


Article

Optimizing Oxygen Exposure during Kombucha Brewing Using Air-Permeable Silicone Bags

Briana Abigail R. Czarnecki ¹, Kortnie M. Chamberlain ¹, Ian M. Loscher ¹, Emily R. Swartz ¹, Lieke M. Black ¹, Emma C. Oberholtzer ¹, Jordan C. Scalia ¹, Bret A. Watson ¹, Lauren E. Shearer ^{1,2}, John N. Richardson ^{1,2} and Jeb S. Kegerreis ^{1,2,*}

¹ Department of Chemistry and Biochemistry, Shippensburg University, 1871 Old Main Drive, Shippensburg, PA 17257, USA; abbiczar@gmail.com (B.A.R.C.); kchamberlain4450@gmail.com (K.M.C.); ianmloscher@gmail.com (I.M.L.); emily.swartz13@gmail.com (E.R.S.); 19lblack@gmail.com (L.M.B.); e.oberholtzer26@gmail.com (E.C.O.); jordan.scalia001@gmail.com (J.C.S.); bret190207@gmail.com (B.A.W.); stainsl@protonmail.com (L.E.S.); jnrich@ship.edu (J.N.R.)

² Cultured Analysis LLC., 1871 Old Main Drive, Shippensburg, PA 17257, USA

* Correspondence: jskegerreis@ship.edu

Abstract: As the commercial and home brewing of kombucha expands to accommodate its increased popularity, novel brewing practices that generate non-alcoholic kombucha in an efficient manner become valuable. The research presented in this work compares kombucha brewed in a glass jar brewing vessel to that brewed in an air-permeable silicone bag. Identical kombucha ferments with various sugar food sources were prepared and placed in each vessel, and variables such as titratable acidity, pH, alcohol by volume, gluconic acid concentration, acetic acid concentration, and sugar content were studied as a function of time. The results indicated that, regardless of the food source, kombucha brewed in an air-permeable bag exhibited more efficient acid production, lower ethanol concentration, and greater sugar utilization relative to equivalent kombucha brewed in a jar.

Keywords: kombucha; fermentation; glucose; fructose; sucrose; silicone; oxygen; ethanol; fermentation methods; microbial action; flavor



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1. Introduction

The first medicinal application of kombucha dates back to ancient China, where the beverage was used to treat digestive issues [1]. The numerous health benefits contained in the acidic and slightly sweet beverage are a direct result of the vitamins, minerals, and other active compounds that make up its composition. Some major benefits consist of probiotic, antibacterial, antioxidant, and antidiabetic properties as well as immune system support [2–4]. Vitamin and polyphenolic compounds present in kombucha are essential for a healthy diet, propagating its popularity from its Chinese roots to all parts of the world.

Kombucha is a mixture of tea, sugar, water, and a symbiotic culture of bacteria and yeast (SCOBY) [5,6]. The fermentation process begins when a sugar source, typically sucrose, is broken down by yeast into glucose and fructose. These sugars are further converted into ethanol and carbon dioxide by yeast. Aerobic bacteria then work to convert ethanol into acetic acid through an oxidation process [5–10]. Bacteria can also convert glucose into gluconic acid. Gluconic acid gives kombucha a sweeter taste in comparison to acetic acid, which leads to an astringent vinegar-like flavor [10,11]. Therefore, maximizing gluconic acid concentration relative to acetic acid concentration is useful for brewers seeking a more palatable kombucha [10]. As acetic acid bacteria thrive in an oxygen-rich environment, while yeasts prefer a more anaerobic environment, increasing oxygen exposure during the fermentation process is hypothesized to increase bacterial activity, allowing the bacteria to convert ethanol into acetic acid more efficiently [12,13]. The efficient conversion of ethanol to acetic acid is thus expected to lower the final ethanol content in

the beverage. Bacteria also convert glucose into gluconic acid faster in an oxygen-rich environment, which limits sugar content while producing more gluconic acid for a better flavor profile. Changing the tea type, SCOBY composition, fermentation time, amounts of starting ingredients, and even temperature could have impacts on the properties of each brew [14–19].

In this work, an air-permeable silicone bag was used to brew kombucha, allowing oxygen to enter from all directions to maximize total oxygen exposure. Our goal was to demonstrate that brewing kombucha in a silicone bag should result in a higher overall acid concentration and lower alcohol and sugar levels compared to kombucha brewed in a traditional glass jar where oxygen can only enter from the top of the vessel.

2. Materials and Methods

To analyze how brewing kombucha in an air-permeable silicone bag compares to a standard glass jar, multiple brews were evaluated in identical environments across equal brewing times. All chemicals used in the analysis of the kombucha samples were obtained from Fisher Scientific (Fair Lawn, NJ, USA), Sigma Aldrich (St. Louis, MO, USA), and Spectrum Chemical MFG Corp (New Brunswick, NJ, USA). The original SCOBY cultures from which all starter and test brews were prepared were purchased from White Labs Brewing Company (San Diego, CA, USA) and Happy Herbalist (Cary, NC, USA).

2.1. Preparation of Fermented Brews

To prepare the fermented brews, the following general recipe was used: 1.5 cups of starter culture (previously brewed kombucha that contains the SCOBY and has a high total acidity), 2 tablespoons of loose black tea leaves (sourced from a local market), 3 L deionized water were combined, along with 150 g of various types and proportions of sugars. Mixtures were allowed to ferment for times up to 49 days at temperatures ranging between 25 and 27 °C.

All brews were monitored for pH, titratable acidity (TA), alcohol by volume (ABV), gluconic acid concentration, and acetic acid concentration [20]. For a subset of brews, sugar (i.e., sucrose, glucose, and fructose) concentrations were acquired using Nuclear Magnetic Resonance (NMR) spectroscopy [21–23]. These data were collectively used to produce fermentation curves to show how these variables change over time.

Kombucha ferments were prepared using the same process for each experiment. The black tea was steeped for ten minutes in a 2 L volume of deionized water. The loose tea was strained out, and the resulting liquid was added to either a silicone bag from Anova Culinary Inc. (San Francisco, CA, USA) or a one-gallon glass jar. Once the tea cooled, the remaining recipe ingredients (sugar and starter culture) were added and mixed with water to reach a final volume of 3 L. The first sample (labeled day 0) was analyzed on the day the kombucha was prepared. An air-permeable fabric secured with a rubber band was used to cover the top of each glass jar. The silicone bags were closed with clamps and suspended in free space to allow for maximum oxygen exposure.

Sample collection was conducted in the same manner for all experiments and across all vessels. Sampling of the kombucha began by gently puncturing or moving the pellicle (the layer of cellulose produced by the SCOBY during the brewing process) to the side if present. Approximately 20–25 mL of broth was collected in a 30 mL beaker. An aliquot of 6 mL was then filtered with a sterile 0.2 micron PES Syringe filter and placed into a sterile capped vial. The unfiltered portion (14–19 mL) was used to determine the pH of the brew, while the filtered sample was used for the remaining measurements. All samples were stored under refrigeration when not in use.

2.2. Measurement of pH

pH was determined using a Cole Parmer pH electrode coupled to a Denver Instruments UltraBasic pH meter (Arvada, CO, USA). The pH electrode was calibrated and gently swirled in the sample before recording the final pH value.

2.3. Determination of Titratable Acidity

Titrateable acidity was determined by potentiometric titration using a standardized 0.100 M solution of NaOH as the titrant. Volumes ranging from 0.5 mL to 1.5 mL of filtered sample were diluted with 25–30 mL deionized water in a 50 mL beaker. A 2.0 mL volumetric buret containing the standardized NaOH was used to titrate the diluted sample. A previously calibrated pH electrode was suspended in the sample, and titration was conducted with stirring to achieve a potentiometric equivalence point.

2.4. Determination of Dissolved Oxygen Concentrations

Dissolved oxygen concentrations ($[O_2]$) were determined using a Hanna instruments Edge Dedicated Dissolved Oxygen Meter (Smithfield, RI, USA). A stir bar was added to the beaker containing a previously obtained sample of kombucha and agitated throughout the determination.

2.5. Quantification of Ethanol

Alcohol by Volume (ABV) was determined using headspace Gas Chromatography utilizing Flame Ionization Detection (GC-FID). 1-Propanol was used as an internal standard, and ultrapure He served as the carrier gas. A FISON GC8000 (Glasgow, UK) with a Restek RTx-BAC PLUS 2 analytical column was run isothermally at 30 °C. Both the injector and the detector were held at 200 °C during the analysis. The sample was prepared by pipetting 1 mL of saturated NaCl, 1 mL of 1-propanol (0.2% by volume), and 100 μ L of either the filtered sample or pre-prepared standards into a headspace vial. Both the headspace vial and gas-tight syringe, purchased from Hamilton Company (Reno, NV, USA), were placed in an oven at 80 °C for 15 min, after which 0.5 mL of headspace gas was drawn up into the syringe and injected. Data were collected and digitized via the PeakSimple Chromatography Data Acquisition System. Sample ABV values were calculated using a standard curve prepared on the same day.

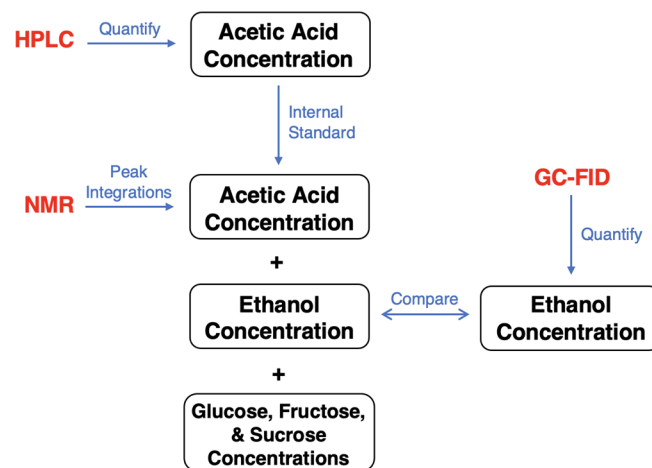
2.6. Quantification of Acetic Acid and Gluconic Acid

High Performance Liquid Chromatography (HPLC) was used to quantify the concentration of acetic acid and gluconic acid in a given sample of kombucha. The measurements were obtained using a Waters Breeze HPLC system consisting of a Waters (Milford, MA, USA) 1525 Binary HPLC pump, a Waters 2487 Dual Wavelength Absorbance Detector, and a Waters 717 plus Autosampler. The analytical column employed was a Restek (Belefonte, PA, USA) Allure Organic Acid 5 μ M (250 \times 4.6 mm) HPLC column. The mobile phase consisted of a 2.4 pH phosphate buffer run isocratically at a flow rate of 0.75 mL per minute. The wavelength of detection was 210 nm. Standard curves were constructed for each analyte and then used to determine unknown concentrations of each acid.

2.7. Determination of Sugar Concentration

A Magritek (Malvern, PA, USA) Spinsolve Ultra (80 MHz) benchtop Nuclear Magnetic Resonance (NMR) spectrometer was used to measure sugar concentrations as a function of time as brewing progressed. The instrument was shimmed to the sample prior to analysis. The instrumental parameters applied were as follows: WET-T2 for water peak suppression, 8 sample scans, repetition time of 15 s, and Carbon-13 decoupling. An NMR sample tube was prepared by rinsing the tube with filtered sample, discarding the rinse, and then filling the NMR tube with a small amount of filtered sample. The resultant peak integrations were obtained using the MestReNova (version 14.3.3-33362) software package.

Acetic acid was used as an internal standard, utilizing its concentration, as previously determined by HPLC. The procedure for sugar quantification and validation is illustrated in Scheme 1.



Scheme 1. The procedure used for the quantification of sugars in samples of kombucha.

Previous quantification of acetic acid concentration in the sample using HPLC allows acetic acid to serve as an internal standard. The peak areas corresponding to specific sugars were determined by integration and compared with those of the internal standard. Through the same process, ethanol concentrations were obtained and compared with GC-FID results to validate the method.

The peaks of sucrose, fructose, and glucose were integrated, and the concentration of each species was determined via Equation (1) [21],

$$[Sugar] = \left(\frac{A_{sugar}}{A_{HAc}} \right) \left(\frac{N_{HAc}}{N_{sugar}} \right) (M_{sugar}) [HAc]_{HPLC} \quad (1)$$

where $[Sugar]$ is the concentration of a sugar (either sucrose, fructose, or glucose) in $\frac{g}{L}$, A_{sugar} is the area of the isolated sugar peak being examined, A_{HAc} is the area of the acetic acid peak used for quantification, N_{HAc} is the number of protons associated with the acetic acid peak chosen, N_{sugar} is the number of protons associated with the sugar peak chosen, M_{sugar} is the molar mass of the sugar in $\frac{g}{mol}$, and $[HAc]_{HPLC}$ is the concentration of acetic acid acquired via HPLC in units of $\frac{mol}{L}$. A similar equation was used to quantify ethanol content in an NMR spectrum,

$$[EtOH] = \left(\frac{A_{EtOH}}{A_{HAc}} \right) \left(\frac{N_{HAc}}{N_{EtOH}} \right) \left(\frac{M_{EtOH}}{\rho_{EtOH}} \cdot 100 \right) [HAc]_{HPLC} \quad (2)$$

where $[EtOH]$ is the concentration of ethanol in the sample in units expressed as a % ABV, A_{EtOH} is the area of the isolated ethanol triplet in the NMR spectrum (Figure 1), N_{EtOH} is the number of protons associated with the corresponding triplet, M_{EtOH} is the molar mass of ethanol, ρ_{EtOH} is the density of ethanol, and the factor of 100 is included to align with the unit being a percentage. The concentration of ethanol calculated using Equation (2) was compared with that determined using GC-FID to validate the results of the NMR technique. An average difference of 0.02% ABV was observed across all measurements taken, justifying the sugar quantification method outlined in Scheme 1.

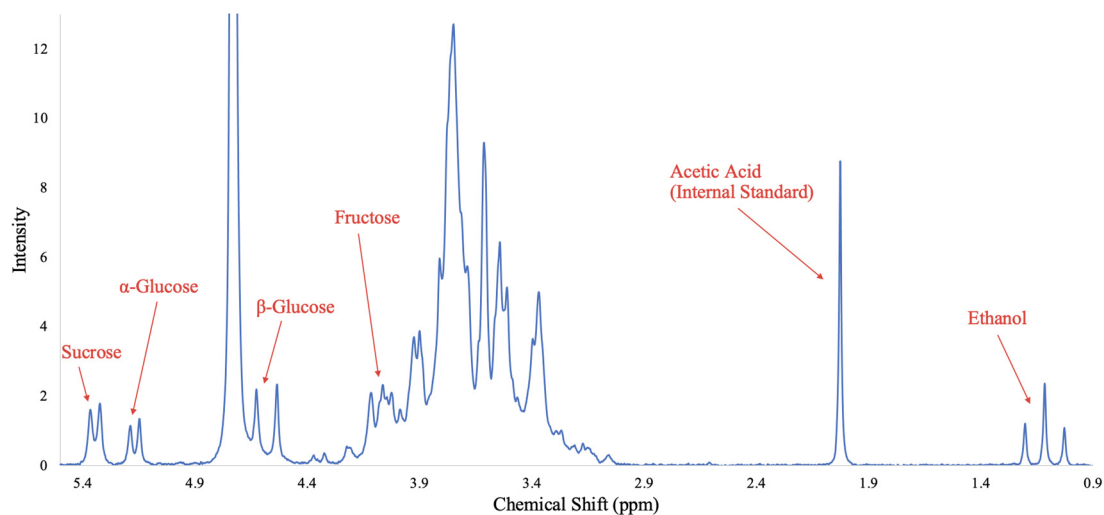
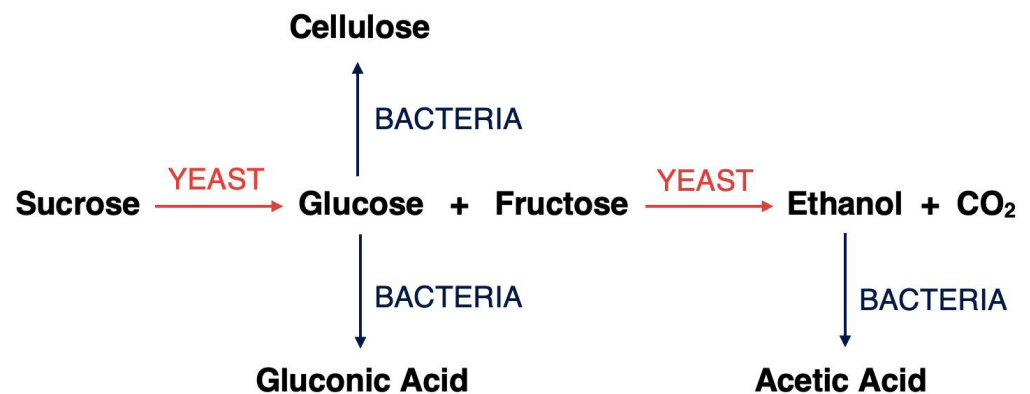


Figure 1. Sample spectrum produced by the benchtop NMR for kombucha with acetic acid acting as an internal standard. Characteristic peaks of the sugars of interest, as well as ethanol, are shown.

3. Results

The primary purpose of this work was to investigate differences in kombucha brewing outcomes between two different vessels of comparable size: a traditional one-gallon jar and an air-permeable silicone bag of the type used for sous vide food preparation. In this study, we further investigated (within the bag/jar combination) different brewing variables, such as food source (i.e., type of sugar) and application of batch-brewing techniques employed by commercial kombucha brewers.

The formation of kombucha during the brewing process comes about as the result of a symbiotic relationship between typical anaerobic yeast strains and aerobic strains of acetic acid bacteria. A simplified version of the process is illustrated in Scheme 2.



Scheme 2. A simplified flowchart showing the different roles of yeast and bacteria as sugar food sources are converted into the main components of kombucha.

Here, yeast serves two important functions: (1) to break sucrose down into the simple sugars glucose and fructose via enzymatic action (invertase) and (2) to undergo fermentation of simple sugars to form carbon dioxide and ethanol via normal fermentation. On the other hand, bacteria then consume ethanol, along with simple sugars, to generate acetic acid, which contributes to the characteristic sour flavor of the beverage. Further, glucose is consumed by bacteria to form gluconic acid as well as cellulose, which makes up the disc-like pellicle found on the surface of the liquid.

3.1. Part 1—Sucrose Food Source

In the first part of our study, we chose to investigate the formation of acids, specifically gluconic and acetic, as well as ABV, in both the bag and jar. In each vessel, the initial brew was prepared using 150 g of sucrose, along with a starter prepared from the original culture provided by the White Labs Brewing Company. The brew period for this part of the study was 35 days. Data were collected as a function of fermentation time to produce fermentation curves for each variable tested; these curves are shown in Figures 2–4. A summary of the pH, TA, and oxygen sensor data is provided in Table 1. We initially hypothesized that the oxygen permeability of the silicone bag would enhance the activity of aerobic acetic acid bacteria, leading to increased acid production and utilization of ethanol [24,25].

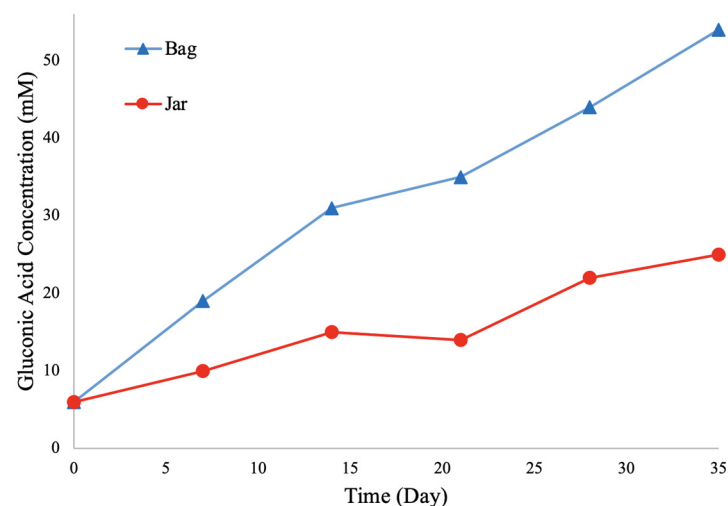


Figure 2. Gluconic acid production throughout a brew cycle for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both samples.

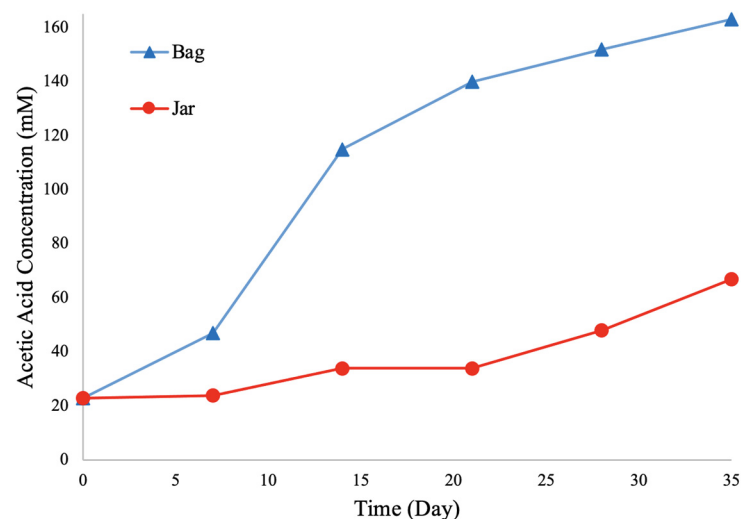


Figure 3. Acetic acid production throughout a brew cycle for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both samples.

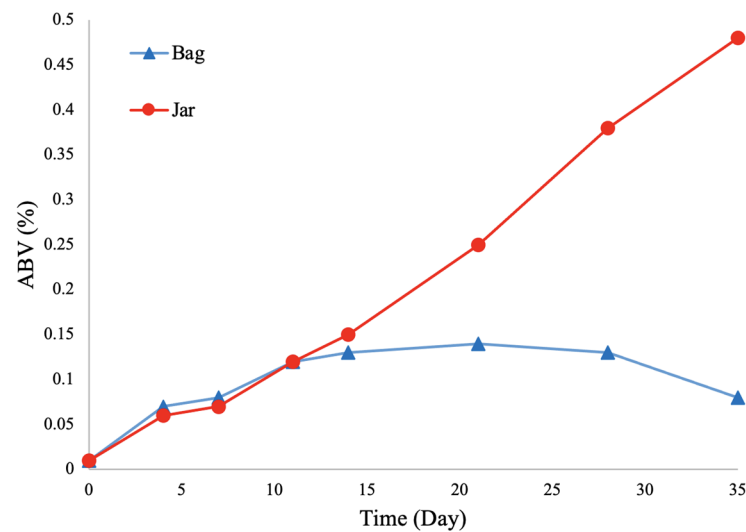


Figure 4. Ethanol production throughout a brew cycle for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both samples.

Table 1. Comparison of changes in pH, TA, and oxygen concentration in Part 1.

Brewing Vessel	pH Day 0 → Day 35	TA (mM) Day 0 → Day 35	[O ₂] (ppm) Day 0 → Day 35
Jar	2.94 → 2.48	29.8 → 117	1.6 → 2.3
Bag	2.97 → 2.30	31.0 → 247	4.4 → 0.9

Table 1 summarizes the total changes in pH, TA, and [O₂] for the kombucha brewed in the bag versus that brewed in the jar over the course of Part 1 of the study. The kombucha brewed in the bag showed a larger total change in each of these variables. A lower pH suggests greater gluconic acid production (as gluconic acid is a stronger acid than acetic acid), a higher TA is indicative of higher acid concentration, and a lower final value of [O₂] suggests oxygen utilization was enhanced in the bag.

Figures 2 and 3 clearly indicate that both gluconic and acetic acids were produced more rapidly in the bag than in the jar across the full 35-day fermentation cycle when sucrose was used as the food source. While the final ratio of acetic acid to gluconic acid was about 3:1 in both vessels, both acids were generated at an increased rate in the bag, with the final TA in the bag being over double that of the jar.

Even more profound are the data shown in Figure 4, which compares the ABV between the bag and jar over the same 35-day fermentation cycle. While the ABV increases nearly identically in both vessels up until around Day 10, a sharp deviation is noted shortly thereafter, in which the ABV associated with the bag rolls over and decreases, while that of the jar continues to increase in a nearly linear fashion. These results speak clearly to enhanced aerobic bacteria activity in the bag relative to the jar, as ethanol is consumed at a much higher rate in the bag beyond Day 14. These results correspond time-wise to the abrupt upward trend in acetic acid formation noted above and are shown in Figure 3.

Decreased ethanol concentration (as noted in the bag) is especially important in kombucha brewing since the resulting beverage is often marketed and sold as a non-alcoholic product. For example, in the United States, kombucha can only be sold as a non-alcoholic beverage if its ABV remains below 0.5%. Therefore, any brewing strategy that can keep the ABV low at any or all stages of the fermentation process could be particularly useful for the commercial kombucha brewer.

The concentrations of different sugars (sucrose, glucose, and fructose) were tracked over time via NMR to investigate sugar depletion as a food source by the SCOBY components. These results, illustrated in Figure 5, show that sugars, in general, were more readily consumed in the bag relative to the jar. In both cases, sucrose was completely utilized, but this occurred a week earlier in the bag relative to the jar. Conversely, the simple sugars glucose and fructose were observed building in as sucrose was depleted. Over time, both glucose and sucrose were gradually consumed, although a sizable bank of each remained over the course of the 35 day ferment, as observed in both vessels. However, both glucose and fructose were consumed at a slightly greater rate in the bag, with glucose being utilized to a greater extent. The higher rate of simple sugar usage in the bag speaks again to a more active bacterial population, which contributes to both pellicle and acid formation, as well as the consumption of ethanol [7,25].

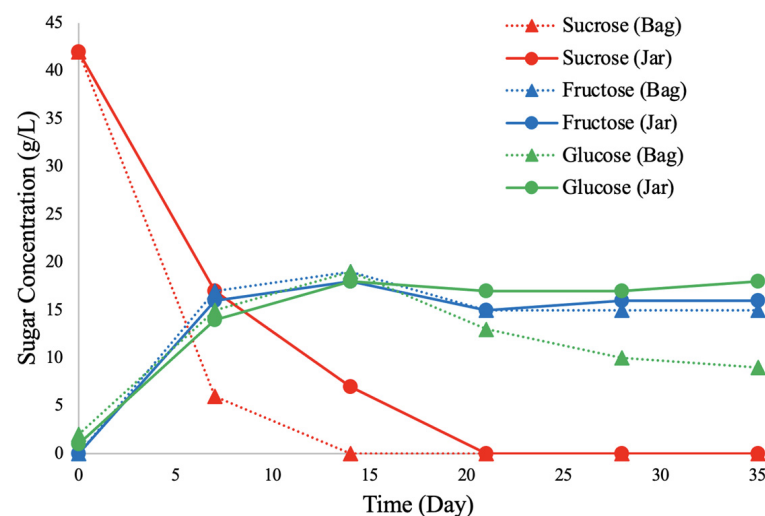


Figure 5. Production and usage of sugars (sucrose, glucose, and fructose) throughout a brew cycle for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both samples.

3.2. Part 2—Glucose and Fructose Food Sources

The second part of this study focused on the investigation of brewing outcomes when only simple sugars (e.g., glucose and fructose) were provided as food sources. This approach mitigated the need for sucrose to be broken down into its simpler components via the invertase reaction, thus making the simple sugars available initially to all of the microbe populations equally. Such an approach mimics kombucha brewing techniques, where an alternate (non-sucrose) food source, such as honey, is utilized.

In our investigations, two different ratios of simple sugars were employed: a 40:60 (m:m) ratio of fructose to glucose and a 70:30 (m:m) ratio of fructose to glucose. As before, each fermentation was allowed to progress in a one-gallon glass jar and also in a comparably sized air-permeable silicone bag. Ferments were monitored for multiple variables over a 26-day brew cycle. Tables 2 and 3 summarize the pH, TA, and [O₂] for Part 2, with the same overall pattern as shown in Part 1 (the bag produces a kombucha with a lower pH, higher TA, and greater oxygen utilization) observable here as well, despite the change in food source.

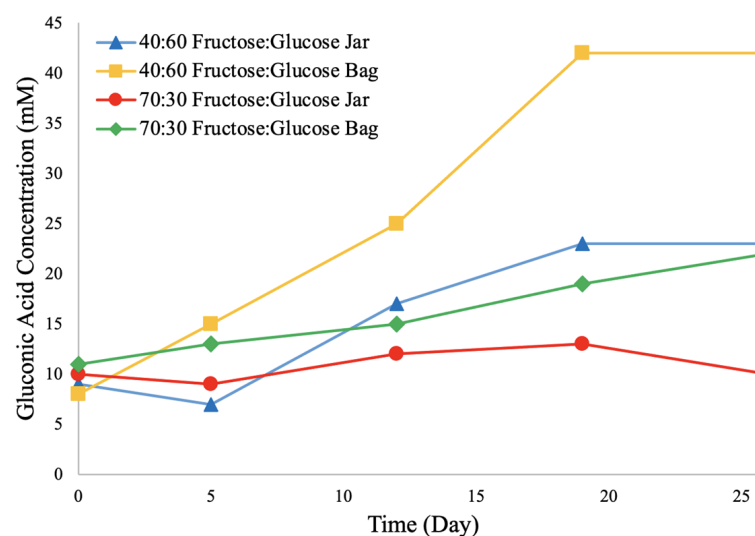
Table 2. Comparison of the changes in pH, TA, and oxygen concentration for the 40:60 fructose:glucose brews in Part 2.

Brewing Vessel	pH Day 0 → Day 26	TA (mM) Day 0 → Day 26	[O ₂] (ppm) Day 0 → Day 26
Jar	3.28 → 2.67	16.0 → 174	5.7 → 5.4
Bag	3.31 → 2.52	3.31 → 2.52	7.0 → 4.8

Table 3. Comparison of the changes in pH, TA, and oxygen concentration for the 70:30 fructose:glucose brews in Part 2.

Brewing Vessel	pH Day 0 → Day 26	TA (mM) Day 0 → Day 26	[O ₂] (ppm) Day 0 → Day 26
Jar	3.25 → 2.76	16.0 → 131	5.4 → 6.8
Bag	3.30 → 2.65	17.0 → 306	6.2 → 4.9

Figure 6 shows gluconic acid production throughout the fermentation in each vessel. In both vessels, the gluconic acid concentration generally increased with time as glucose was consumed, with the highest concentration resulting from the 40:60 fructose:glucose mixture in the bag. Conversely, the lowest production of gluconic acid during the brewing cycle resulted from the 70:30 fructose:glucose mixture in the jar.

**Figure 6.** Gluconic acid production throughout a brew cycle for kombucha brewed in either a standard glass jar or air-permeable silicone bag. The starting sugar ratio for kombuchas was either 40:60 fructose:glucose or 70:30 fructose:glucose.

Unsurprisingly, gluconic acid formation was favored by the 40:60 fructose:glucose sugar ratio and by brewing in the silicone bag, which allowed for greater oxygenation of the brew mixture [25]. For example, the 70:30 fructose:glucose bag mixture contains far less glucose than the 40:60 fructose:glucose jar mixture, yet at Day 26, their gluconic acid concentrations are nearly identical. This observation demonstrates the enhanced usage of glucose in the bag relative to the jar.

Figure 7 illustrates how ABV varied over the same 26-day fermentation period. Regardless of the initial sugar composition, ABV showed a drastic roll-over just beyond Day 12 for both bag brews, whereas both jar brews continued to generate ethanol in a nearly linear fashion throughout the brew cycle, thus closely resembling the time-dependent behavior seen earlier in Figure 4 when sucrose alone was used as a food source.

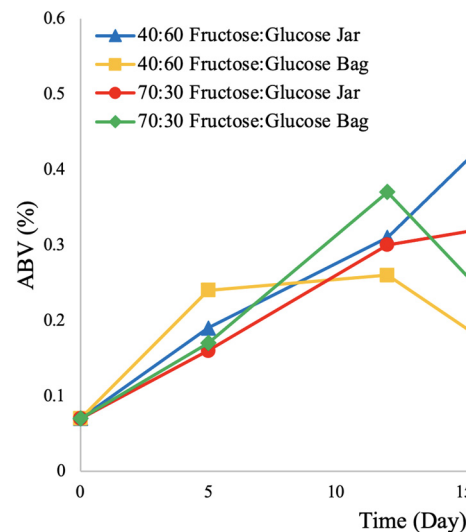


Figure 7. Ethanol production throughout a brew cycle for kombucha brewed in either a standard glass jar or air-permeable silicone bag. The starting sugar for the kombuchas was either 40:60 fructose:glucose or 70:30 fructose:glucose.

Figure 8 shows glucose usage throughout the brew cycle for both ratios of sugars fermented in the jar and bag. Focusing on the relative slopes of the near-linear decays, it is apparent that the bag brews showed considerably faster utilization of glucose over the course of the brew cycle, with the highest usage of glucose corresponding to the 40:60 fructose:glucose mixture.

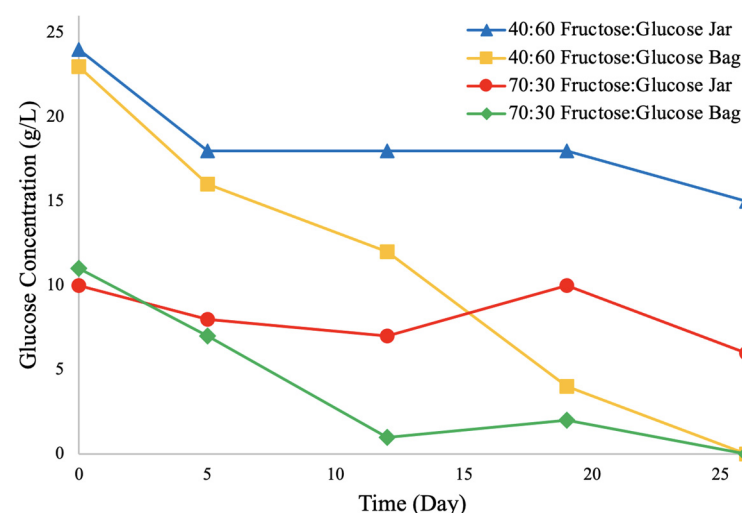


Figure 8. Glucose consumption throughout a brew cycle for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. The starting sugar for the kombuchas was either 40:60 fructose:glucose or 70:30 fructose:glucose.

A similar trend is shown in Figure 9, which illustrates how fructose was utilized over time during the 26-day fermentation process. Again, near-linear fructose usage was observed in all vessels, with relative rates of utilization showing that the bag brews consumed fructose at a higher rate than the comparable brews prepared in jars. In the specific case of the 40:60 fructose:glucose mixture, all of the fructose was consumed by Day 26 of the fermentation cycle.

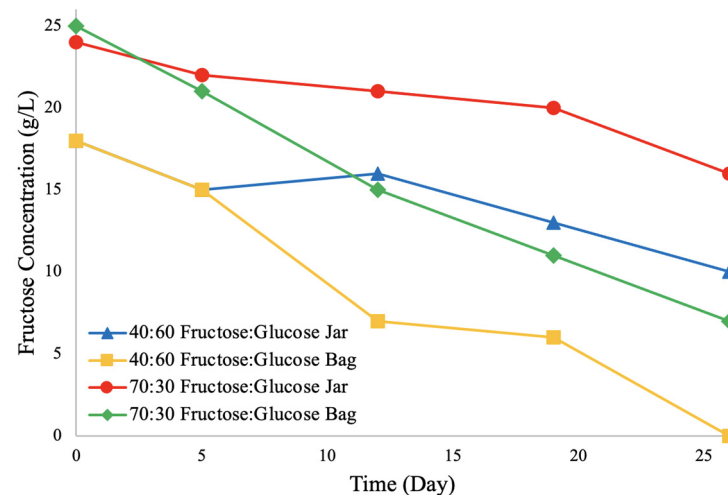


Figure 9. Fructose utilization throughout the brew cycle for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. The starting sugar for the kombuchas was either 40:60 fructose:glucose or 70:30 fructose:glucose.

Similarly, as shown in Figure 10, the acetic acid concentration increased over time, corresponding to the decay in fructose concentration, as shown previously in Figure 9. Again, acetic acid formation occurred most rapidly and ended with the highest concentrations in the bag brews relative to the jar ferments.

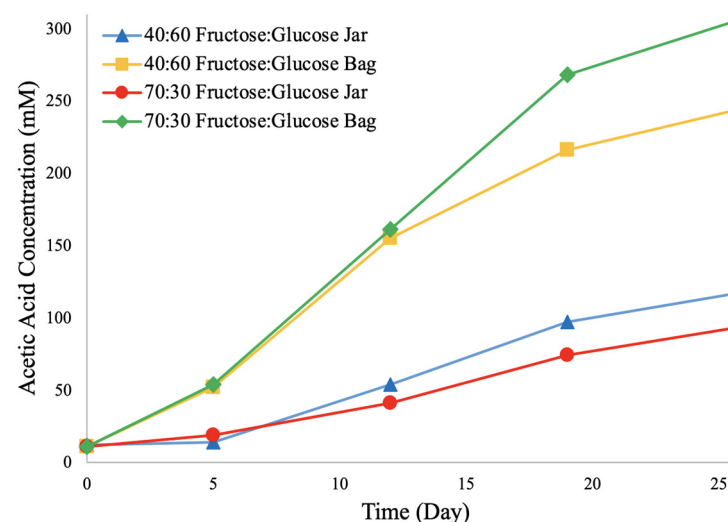


Figure 10. Acetic acid production throughout a brew cycle for kombucha brewed in either a standard glass jar or air-permeable silicone bag. The starting sugar for the kombuchas was either 40:60 fructose:glucose or 70:30 fructose:glucose.

Taken together, the results of the second phase of this work collectively indicate that even when sucrose was omitted as a food source and replaced with simple sugars alone, the bag ferments yielded over time smaller residual sugar concentrations, higher acid concentrations, and lower ABV values when compared to an identical brew prepared in a jar. As stated previously, the data indicate that the more highly oxygenated environment provided by the air-permeable bag facilitates aerobic bacterial activity over anaerobic yeast activity.

3.3. Part 3—Mimicking a Batch-Brewing Process

The third and final phase of this work was an investigation of how brewing in a silicone bag vs. a jar varied when multiple re-starts of the fermentation process were performed. This procedure was chosen to mimic the so-called batch-brewing process used by many commercial and home kombucha brewers. In this type of brewing, a portion of the mature kombucha ferment is harvested for flavoring and use, while the remaining portion is retained for use as a starter culture for the next batch. This process is then repeated over multiple fermentation cycles. In our case, we chose to enact three cycles consisting of an initial 21 day ferment, followed by two subsequent 14 day brew cycles. Between each cycle, we harvested 50% of the volume of the ferment produced on the terminal day of the cycle, leaving 50% of the volume behind to start the next fermentation cycle. For this part of the study, 50 g of sucrose was used at the beginning of each cycle as the food source in each vessel. The initial starter used was propagated from the original culture sourced from Happy Herbalist. ABV and acid production were measured at regular time intervals across the total duration of the study.

Figures 11 and 12 show gluconic and acetic acid production, respectively, as a function of brewing time. In both figures, abrupt decreases in acid concentration are noted at days 20 and 35, which result from dilution with sweet tea at each point of re-start. Prior to each dilution, an increase in each acid concentration is noted, with the bag slowly outpacing the jar prior to the first dilution. However, after subsequent re-starts, the bag rapidly accelerates the production of both gluconic and acetic acids, well exceeding the concentrations present during the previous cycle. In contrast, while both acids increase in concentration in the jar, it is only to the extent of maintaining the concentration of each acid prior to each new cycle.

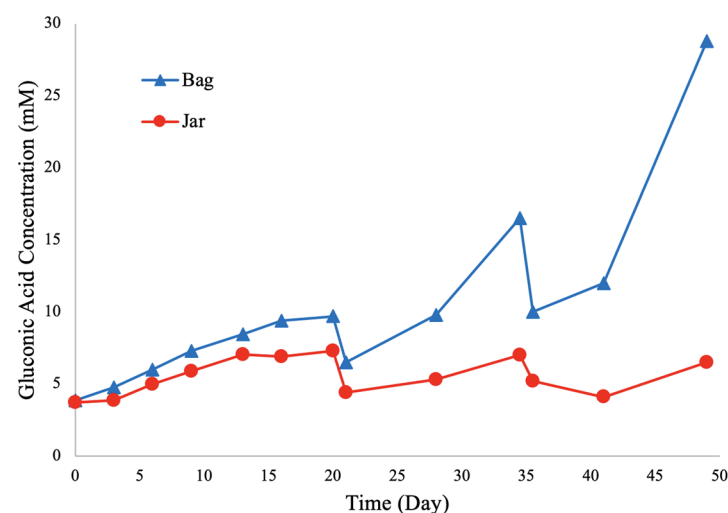


Figure 11. Gluconic acid production throughout multiple brew cycles in kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both samples.

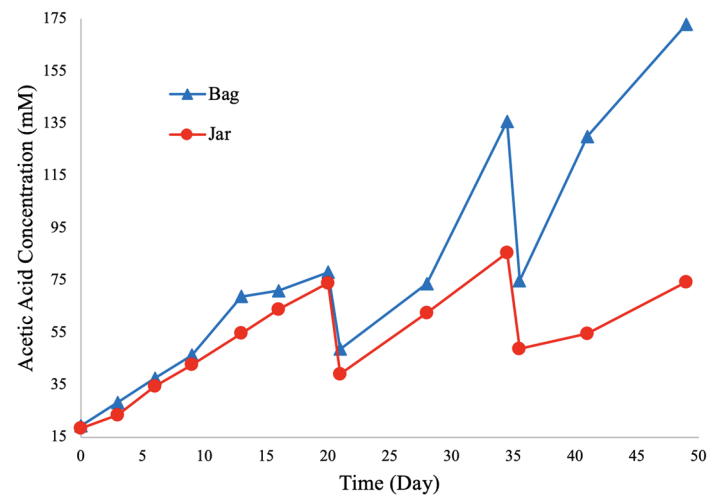


Figure 12. Acetic acid production throughout multiple brew cycles for kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both kombuchas.

Finally, Figure 13 shows the production and consumption of ethanol over the duration of the fermentation process. While the bag produces less ethanol at all points during fermentation, the ethanol concentration is observed to decrease much more drastically after the first re-start, essentially becoming unmeasurable by Day 49. In contrast, the ethanol content in the jar remained high at approximately 0.7% at the same time point.

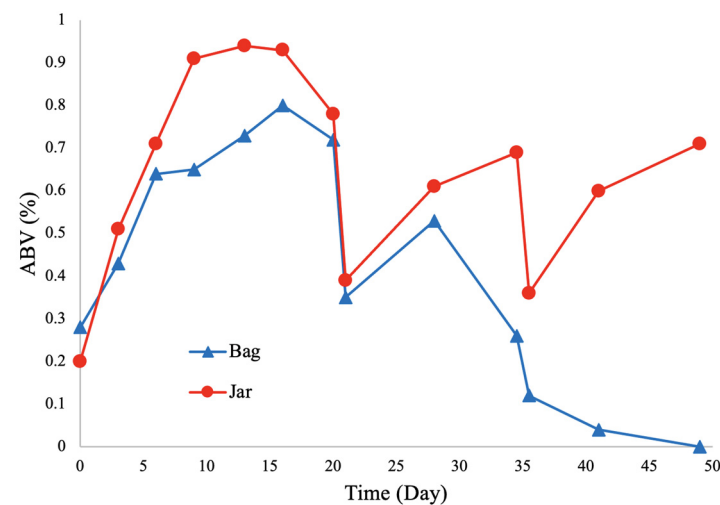


Figure 13. Ethanol production throughout multiple brew cycles in kombucha brewed in either a standard glass jar or an air-permeable silicone bag. Sucrose was used as the starting sugar for both kombuchas.

The combination of efficient sugar usage, rapid acid production, and low ABV all point toward the bag as a means of producing flavorful, alcohol-compliant kombucha in a shortened fermentation time. Further, increased concentrations of gluconic acid (as seen in the bag) can lead to a less acerbic, more accessible, and pleasant sour flavor in commercial kombuchas [10]. Another possible application of bag brewing could be the rapid production of an effective starter culture that could be used to produce kombucha using larger or more traditional brewing vessels produced from materials such as glass or stainless steel.

4. Discussion

We believe that the effectiveness of the silicone bag as a brewing vessel stems from a combination of factors that allow aerobic bacteria to inhabit an environment that facilitates their activity, thus leading to the desirable fermentation measurables that we have noted above.

Firstly, as we have emphasized earlier, a large factor contributing to the bag's success must be its overall air permeability. Whereas in a jar, oxygen can only enter from the exposed top, the bag allows for oxygen to enter from all directions; the overall gas-permeable surface area of the bag far exceeds that of a glass jar of equal volume. This increase in oxygen availability in turn, accelerates the activity of the aerobic *Acetobacter* strains present in kombucha [5,24]. The activity of the yeast must be considered as well. Normally, under anaerobic conditions, yeast carry out fermentation to form ethanol. Increasing oxygen exposure would be expected to shift the yeast to respiration, thus reducing ethanol production via the Pasteur effect [25]. Alternatively, under aerobic conditions and in the presence of excess sugar, yeast can generate ethanol via the Crabtree effect [26]. In the case of brewing kombucha in an air-permeable bag, it is possible that the Crabtree effect is dominant based on our observations of increased acetic acid production, which requires a proportional production of ethanol. Further evaluation will be required to confirm this hypothesis.

The second consideration involves the enhanced formation of pellicles throughout the inner surface area of the bag that is in contact with the kombucha culture broth. It has been well established that pellicle formation allows for continued bacterial exposure to an aerobic environment while also providing a layer of protection for the components of the SCOBY [27,28]. Studies have also shown that the region of the pellicle at the liquid interface is highly rich in bacteria, more so than in the broth alone [29]. This concentration of bacteria across a large surface area creates an environment in which the mass transport of sugars to these active surfaces is greatly enhanced, thus leading to greater bacterial activity. This idea is supported by the reported dissolved oxygen concentration measurements in the bag, which indicate a larger oxygen content early in the brew cycle relative to the comparable jar. However, over longer fermentation times, the measured oxygen concentration in the bag became smaller than that found in the jar. This result could stem from pellicle growth in the bag limiting the accessibility of oxygen to the system or higher efficiency of oxygen consumption as fermentation progresses. Taken together, our results indicate clearly that an air-permeable silicone bag is a highly efficient vessel in which to brew kombucha.

5. Conclusions

The use of the air-permeable silicone bag produced a kombucha that had significantly different and more favorable measured parameters than kombucha brewed in a jar, which may make it a preferable tool for brewers in the future. When using a pure sucrose food source, the bag was found to produce gluconic and acetic acid far more efficiently than the jar while also maintaining a low ABV that eventually decreased with time. These findings suggest that the bag could be utilized to produce a product that achieves acid endpoints more effectively, particularly in the case of creating a kombucha starter. An effective starter, crucial for initiating a new kombucha brew, generally exhibits high acid concentrations and low alcohol levels, which is an increasingly important consideration from a health perspective for consumers and producers of the beverage [30,31].

Results from the investigation focusing on the variation of sugar food sources found that kombucha brewed in a bag with a larger glucose concentration (relative to fructose) produced a more favorable acid profile, minimized alcohol content, and resulted in the lowest total sugar content at the end of the brewing period.

Finally, when the silicone bag was used in a simple batch-brewing scenario, acid production was maximized, while ethanol production was minimized over several re-start cycles, even though a different starting culture was utilized.

These results together indicate that using a glucose-favored simple sugar mix in conjunction with an air-permeable silicone bag may aid kombucha brewers in brewing a

more optimal product over a shorter brewing cycle, thus leading to enhanced production of the product.

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