

The affect of temperature and pH on the food safety of kombucha tea

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Abstract

Objectives: Kombucha tea is becoming an increasingly popular food item within the Vancouver area. The tea is prepared through fermentation at room temperature during which acidic by-products are produced lowering the overall pH of the tea. Though the pH eventually reaches levels below 4.6, many health authorities prevent the sale of kombucha in farmers markets due to potential food safety issues. The initial pH before fermentation is around 5.5 and is then left at room temperature to ferment. As a result, this process potentially could allow for food borne illness causing organisms to survive and proliferate within the sugared tea. This research project will investigate the relationship of pH and time during fermentation at both room and refrigeration temperatures. Fermentation within a refrigerator could provide a safer alternative fermentation method

Methods: The pH was measured using a pH meter for 30 samples at both room and refrigeration temperatures providing a total of 60 samples. The pH was measured periodically every twelve hours for a total of 120 hours. The data was analyzed using a linear regression model to determine if the pH change over time was statistically significant. The time at which the pH dropped below 4.6 was also noted for food safety purposes

Results: At room temperature the pH steadily decreased in a linear fashion throughout the entire sampling period, dropping below 4.6 within 12 hours. The pH decreased in a nearly identical fashion when fermented in a refrigerator for the first 72 hours of sampling. After the 72 hour mark the pH stabilized at approximately 3.75, whereas the pH at room temperature continued to decrease down to 3.10 after the full sampling period

Conclusion: The results indicate that kombucha tea becomes a non-potentially hazardous food within the first 12 hours of fermentation. The pH dropped below 4.6 after 12 hours at which point no food borne illness causing bacteria are able to survive and proliferate within the tea. The observed decrease in pH during the first 72 hours within a refrigerator is unlikely to have resulted from the fermentation process and therefore is not a feasible practice. Fermentation at room temperature appears to be a relatively safe process if home brewers are able to measure the pH change and carry out the process in a sanitary manner

Keywords: Kombucha, fermentation, pH, temperature, refrigeration, tea

Introduction

Kombucha is a fermented tea product prepared by placing a symbiotic culture of bacteria and yeast (SCOBY) into a mixture of green or black tea, sugar and other flavorings (Jayabalan, 2009). The mixture is then stored at room temperature for typically 7-14 days during which the bacterium *Gluconacetobacter xylinus* converts alcohols produced by the yeast into

various acids, including acetic acid (Jayabalan, 2009). The production of these acids lowers the pH of the mixture until the desired flavor and acidity level is reached. This process would initially appear to be potentially hazardous in terms of food safety. As noted, the brewing process is conducted at room temperature for over a week and involves different species of

bacteria and yeast. However, when a food product reaches a pH of below 4.6 it is considered non-potentially hazardous as harmful bacteria will not be able to survive or proliferate in these conditions (BCCDC, 2015). The rate at which the pH lowers below 4.6 will be indicative of the relative food safety. Therefore, in this study the pH levels of Kombucha tea will be measured periodically throughout the brewing process to determine its safety in regards to health.

Kombucha has become an increasingly popular food item with sales growing by \$400 million from 2005 to 2014 within the US (Narula, 2015). This popularity has extended into farmers markets where many brewers are now attempting to brew and sell homemade kombucha. The food safety risks are also not entirely established and this has prompted Health Authorities within Metro Vancouver to prevent the sale of Kombucha and other fermented products within these farmers markets unless prepared in a commercial facility (Corder, 2015). This experiment is being conducted to provide health authorities and environmental health officers with more information on the risks associated with brewing kombucha.

To further the scope of this experiment, the pH will be measured during the fermentation process at both room and refrigeration temperatures. Kombucha is typically brewed at room temperature as noted above. However, many of the potential risks associated with brewing kombucha could be eliminated if fermentation was possible within a refrigerator. Temperature abuse would no longer be an issue and contamination of the product would be prevented. This safer practice would also be more acceptable from a health authorities perspective and would be an effortless adjustment for home brewers to make. In the event that fermentation in a refrigerator is not possible, this experiment will still provide information on the pH changes at room temperature.

Evidence Review

Antimicrobial activity of kombucha tea

Sreeramulu, Yang, and Wieger (2000) determined that several organisms associated with food borne illnesses were sensitive to a Kombucha solution which had reached a pH of 2.5. Additionally, the study noted that even when the pH was made neutral and the solution was thermally denatured there was still an antimicrobial affect exerted against the bacteria indicating the presence of other antimicrobial compounds. This was supported by a study in which Greenwalt, Ledford and Steinkraus (2000) inoculated organisms associated with food borne illnesses on an agar media containing an absorbent pad soaked in Kombucha. The antimicrobial activity against most of the pathogens was found to be significant and was thought to be due mainly to the acetic acid content of the Kombucha. Finally, a study by Cortesia (2014) focused on the antimicrobial properties of acetic acid against mycobacteria (Causative agent of tuberculosis). A 30 minute exposure of mycobacteria to a 6% solution of acetic acid considerably reduced the number of viable bacteria.

pH change over time

Studies measuring the pH tend to make general statements regarding the pH change over time due to the number of variables which can influence the rate of the pH decrease. One study demonstrated that the pH decreases from about 5.44 to 3.0 in approximately five to seven days (Jayablan, 2014). A report from Utah State University considers Kombucha contaminated if the pH doesn't reach 4.6 or below within 7 days (Nummer,2013). An interesting observation in the majority of the studies is the sudden decrease in the pH within the first hour. One study noted that the starting pH of their kombucha tea was at 3.75, while another had a starting pH of 5 (Sievers, 1996). These findings indicate that the SCOBY itself may release acidic compounds from a previous brew regardless of any fermentation which has yet to take place. This effect was similarly observed in this experiment

when within the first hour the pH had already dropped from 5.5 to 4.75.

The effect of temperature on fermentation

During the fermentation process the Kombucha is brewed at room temperature. For instance in a study by Jayabalan, Mariumtu, and Swaminathan (2006) kombucha was brewed at 25 degrees Celsius. This temperature is favorable as it allows for steady production of acidic products including acetic acid. For this reason Kombucha is only refrigerated during storage when changes in acidic content are to be halted. However, during fermentation the room temperature will be also favorable for the proliferation and survival of disease causing organisms. Another study showed that during storage of Kombucha at refrigeration temperatures the number of bacteria and yeast cells decreased steadily over the course of 14 days (Fu, 2014). Since some of the bacteria and yeast remained viable during refrigeration temperatures one can assume that the pH will continue to lower, albeit at much slower rate.

Health Hazards

The primary hazard that health authorities are concerned about is shelf life and contamination (Corder, 2015). However, another issue is over-fermentation results in an excess quantity of acetic acid or chemical leaching (Jayabalan, 2014). The BCCDC developed a food safety plan regarding kombucha which mentioned cases in which people suffered from acidosis caused by drinking too much over-fermented Kombucha (BCCDC, 2015). The highly acidic brew can cause perforations in the intestinal tract of susceptible people and has resulted in a death by acidosis (Sunghee, 2009). The highly acidic environment can also leach chemicals out of storage materials (Sabouraud, 2009). Therefore, kombucha should only be stored in glass or food grade plastic containers to avoid heavy metal or chemical leaching and subsequent poisonings.

Methods

A basic recipe was used for this experiment which includes only water, tea, sugar and a kombucha culture also known as a symbiotic culture of bacteria and yeast (SCOBY). Four liters of water was brought to a boil and then one cup of sugar and four bags of black tea were added. The tea bags were removed after five minutes of brewing and then discarded. After the sugared tea had cooled down to about room temperature it was equally divided into one liter glass sampling containers. The total volume of tea in each container was 750 ml. The SCOBY's were then cut to equal sizes using a sanitized knife to ensure the fermentation would occur at an identical rate. These cut up SCOBYs were then added individually to each sampling container. The containers were then sealed using cheese cloth held tightly to the container with an elastic band wrapped around the opening.

When measuring the pH, a glass pipette was used to periodically draw a small volume of the kombucha solution from each glass container. These small samples were subsequently placed into disposable plastic containers and then the pH was measured using a calibrated ExTech waterproof palm pH meter. The pH meter was calibrated before each sampling period to validate its accuracy. The pH meter measured both the pH and temperature simultaneously, both of which were recorded.

The room temperature fermentation was carried out at 20 degrees Celsius and at 4 degrees Celsius within a refrigerator. These temperatures were sustained throughout the entire sampling period. Exposure to light was limited and the sampling areas were only disturbed during measurements.

Results

At room temperature the pH data set had a mean of 3.76, a median of 3.71, and a standard deviation of 0.514. At refrigeration temperature the pH data set had a mean of 3.96, a median of

3.77, and a standard deviation of 0.352. The average pH of both of the data sets are under a 4.6 indicating that fermentation may be feasible at both of these temperatures. These results contradict the initial literature review as refrigeration temperatures will typically halt any fermentation processes. The mean, median, mode and standard deviation information is summarized below:

	pH at 20 °C	pH at 4 °C
Mean	3.76	3.96
Median	3.71	3.77
Mode	N/A	N/A
Standard Deviation	0.514	0.352

The average pH at each sampling period is presented in a table below. Note the stabilization of the pH at 4 °C after the 72 hour mark. Conversely, the pH continues to decrease in a linear fashion at room temperature:

Time (Hours)	Mean pH at 4 °C	Mean pH at 20 °C
1	4.78	4.76
12	4.47	4.44
24	3.99	4.14
36	3.87	3.99
48	3.76	3.81
60	3.7	3.71
72	3.69	3.62
84	3.69	3.41
96	3.71	3.23
108	3.68	3.14
120	3.7	3.08

Inferential Statistics

30 samples at both room and refrigeration temperature were taken for a total of 60 samples. Two sets of hypothesis were

developed as the pH was measured over time at two different temperatures:

Room Temperature

Null hypothesis (H_{OR}):

In 120 hours there will be no change in the pH at room temperature

Alternative hypothesis (H_{AR}):

In 120 hours there will be a change in the pH at room temperature

Refrigeration

Null hypothesis (H_{OR}):

In 120 hours there will be no change in the pH at refrigeration temperatures

Alternative hypothesis (H_{AR}):

In 120 hours there will be a change in the pH at refrigeration temperatures

Linear regression analysis was carried out in SAS using an excel spreadsheet prepared earlier. After 72 hours At room temperature the t-value was -57.24 with a p-value less than 0.0001 and therefore the association between time and pH is statistically significant (Barr, 2015). The R-squared value was very high at 0.9403 indicating that 94.03% of the variation in values for pH can be accounted for by knowing time (Heackcock & Karakilic, 2015). Therefore, the null hypothesis is rejected as there was a statistically significant change in the pH at room temperature. Linear regression analysis was then carried out post 72 hours producing similar results at room temperature. The pH continued a linear decline to a final pH of approximately 3.1 after 120 hours. The association between time and temperature was statistically significant throughout the entire sampling period at room temperature.

Similarly, at refrigeration temperatures the t-value was -45.86 with a p-value which was less than 0.0001 and therefore the association between time and pH was also statistically significant at refrigeration temperatures before 72 hours. The R-squared value was 0.9100 which indicates that 91% of the variation in values for pH can be accounted for by knowing time. In other words, there is a quantifiable

relationship between pH and time when kombucha is fermented in a refrigerator. Therefore, at refrigeration temperature the null hypothesis would also be rejected as there was a statistically significant change in the pH over time highlighted by the very low p-value. However, after the 72 hour sampling period the pH stabilized and no longer decreased. When a linear regression model was applied to the pH change over time within a refrigerator after 72 hours the p-value was 0.8697. This indicates that the association between time and pH is not statistically significant when applied to the post 72 hour sampling period and the null hypothesis cannot be rejected.

These results are summarized below. Note the shift in the p-value and r-squared value at refrigeration temperatures after the 72 hour sampling period:

	1-72 Hours		72-120 Hours	
	P-Value	R-Squared	P-Value	R-Squared
Room	<.0001	0.9403	<.0001	0.9643
Refrigerator	<.0001	0.9096	0.8697	0.0002

Discussion

The pH continued to decrease at room temperature after 72 hours resulting in a final pH of around 3.1 after 120 hours but had conversely stabilized within the refrigerator. Statistical analysis had shown that after the 72 hours mark, the pH change over time was no longer statistically significant at refrigeration temperatures. This shift after the 72 hour mark was of particular significance as it potentially explains the unexpected initial results. It was unusual to observe the decrease in pH over time within a refrigerator as typically refrigeration temperatures are used to halt fermentation and extend the shelf life of kombucha (SungHee, 2009). However, during the 72 hours of this study the pH within a refrigerator steadily decreased at a nearly identical rate to that of the pH at room temperature. There are two explanations as to how the pH was decreasing within a refrigerator. The first is that the

fermentation wasn't immediately halted and continued until about the 72 hour mark. This scenario is unlikely and is not supported by any previous studies. The more likely explanation is that the kombucha released acidic liquids into the newly prepared tea over time regardless of any fermentation that may have taken place. The SCOBY is quite absorbent and much of its mass comes from the liquid it absorbs from its direct environment. Additionally, every time a new batch of kombucha is prepared, a SCOBY is taken from a previously fermented batch and transferred into a fresh container of tea. The previous kombucha batch would have already reached a low pH and so this low pH solution would have been absorbed in the SCOBY. When the SCOBY enters the new container of tea, it would slowly release its acidic contents over time which would result in the observed pH decrease. This pH decrease would therefore occur regardless of temperature differences. After about the 72 hour mark, the pH continued to decrease at room temperature but had reached a steady state within the refrigerator. Perhaps the acidic contents stored within the SCOBY had fully been released into the new batch of tea and now the observed decrease in pH was directly due to fermentation. This coincides with previous studies which have demonstrated that the pH can decrease from 5.44 to about 3.0 in roughly five to seven days (Jayabalan, 2014).

This explanation is further supported by the fact that the pH had already dropped from 5.44 to about 4.75 in just one hour. This quite significant decrease in the pH is unlikely a result of fermentation alone and it is more reasonable to assume that acidic contents had been released from the SCOBY itself. Previous studies involving the fermentation of kombucha have also observed similar findings. One study which involved fermenting kombucha noted that the starting pH was 3.75, while another had a starting pH of about 5 (Sievers, 1996). These differences in the initial pH are due to many factors, but can partly be explained by the SCOBY itself. The size of the SCOBY and the previous batches pH will certainly affect the starting pH of a new batch. Additionally, some studies choose to add a certain volume of previous kombucha tea to the new batch to

further reduce the initial pH and provide a more hospitable environment for the SCOBY (Sievers, 1996). Regardless of the affects of temperature, the pH had decreased below 4.6 within twelve hours at both room and refrigeration temperatures resulting in a safe product. The kombucha is no longer potentially hazardous as no food borne illness causing bacteria are able to proliferate or survive within such an environment. Fermentation at room temperature is therefore feasible, whereas refrigeration can be used to instead halt fermentation and extend the shelf life of the product or prevent over-fermentation.

Recommendations

The main issue from a food safety standpoint is primarily related to sanitation and avoiding contamination during the initial preparation. Care has to be taken by the brewer to ensure that all of the equipment and food contact surfaces used are properly disinfected. Additionally, the brewer must ensure they practice good hand washing practices and protect the newly prepared tea from mold spores. Protecting against mold spores is accomplished by leaving the tea and SCOBY uncovered for as little time as possible. Simply leaving the breathable lid on the container until the kombucha is ready to be consumed or bottled avoids this issue.

For a home brewer to sell kombucha in a farmers market they need to prove that they are well educated on the importance of pH and the potential for mold contamination. The home brewer can provide documented step by step instructions on how they prepare the kombucha, how they test the pH over time and their sanitation practices. Several health authorities and the BCCDC have prepared short documents which detail the risks and recommended steps to take during the preparing of kombucha (BCCDC, 2015). Environmental health officers (EHO) can provide these documents to home brewers if they lack the food safety knowledge regarding kombucha brewing.

Other recommendations include labelling requirements that state if the product is

unpasteurized, provide storage instructions, and recommended daily intakes. Since the finished product still contains some viable organisms the product should be refrigerated to halt any further fermentation and prevent the product from getting too acidic. As noted previously from the literature review, adverse health effects were associated with the over-consumption of kombucha that was more acidic than usual (SungHee, 2009). To avoid this scenario, the label should also state that consumption of this product should be limited to one bottle a day.

Future Research Suggestions

To further the understanding of preparing kombucha safely several components of this study can be expanded on. The pH decrease at refrigeration temperatures was unexpected and future studies can attempt to replicate or refute this finding. The affect of temperature on the change in pH can be further understood by measuring the pH change at various temperatures. This study measured the pH change at a room temperature of 20 degrees Celsius which constitutes the lowest recommended temperature for brewing kombucha. Using the lowest temperature allowed for insight into a worst case scenario operation in which fermentation would hypothetically be at its slowest. Future studies can ferment within the kombucha's more optimal temperature range of 25 - 30 degrees Celsius.

This study concluded that the main focus should be placed on brewer knowledge to ensure kombucha safety. It is more important to ensure the brewer is aware of the risks involved and is taking the necessary steps to avoid contaminating the final product. Future studies could assess home brewers knowledge through the application of a survey. Questions on the importance of pH, sanitation and controls can be integrated into this survey. It would be useful to understand how educated home brewers are on this subject. This survey could also be given to EHOs to test their knowledge on kombucha preparation. Kombucha is still a relatively new food product and the procedures used to make kombucha are quite unique. EHOs would be more prepared to assess the home brewers

ability to produce kombucha if they themselves were more educated on the subject.

Conclusion

Kombucha appears to be a relatively safe product as the pH dropped to below 4.6 within 12 hours at both temperature conditions. The pH decreased in a linear fashion at room temperature reaching a final value of approximately 3.1 after 120 hours. The pH decreased in a similar fashion when fermentation took place within a refrigerator up until the 72 hours mark. After this sampling period the pH stabilized and remained essentially the same for the remainder of the experiment. This unexpected initial decrease of the pH in the refrigerator was postulated to have not been a result of fermentation. The acidic tea prepared from a previous brew may be transferred into a new batch as the SCOBY is highly absorbent. Therefore, fermentation is not feasible in a refrigerator but instead provides a means in which the fermentation can be halted. This will extend the shelf life of the product and prevent over acidification of the kombucha. Focusing on the brewers knowledge of food safety regarding kombucha and sanitary practices appears to be more important than the fermentation

temperatures. The brewer must measure the pH over time and ensure that a pH below 4.6 is eventually reached. The brewer must also practice effective sanitation of the equipment and preparation areas to prevent contamination of the initial product which has a pH above 4.6. BCCDC and health authorities have developed guidance documents specifically for preparing kombucha in a safe manner. These documents may be provided to home brewers who can then use them as a resource in developing a food safety plan specifically for kombucha. An approving authority can then assess the brewers capability in safely preparing the product.

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Competing Interests

The authors declare that they have no competing interests

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