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Paper No. CSBE13-118

Effects of Flavoured Tea on the Products of Kombucha Fermentation

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Written for presentation at the
CSBE/SCGAB 2013 Annual Conference
University of Saskatchewan, Saskatoon, Saskatchewan
7-10 July 2013

ABSTRACT Kombucha, a beverage fermented by a symbiotic colony of bacteria and yeast (SCOBY), has been traditionally consumed by Asian and Eastern European cultures for centuries. As consumer demand for kombucha grows in North America, there is a need to control, predict and optimize the fermentation process. This study aims to evaluate the effect of the tea variety used in the sweet-tea substrate on the development of organic acids and ethanol in the final product. The presence of essential oils and reduced caffeine level in lavender pomegranate green tea has been anecdotally observed to detrimentally affect the SCOBY metabolism and growth rate. The fermentation of black and lavender pomegranate green tea (1.5 g/L) and sucrose (70 g/L), acidified with vinegar (1% v/v) was monitored. The experiment measured the temperature, pH and total acidity of the tea each day as well as the change in biomass weight over the fermentation period. There was no significant difference in the titratable acidity and pH between the two teas used, but the black tea yielded a noticeably higher biomass growth rate. Further research is being conducted to track the change in concentration of carbohydrates and ethanol in order to determine the impact of differing biomass yield on the products of kombucha fermentation.

Keywords: Kombucha, SCOBY, bioreactor, fermented beverages, green tea

INTRODUCTION Kombucha is a traditional beverage originating from various regions in Asia (Tietze 1995). In keeping with the centuries old practice, kombucha is produced through aerobic fermentation of sweetened black tea. Kombucha is an acidic, mildly alcoholic beverage that many enjoy for its taste and perceived health benefits (Malbaša, Lončar et al. 2008). Some anecdotal health benefits of kombucha include reduced risk of cancer, lengthened lifespan, improved arthritis symptoms, and strengthened kidney function, but most of the health claims have not been scientifically proven (Tietze 1995, Dufresne, Farnworth 2000, Greenwalt, Steinkraus et al. 2000).

Fermentation of the tea is accomplished by a symbiotic colony of bacteria and yeast, or SCOBY, assembled in a cellulose mat or pellicle. In the production of kombucha, black tea is sweetened with 50-150g/L of sugar prior to being acidified with an aliquot of kombucha from the previous batch

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or other buffering acid. The acidified sweet tea solution is then inoculated with a SCOBY from a previous fermentation, prior to aerobic incubation for 7-10 days (Tamang, Kailasapathy 2010) . Currently, methods of brewing kombucha are heavily influenced by traditional, unscientific techniques.

Many different types of tea are used as the raw material for creating the brewing medium, but all teas varieties are categorized as *Camellia sinensis* species (Katiyar, Mukhtar 1997) . Popular tea varieties include black, green, white, and oolong. Black and oolong teas are oxidized and have higher polyphenol oxidase activity, which causes their dark colouration. Green and white teas are unoxidized, hence the light coloration of the beverage they produce (Wang, Helliwell 2001, Graham 1992, Dufresne, Farnworth 2000) . Oxidation of the tea occurs when the leaves are exposed to open air allowing the polyphenols, specifically the catechins, within the leaf to oxidize (Tamang, Kailasapathy 2010) .

In addition to the type of tea used, the sugar concentration used in brewing kombucha must also be taken into consideration. Sugar sources such as white sugar, cane sugar, or molasses are most commonly used as the carbon source for the SCOBY (Malbaša, Milanović et al. 2009, Malbaša, Lončar et al. 2008, Lončar, Malbaša et al. 2001) . A 2008 study by Malbasa et al. indicates that the best kombucha product is achieved with a sucrose concentration of 70 g/L. This concentration of sugar gives the optimal carbon source to achieve the proper pH, and composition of acids (Malbaša, Lončar et al. 2008) . SCOBYs used for kombucha production tend to contain *Brettanomyces bruxellensis*, *Candida stellata*, *Schizosaccharomyces pombe*, *Tortaspora delbrueckii*, and *Zygosaccharomyces bailii* (Jayabalan, Marimuthu et al. 2007, Sievers, Lanini et al. 1995) . During production, the SCOBY is placed on top of the liquid where the comprising microorganisms consume the sugar and convert it to acids and alcohol. Yeasts are mainly involved in the alcoholic fermentation of the fermentable sugars to ethanol, and the acetobacter and gluconobacter bacteria convert the ethanol to acetic acid and additional metabolic products.

Although kombucha is a drink that dates back many centuries in Asia, there has been limited research conducted on the best brewing techniques. There are many aspects of brewing requiring exploration, including the impact of the type of tea on the brewing time, fermentation products and sensory qualities of the resultant kombucha.

MATERIALS AND METHODS

Microorganisms A local kombucha culture, or SCOBY, was obtained from Live Kombucha, in Guelph, Ontario. After receiving the SCOBY, it was grown for 14 days to increase its biomass and reduce the lag phase once it was divided into the six jars for the experiment.

Substrate In the experimental trial, the tea for each tea variety was prepared the same way, using the same equipment, except for variation in the type of tea. Loose-leaf orange pekoe black tea, loose-leaf lavender pomegranate green tea and granulated white sugar were purchased from the local bulk food store. Tap water was brought to a boil in a lidded stainless steel pot and removed from the heat, just prior to addition of tea (1.5g/L). The tea was allowed to steep for 15 minutes, and poured through a wire sieve to remove the tea leaves. Granulated sugar (70g/L) was added to the filtered tea and stirred with a sterile metal spoon to dissolve. After cooling to room temperature, 4L capacity glass jars (sterilized with 5.25% hypochloric acid) were filled with 3L of the tea. Each jar was acidified with 35mL vinegar (5% acetic acid) and inoculated with the matured tea fungus pellicle.

Fermentation and Analysis The bioreactors were covered with sterile cheesecloth and fermentation at 22C \pm 6°C was monitored. Sampling of the fermentation broth was conducted every day for a period of 16 days by means of a spigot located at the base of the bioreactors. During

fermentation the pH value, titratable acidity, and temperature were measured. The weight of the surface-laying SCOBY in each jar was taken at the start and end of the fermentation period.

The pH values were measured using an electronic pH meter calibrated at pH 4.0 and 7.0. The titratable acidity was determined according to (OVI 1990). The kombucha was degassed and a 20ml aliquot was titrated with 0.1M NaOH using phenolphthalein indicator. The titratable acidity was expressed in grams of acid per litre of sample.

RESULTS The experiment conducted analyzed the effect of the variety of tea on the pH, titratable acidity, temperature and resultant biomass of the kombucha over the course of the fermentation period. It was hypothesized that utilization of a flavoured, white tea would have a detrimental effect on the metabolism of the SCOBY and result in slower formation of organic acids.

pH and Temperature

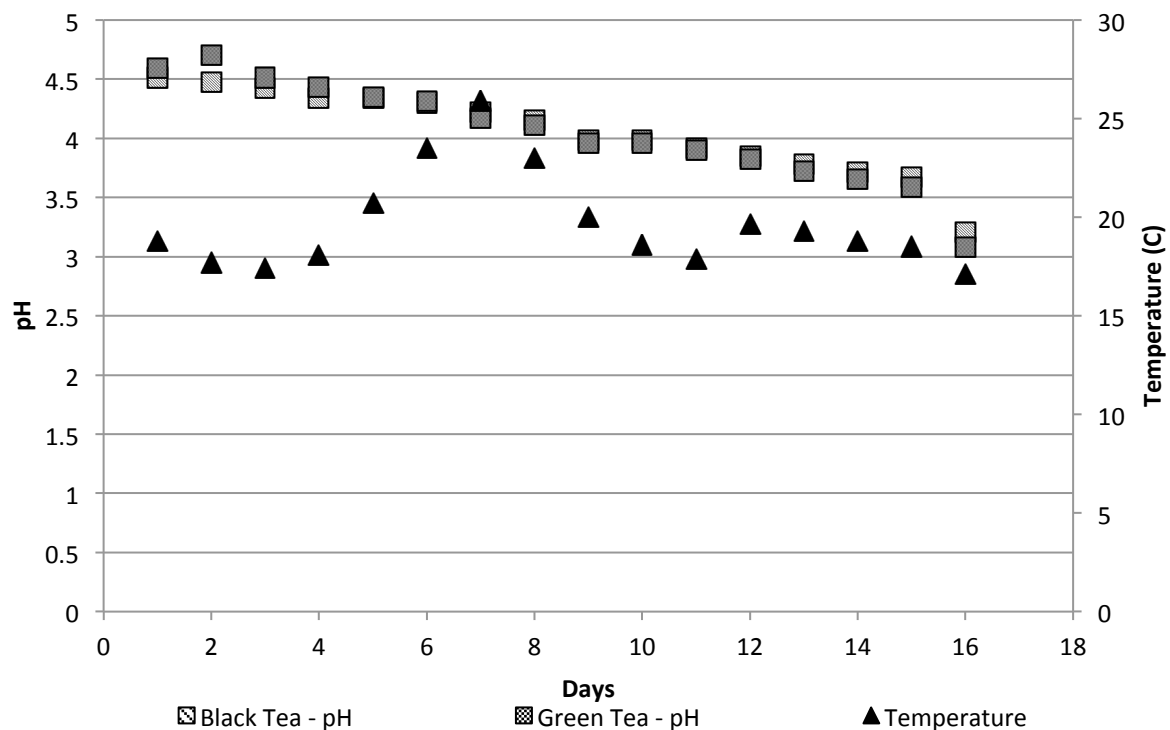


Figure 1. Change in the average pH and temperature of the black and green tea with respect to number of fermented days.

The lab in which the fermentation process occurred in had an ambient temperature that was slightly cooler than the ideal fermentation temperature. For this reason, the overall fermentation process during experimentation was slow progressing. As can be noticed from figure 1, between days 5 and 7, the temperature increased from 17°C to 26°C. During those days, it can be noticed that the rate at which the pH of the tea was changing also increased. Similarly, during days 7 to 9, the temperature dropped back to 17°C, and similarly the rate of change of the pH also decreased. This relationship reinforces the concept that the total fermentation time of the process is dependent on temperature, and maintaining a consistent temperature during the process would be vital in getting consistent product.

pH and Titratable Acidity

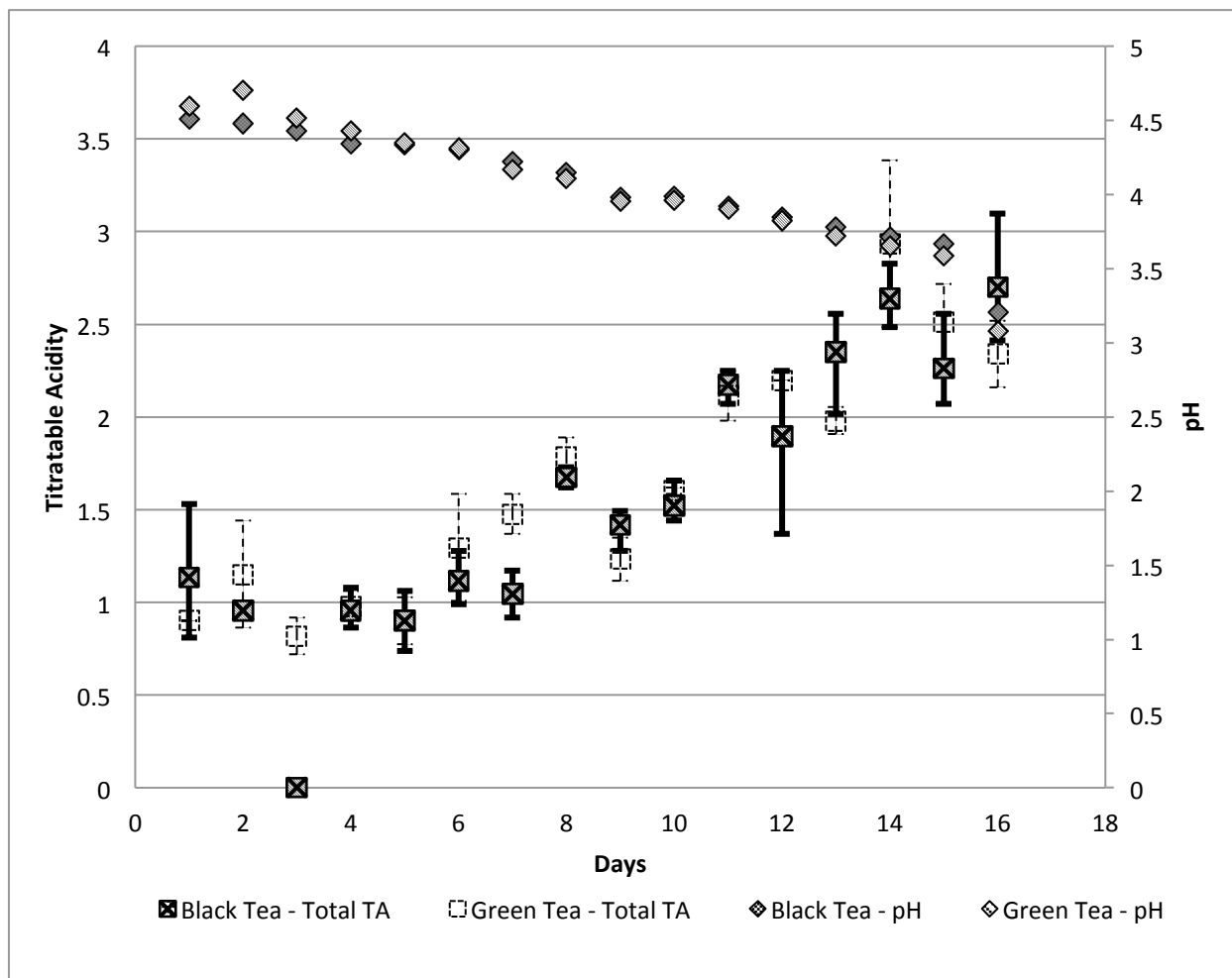


Figure 2. The change in total titratable acidity and pH of black and green tea.

pH is a logarithmic measure of free hydrogen ions in a solution, while titratable acidity, is the measure of both the free and bound hydrogen ions. pH is more important in terms of microbiological activity, while titratable acidity is often linked to the acidic mouth-feel of a product (Boulton 1980). It is for this reason, both the pH and the titratable acidity of the samples were tested.

Results from the experiment indicate that the pH and the titratable acidity of the samples increase as the fermentation progressed. The fluctuation of the titratable acidity trend for both the black and the green tea in figure 2 is believed to be due to errors that may have occurred during titration. As two different people titrated the samples, titrating till the correct shade is reached, may not have been consistent. Moreover the water of used for titration was boiled, and as such the temperature may have also played a role in the results.

Volatile and Non-Volatile Acidity

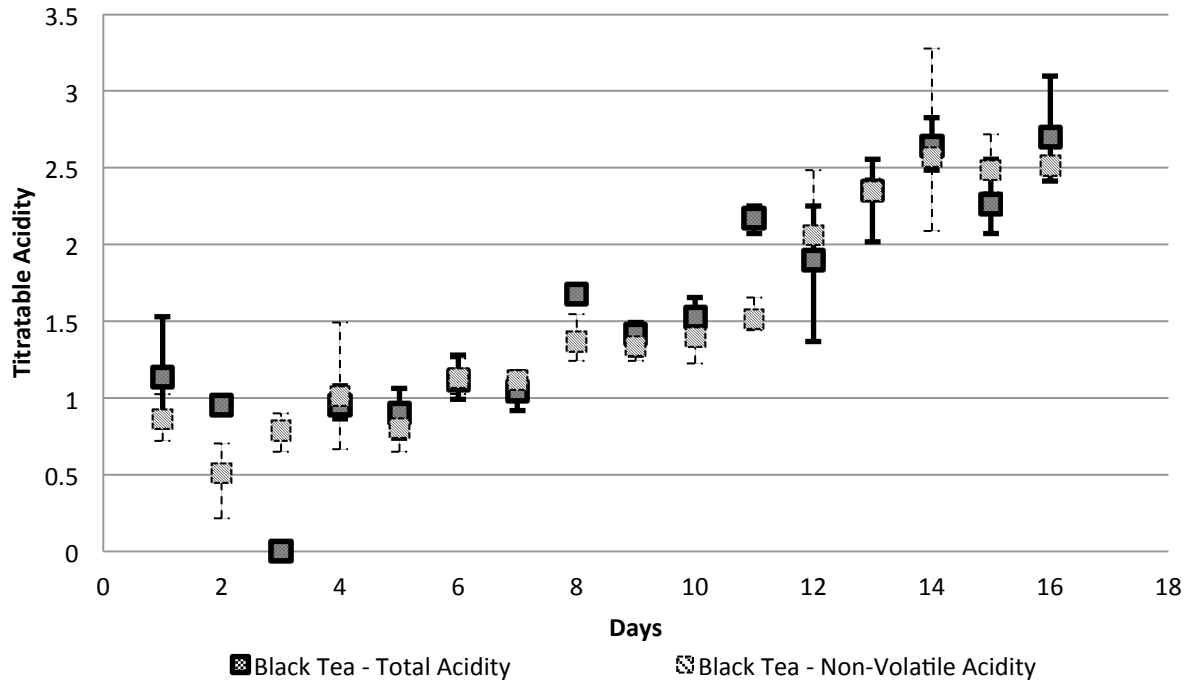


Figure 3. Total titratable acidity and non-volatile titratable acidity of black tea.

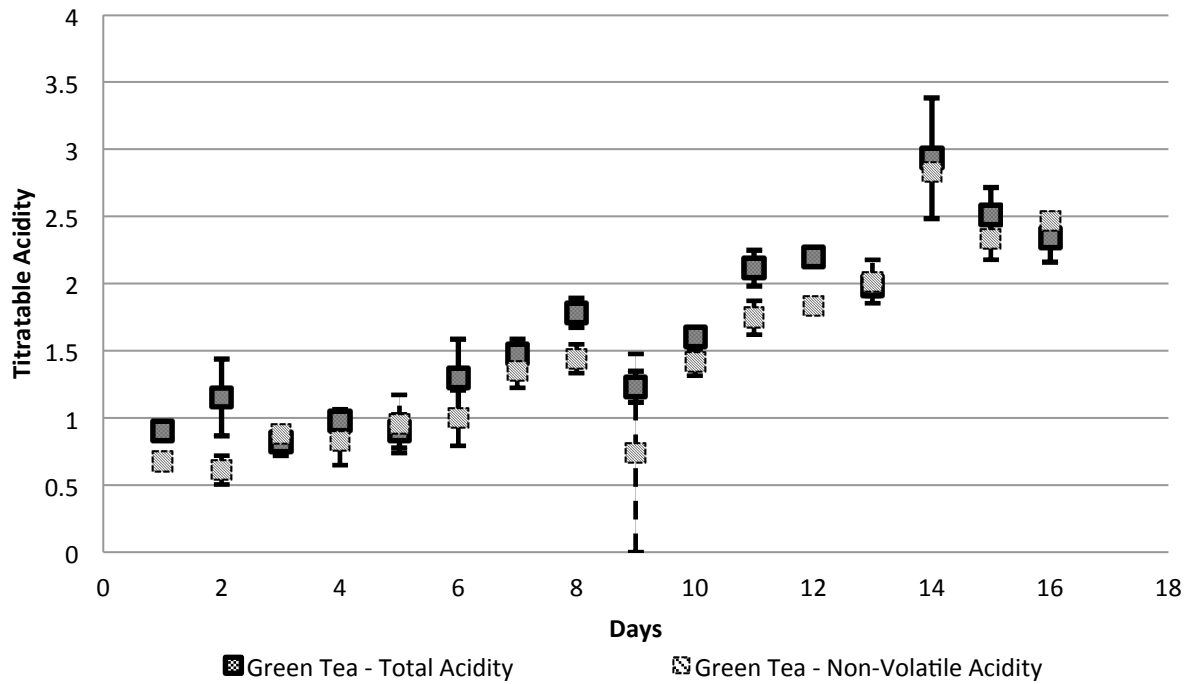


Figure 4. Total titratable acidity and non-volatile titratable acidity of green tea.

Figures 3 and 4 indicate no discernible trend between the non-volatile acidity and the total acidity. This is believed to be because to find the non-volatile acidity, the samples had to be boiled, and if the samples were not boiled to an adequate degree, there would be a certain amount of volatile acidity within the sample. In general, the non-volatile acidity should follow the same trend as that of the total acidity, but at a lower level (Greenwalt, Steinkraus et al. 2000) .

Biomass

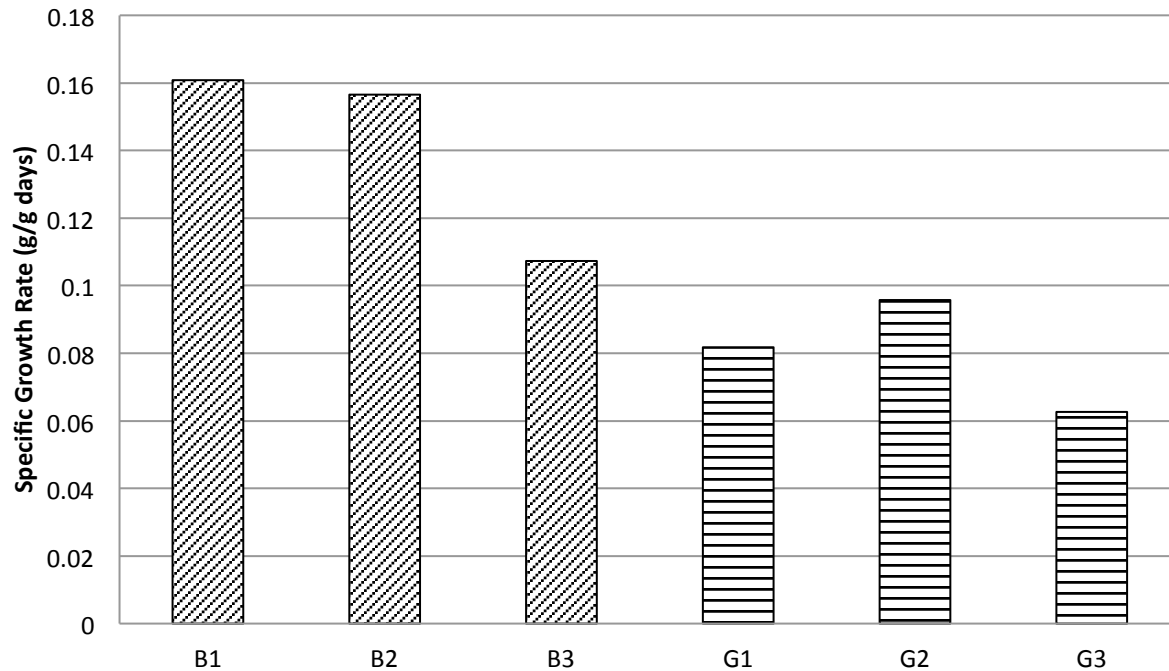


Figure 5 - Specific growth rate of biomass in green and black tea samples over the 16 day fermentation period.

The specific growth rate of the biomass was calculated by measuring the initial and final mass of the biomass. As can be seen, for the black tea had a higher growth rate when compared to the green tea. From figure 5, B3 had a lower growth rate than that of B1 and B2, but the pH and titratable acidity of all of the samples black tea samples were similar. Similarly, while G2 and G3 had similar titratable the growth rate varied significantly. This variability can be attributed to the SCOBY containing some amount of tea, which could not be removed without damaging the SCOBY. One point of note is that on average, the growth rate of the biomass in the black tea was significantly higher than that of the growth rate of the biomass in the green tea.

CONCLUSION Kombucha is a beverage that many cultures enjoy for its pleasant flavour and touted health benefits. As shown by increasing sales over the past several years, and predicted further growth of the industry, kombucha brewers will benefit increasing their output volume and process control. The tea substrate is a key determinant in the success of the fermentation, resultant chemical constituents and taste of the kombucha. The experiment investigated the impact that using different types of tea has on the growth and metabolism of the SCOBY. It was found that while there was no significant difference in the titratable acidity and pH between the two teas used, the black tea yielded a noticeably higher biomass growth

rate. Further research is being conducted to track the amount of carbohydrates and ethanol in order to determine the impact of differing biomass yield on the products of kombucha fermentation. Resultant experimental data will also be used to develop a kinetic model to compare and predict the rate of fermentation. A predictable and controllable fermentation process will benefit commercial kombucha producers and reduce their risk of fermentation failure.

Acknowledgements. This research is funded by University of Guelph, School of Engineering with support from the Department of Food Sciences and Live Kombucha in Guelph, Ontario.

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