warmer temperatures will yield kombucha unfit for human consumption.
Microbiological Composition

The microbial composition of kombucha varies. Geographic & climactic conditions of cultivation and the local species of wild bacteria & yeast all help to determine the exact microbial composition of kombucha (Mayser et al., 1995). It is with this rationale in mind that the design of this study contains a microbiological component, in which the microbial composition of the specific kombucha being used throughout this study could be determined.

This component of the study began with a sample of brewed kombucha, Sample K1. Sample K1 is one of four samples of kombucha brewed as part of this study and was selected for the microbiological component study due to its favorable sensory properties; Sample K1 smelled sour, tasted sweet, was carbonated, and appeared a clear to opaque yellow color, all of which are desirable traits of kombucha. A sample of K1 was used to identify the microbial composition of the kombucha used in this study. Bacteria and yeast were isolated from this broth and used to conduct the microbial identification processes of this study.

Materials

The microbiological component of this experiment was conducted using Kombucha sample K1. Nutrient agar plates, YEPD plates, swabs, inoculator loops, and Bunsen burners were used for the isolation of the bacteria and yeast. Incolulater loops, Bunsen burners, isopropyl alcohol, crystal violet, iodine, safranin, distilled water, and glass slides were used to perform a gram stain. Sterile water, an Eppendorf centrifuge 54145, a vortex, and pipettes were used to extract bacterial
DNA. Bio-rad Instagene Matrix, SNA primers, Fermentas PCR Master Mix were used to conduct the polymerase chain reaction. Lastly, were used to conduct agarose gel electrophoresis.

**Methods**

**Isolation**

A sample of K1 broth was used to inoculate onto nutrient agar and YEPD plates, in an attempt to isolate a pure culture. The treated plate was allowed to incubate upside down at 37°C for two days. Using the discontinuous streaking method, the growth from the plate was transferred to a new nutrient agar plate or YEPD plate and again allowed to incubate at 37°C for two days.

When grown in culture, bacteria and yeast are easily distinguishable by size (Jarrell et. al, 2000). Two distinct growths were observed; In culture, the bacteria were observed as small, colony forming units, while the yeast were observed as thicker streaks of microbial growth. The isolation process was repeated 3 times, yielding separate pure cultures of bacteria on nutrient agar plates and yeast on YEPD plates. The continuous streaking plates were then prepared for bacteria and yeast stocks.

**Gram Stain**

Using the isolated pure bacteria culture, a smear was prepared with the use of an inoculation loop, distilled water, and a glass slide. The sample was heat fixed to the slide and a gram stain was performed. The smear was flooded with the stain crystal violet for one minute. After the excess crystal violet was poured off, the smear was washed gently with distilled water and allowed to air-dry for 30 seconds. The smear
was flooded with iodine, a mordant, for one minute. After the excessive iodine was poured off, the smear was washed gently with distilled water and allowed to air-dry for 30 seconds. The smear was washed with a gentle flowing stream of alcohol, a decolorizing agent, for roughly 30 seconds. The smear was washed gently with distilled water and allowed to air-dry for 30 seconds. The smear was flooded with the counterstain safranin for one minute. The smear was washed gently with distilled water and allowed to air-dry for 30 seconds. The smear was blotted gently with blotting paper. Each slide was then observed using a compound light microscope and the results of the gram stain were recorded.

**Bacterial DNA Extraction**

Using the isolated bacteria culture form the K1 sample of kombucha, the DNA of the bacteria was extracted using the Chelex method. This method was selected for its speed and ease of use. This began with 500 microliters of sterile water, which was inoculated with a swab of bacteria from K1 bacterial pure culture. After cells were suspended, it was centrifuged using Eppendorf centrifuge 54145 at 12000 rpm for a period of 2 minutes. The water was then removed using a pipette and the sample re-suspended in 200 microliters of bio-rad instagene matrix, vortexed for a period of ten seconds, and finally incubated in water at 56 degrees C for a period of 2 hours.

When removed from the 56°C water bath, the samples were then vortexed for 15 seconds. The samples were then introduced into a new water bath, and allowed to incubate at 100°C for a period of ten minutes. The samples were then
removed and centrifuged for 3 minutes at 10,000 rpm. The supernatants were then separated and stored in new tubes. All samples were stored at -20°C.

**Polymerase Chain Reaction**

The universal primers LbLMA1 forward primer and R16-1 reverse primers were used to PCR general lactobacillus species (Dubernet et al., 2002). Primer ACIDO forward primer and R16-1 reverse primer is used to PCR specifically L. Acidophilus; Primer CASEI for forward primer and R16-1 reverse primer for L. casei and Primer LAC for forward primer and R16-1 reverse primer for common lactobacilli.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>LbLMA1</td>
<td>CTCAAAACTAAACAAAGTTTC</td>
</tr>
<tr>
<td>R16-1</td>
<td>CTTGTACACACCGCCCGTCA</td>
</tr>
<tr>
<td>ACIDO</td>
<td>TGAACCAACAGATTCA</td>
</tr>
<tr>
<td>CASEI</td>
<td>CTGAGATTCACCTAAAAACG</td>
</tr>
<tr>
<td>LAC</td>
<td>TGACGACARCCARGCAACCA</td>
</tr>
</tbody>
</table>

Table 1. Selected Primer, DNA sequences used for PCR

The DNA isolated from the chelex method was used to carry out PCR-based assay. 12.5 uL of Fermentas Master Mix was pipetted into a sterile 0.2 ml PCR reaction tube along with 5.5 ul of sterile water, 1 uL Forward Primer and 1 uL Reverse Primer, and 5 uL of DNA extract. The other set of the PCR reaction have the same amounts of master mix and primers, except the DNA sample was 1 ul with 9.5 ul sterile water. The reaction tubes were placed into the thermal cyclers with the following conditions: Initial denaturation for 2 minutes at 95°C, denaturation for 1 minute at 95°C, annealation of primers for 30 seconds at 50°C, extension of DNA for 2 minutes at 72°C, repetition of the previous 4 conditions for 35w cycles, final extension for 10 minutes at 72°C, and finally cooling at 4°C.
**Agarose Gel Electrophoresis**

The agarose gel was prepared by adding 0.8 gram of agarose powder and 1 ul of ethidium bromide to 100 ml buffer solution. The total solution was then melted in microwave until it became clear in color and allowed to cool to 55°C. The mixture was poured into a casting tray and allowed to solidify. The gel comb was placed into one end of the mixture after it was poured in order to produce the wells. Once solid, the gel was placed into the electrophoresis chamber, and covered with a buffer solution.

6 µl of 6X Sample Loading Buffer was then added to each 25 µl PCR reaction. The samples were dyed and centrifuged. 15 µl of each sample was carefully pippetted into separate wells in the gel. 15 µl of the DNA ladder standard was pipetted into lane 1 of the gel. The gel was run at approximately 100 to 115 V for about an hour. The gels were analyzed under UV light using a Kodak Image Station 440CF (Perkin Elmer Life Sciences, Waltham, MA).
Nutrition & Food Science

Materials

The materials for the food science component of this experiment include a sample of Kombucha Culture - A SCOBY (Symbiotic culture of bacteria and yeast), 4 400ml glass beakers, 56 8.5oz Glass bottles with caps, shrink wrap, two incubators, and a refrigerator. The kombucha used in this experiment kombucha was brewed with the use of distilled water, green tea, and sugar. Lastly, to measure the specific gravity of the samples throughout the experiment, hydrometers were used.

Methods

The dependent variables of this experiment are fermentation time & incubation temperature. The independent variable is alcohol percentage of the broth.

The methodology for this component of the study began with the traditional preparation of kombucha tea: 5 cups of distilled water was brought to a roaring boil, then allowed to cool for a period of 5 minutes. 2 Lipton brand green tea bags were allowed to steep in the hot water for ten minutes. The tea bags were then removed and discarded. 2000ml distilled water, 200g of white sugar, and 125ml of mother broth (previously fermented tea) were added to create a green tea mixture.
Kombucha 1 (K1) 1.028
Kombucha 2 (K2) 1.028
Kombucha 3 (K3) 1.028
Kombucha 4 (K4) 10.28

Table 2. Basal Specific Gravity Readings of all Kombucha samples
*The temperature of the liquid at the time of recording affects the reading. The readings displayed represent the specific gravity as it has been adjusted for the temperature of the liquid being tested.

The green tea mixture was prepared uniformly in 4 400ml glass beakers. A symbiotic culture of bacteria and yeast (SCOBY) weighing 100g was laid on the surface of the liquid in each beaker, and then covered with a thin cloth. 2 batches were then incubated at 26°C and 2 batches were incubated at 35°C, where they were allowed to ferment for a period of fourteen (14) days. The initial fermentation time of 14 days was incorporated into the design of this study as a middle ground between home brewed practices, and commercially brewed practices. On the 14th day of fermentation, the beakers were removed from the incubators, and each starter SCOBY was removed from the beakers. The specific gravity of each kombucha mixture was recorded.

All 4 kombucha samples were transferred into 8.5oz glass bottles and sealed. The excess broth was bottled and stored for used in the microbiological component of this study. Unused samples were later discarded. 12 bottles of each sample were stored at 4°C, 12 of each bottle were stored at 26°C, and 12 bottles of each sample were stored at 35°C. A total of 36 bottles were utilized for data collection. Each week, one bottle of each sample, at each temperature was opened and the specific gravity of the contents recorded.
Results

The initial step of discontinuous streaking of sample K1 onto a nutrient agar plate yielded a pure sample of bacteria isolated in culture. Discontinuous streaking of sample K1 onto YEPD plate yielded a pure sample of yeast in culture.

Figure 1. Discontinuous streak from kombucha sample K1 isolated in culture.

Figure 2. Isolated bacteria from kombucha broth in culture
The slides created from K1 isolated bacteria and yeast cultures through the gram stain procedure yielded the observation of gram-positive bacilli (Figure 3) and large yeast cells (Figure 4).

Through bacterial DNA extraction, PCR using primer sequence TGACGACARCCARGCACCA and amplification of the PCR through agarose gel electrophoresis revealed the bacteria to be a lactobacillus.
Observations

When fermented in an ideal environment, kombucha forms a new macroscopic colony, which floats atop of the broth and takes the shape of the brewing container. This new growth is often referred to as a “baby” and has been described as a floating cellulosic pellicle layer at the top of the broth (Chu & Chen, 2006). Visual observations showed that samples K1 & K2 formed such a new layer after 14 days of fermentation, and again when bottled and stored at room temperature. However, such new colonies were not observed when brewed at any other temperature, nor were they observed at all in samples K3 or K4 at any time throughout the brewing process.

Figure 3. New SCOBY produced in sample K1 at 56 days, measuring 2mm thick

Sensory observations regarding each sample were recorded. It was found that storage of all samples in 35°C yielded foul smelling, brown colored broth, which
produced little or no carbonation. Storage of all samples in 26°C yielded sweet-smelling, yellow colored broth, which produced carbonation. Samples incubated in 26°C decreased in color during the initial 14-day fermentation; what was initially a slightly brown-colored liquid became more opaque and slightly yellow in color. These observations support the findings of Chu & Chen (2006) that components in the tea undergo microbial biotransformation or degradation during the kombucha fermentation process into smaller molecules.

**Data Collection**

Numerical data was collected through the measurement of the specific gravity of the liquid. This was done with the use of a hydrometer. When sugar is present (before fermentation) the hydrometer floats higher in the more dense liquid. After fermentation, the sugar is converted to alcohol, and the hydrometer will float lower. The difference in readings allows the determination of the percentage of alcohol in the kombucha. Alcohol percentage was recorded at the beginning of the study and every fourteen days thereafter for a period of 6 weeks.

The specific gravity of each mixture was recorded over the course of 56 days or 8 weeks, yielding the following data:
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>14 Days</th>
<th>28 Days</th>
<th>42 Days</th>
<th>56 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1-4°C</td>
<td>1.028</td>
<td>1.027</td>
<td>1.025</td>
<td>1.025</td>
<td>1.024</td>
</tr>
<tr>
<td>K1-26°C</td>
<td>1.028</td>
<td>1.027</td>
<td>1.027</td>
<td>1.027</td>
<td>1.024</td>
</tr>
<tr>
<td>K1-35°C</td>
<td>1.028</td>
<td>1.027</td>
<td>1.025</td>
<td>1.025</td>
<td>1.025</td>
</tr>
<tr>
<td>K2 - 4°C</td>
<td>1.028</td>
<td>1.028</td>
<td>1.026</td>
<td>1.026</td>
<td>1.026</td>
</tr>
<tr>
<td>K2- 26°C</td>
<td>1.028</td>
<td>1.028</td>
<td>2.028</td>
<td>1.028</td>
<td>1.028</td>
</tr>
<tr>
<td>K2-35°C</td>
<td>1.028</td>
<td>1.028</td>
<td>1.027</td>
<td>1.027</td>
<td>1.027</td>
</tr>
<tr>
<td>K3-4°C</td>
<td>1.028</td>
<td>1.026</td>
<td>1.030</td>
<td>1.030</td>
<td>1.030</td>
</tr>
<tr>
<td>K3-26°C</td>
<td>1.028</td>
<td>1.026</td>
<td>1.035</td>
<td>1.035</td>
<td>1.035</td>
</tr>
<tr>
<td>K3-35°C</td>
<td>1.028</td>
<td>1.026</td>
<td>1.029</td>
<td>1.028</td>
<td>1.028</td>
</tr>
<tr>
<td>K4-4°C</td>
<td>1.028</td>
<td>1.026</td>
<td>1.034</td>
<td>1.031</td>
<td>1.032</td>
</tr>
<tr>
<td>K4-26°C</td>
<td>1.028</td>
<td>1.026</td>
<td>1.038</td>
<td>1.038</td>
<td>1.038</td>
</tr>
<tr>
<td>K4-35°C</td>
<td>1.028</td>
<td>1.026</td>
<td>1.035</td>
<td>1.035</td>
<td>1.035</td>
</tr>
</tbody>
</table>

Table 3. Specific gravity readings for all Kombucha samples, taken from Day 0 through Day 56 of the experiment.

Unexpected numbers in specific gravity were recorded for kombucha samples K3 and K4; the numbers recorded for K3 and K4 indicated a loss of liquid. It was hypothesized that the specific gravity measure would steadily decrease, as was recorded for samples K1 and K2, signifying a difference in the composition of solids in the liquid and therefore potentially, the production of alcohol.

The specific gravity was then converted into Alcohol by Volume percentage (ABV%) using the following equation:

\[(\text{Original Gravity} - \text{Final Gravity}) \times 131.25 = \text{ABV}\%\]
<table>
<thead>
<tr>
<th></th>
<th>14 Days</th>
<th>28 Days</th>
<th>42 Days</th>
<th>56 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1-4°C</td>
<td>0.13%</td>
<td>0.42%</td>
<td>0.42%</td>
<td>0.55%</td>
</tr>
<tr>
<td>K1-26°C</td>
<td>0.13%</td>
<td>0.13%</td>
<td>0.13%</td>
<td>0.52%</td>
</tr>
<tr>
<td>K1-35°C</td>
<td>0.13%</td>
<td>0.42%</td>
<td>0.42%</td>
<td>0.42%</td>
</tr>
<tr>
<td>K2 - 4°C</td>
<td>0%</td>
<td>0.29%</td>
<td>0.29%</td>
<td>0.29%</td>
</tr>
<tr>
<td>K2- 26°C</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>K2-35°C</td>
<td>0%</td>
<td>0.16%</td>
<td>0.16%</td>
<td>0.16%</td>
</tr>
</tbody>
</table>

Table 4. Total Percent Alcohol by Volume for Kombucha samples K1 & K2, taken from Day 14 through Day 56 of the experiment, adjusted for temperature.
Discussion

Findings

Lactobacillus was identified as the bacteria in sample K1 of kombucha. In many fermentation processes, the presence of bacteria is deemed a contamination as it results in levels of lactic acid, acetic acid, and other environmental conditions that inhibit the production of alcohol by the yeast (Ingledew, 1999). Fermented teas such as kombucha, however, are unique in their normal fermentation process due to the desired presence of both a bacteria and yeast to yield the end resulting brew. Through this symbiosis, the yeast ferments the sugar into ethanol, which is oxidized by the bacteria (Teoh, et al., 2004). When kombucha is brewed under favorable conditions, alcohol will be produced, due to the presence of yeast. However, temperature does not seem to be the only determining factor in the production of alcohol in properly brewed kombucha. Rather, bacterial concentration must also be taken into account when brewing. The concentration of bacteria in a particular sample may contribute to the varying levels of alcohol recorded. Though the levels varied, the overall totals observed remained within 0.5% of each other.

In addition to the inhibition of alcohol production, the presence of lactobacillus confirms that kombucha is a probiotic beverage. And so, regular consumption of kombucha may offer many of the same health benefits as consumption of any probiotic. And so, kombucha may be used as an alternative to dairy-based probiotic foods, such as yogurt, for inclusion in dairy-free diets.

Kombucha is best brewed when prepared in room temperature. The sensory qualities of each broth may have been more greatly influenced by temperature than was the
production of alcohol. Differences in color, odor, taste, and smell suggest that when stored at 35°C, the kombucha produced is not “healthy” and so it is recommended that kombucha be stored at refrigerated or room temperatures.

**Implications in Marketing & Consumer Health**

It is reported that half of Americans actively take a dietary supplement (Reedy, et al., 2005), including the frequent use of probiotics, such as kombucha. However, those consuming mass-produced kombucha have been doing so with no knowledge of how much alcohol they are consuming with each sip. Certain groups may be negatively affected by the accidental consumption of alcohol, such as alcoholics, children, pregnant women, and people operating heavy machinery.

This study illustrates the variation of alcohol levels that may occur due in part to brewing conditions in the production of kombucha. Due to the potential discrepancies, it is recommended for further study that the ABV% of commercially prepared kombucha be closely monitored. This scrutinization may aid in the prevention of accidental alcohol over-consumption by way of kombucha by these at-risk groups.

In this study, no sample of kombucha produced more than 0.5% alcohol within 6 weeks of storage, when stored at any temperature. It was not until 8 weeks post-fermentation and post-bottling, that alcohol production surpassed the FDA requirement to be sold as a non-alcoholic beverage. And so, a shelf life of 6 weeks can be recommended to consumers for commercially produced kombucha. After such time, the brew does not expire or “go bad”, as the contents are protected by the fermented symbiotic ecosphere inside; Kombucha exhibits antimicrobial activity against a wide range of bacteria. Kombucha has been shown to contain levels of usinic acid, acetic acid, bacteriocins, and
tea-derived phenolic compounds, all of which may act as antimicrobial compounds (Teoh, 2004). The presence of bacteria and yeast in the broth, and not solely in the pellicle layer, permit fermentation and alcohol production to continue after the “mother” SCOBY has been removed and after bottling has occurred. And so, the potential for variation in alcohol content should be disclosed to potential consumers on the label of commercially produced kombucha.

This pilot study ultimately presents identification of the bacteria of kombucha, offers a standard means of preparation for the brewing process, suggests a new initial fermentation time, and puts forward a preliminary observation of the rate of alcohol production, thus adding to the collective pool of knowledge about kombucha.
References


