Current Research in Food Science 8 (2024) 100694

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Perspectives from CO+RE

Elevated abundance of *Komagataeibacter* results in a lower pH in kombucha production; insights from microbiomic and chemical analyses

Aghogho Ohwofasa [a,b], Manpreet Dhami [c], Christopher Winefield [a], Stephen L.W. On [a,b,*]

[a] Department of Wine, Food and Molecular Biosciences, Lincoln University, Lincoln 7647, New Zealand
[b] Centre of Foods for Future Consumers, Lincoln University, Lincoln 7647, New Zealand
[c] Manaaki Whenua - Landcare Research, Lincoln, New Zealand

ARTICLE INFO

Keywords:
Kombucha
Komagataeibacter
SCOBY
Metabarcoding
Microbial diversity

ABSTRACT

Kombucha consumption has grown rapidly worldwide in the last decade, with production at both small- and large scales. The complex fermentation process involves both bacterial and yeast species, but little is known regarding the progression of microbial development during production. We explored the microbial diversity of multiple batches across two kombucha types, i. e commercial scale versus laboratory-made (hereafter "home") kombucha brew using metabarcoding to characterize both fungal and bacterial communities. We found the microbial community of the commercial kombucha brew to be more complex than that of the home brew. Furthermore, PERMANOVA uncovered significant compositional differences between the bacterial ($F = 2.68$, $R^2 = 0.23$, $p = 00.001$) and fungal ($F = 3.18$, $R^2 = 0.26$, $p = 00.006$) communities between batches. For the home brew, both alpha and beta diversity analyses revealed no significant differences between all batches and replicates. When the microbial diversity of the home and commercial kombucha types were directly compared, the former had higher proportions of *Ammoniphilus* and *Komagataeibacter*. The commercial kombucha on the other hand were high in *Anoxybacillus*, *Methylobacterium* and *Sphingomonas*. For the fungal communities, the most dominant fungal genera detected in both kombucha types were similar. Linear model revealed significant correlations between some microorganisms and the sugars and organic acids assayed in this study. For example, rising glucose levels correlated with an increase in the relative abundance of *Komagataeibacter* ($F = 7.115$, Adj. $R^2 = 0.44$, $p = 00.0003$). We believe these results contribute towards achieving a better control of the kombucha fermentation process and may assist in targeted product diversification.

1. Introduction

Kombucha is a beverage produced by fermenting sweetened black tea using a complex of bacterial and yeast species (Sun et al., 2015). Due to the rich presence of bioactive compounds, it has been referred to as a functional food with numerous health benefits (Cardoso et al., 2020; Diez-Ozaeta and Astiazaran, 2022). There are numerous theories pertaining its origin but all have pointed to the fact that it has been consumed in different countries such as India, China, Japan, Russia, Korea and Philippines for 2000 years (Torán-Pereg et al., 2021). Recently, the demand and popularity of kombucha has increased globally and is projected to gain a market value of USD 3.5–5 billion by 2025 (Batista et al., 2022; Kim and Adhikari, 2020).

Fermentation in kombucha can take anywhere between 8 and 14 days at temperatures ranging from 18 to 28 °C (Grassi et al., 2022). It begins by the addition of some previously fermented tea followed by inoculation of a starter culture commonly referred to as Symbiotic Culture Of Bacteria and Yeast - SCOBY (Kapp and Sumner, 2019). The microbial composition of the starter culture used can vary, depending on factors like its origin, geographical location, condition of the environment and metabolites synthesized during fermentation (Laavanya et al., 2021). Globally, different research groups have explored kombucha with the aim of describing the microbial populations associated with its fermentation. Andreson et al. (2022) used metagenomic methods to outline the microbial composition and further assessed the sensory and chemical profiles of several brands of commercial kombuchas from the market: all products differed in terms of their microbiome, chemical and sensory properties. Similarly, Barbosa et al. (2021) analysed the microbial communities associated with kombucha fermentation produced using black and green teas, and found the dominant bacterial genera

* Corresponding author. Department of Wine, Food and Molecular Biosciences, Lincoln University, Lincoln 7647, New Zealand.
E-mail address: stephen.on@lincoln.ac.nz (S.L.W. On).

https://doi.org/10.1016/j.crfs.2024.100694
Received 18 October 2023; Received in revised form 4 February 2024; Accepted 5 February 2024
Available online 18 February 2024
2665-9271/© 2024 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

across all fermentation times proved to be *Komagataeibacter*. Reports of *Komagataeibacter* being the most abundant bacterial genus in Kombucha is a relatively common phenomenon due to its role in producing the solid phase pellicle (Harrison and Curtin, 2021). Further, the fungal communities were dominated by *Zygosaccharomyces*. These taxa have previously been identified to dominate other kombucha communities. (Coton et al., 2017; Teoh et al., 2004). However, unlike the bacterial genus, there seems to be a high variability in the dominant fungi genera reported by various studies (Harrison and Curtin, 2021). Other genera that have been reported to be dominant includes *Brettanomyces* (Marsh et al., 2014) and *Pichia* (Reva et al., 2015).

One study in New Zealand analysed kombucha from a sensory and consumer aspect and carried out an analysis of different novel kombuchas that were produced using ingredients such as Kawakawa (*Piper excelsum*) leaves, hops and black pepper (Alderson et al., 2021); each one gave rise to unique sensory characteristics but the kombucha made with hops was the most preferred. Besides sensory analysis, several studies have been done in terms of microbial analysis. Wang et al. (2022a) identified and characterized the dominant acetic acid bacteria (AAB) and yeast strains found in kombucha products sold in New Zealand.

Self- or Homemade kombucha is becoming increasingly popular. This is supported by the increase in the brands of kombucha starter kits which are targeted for these brewers (Brewer et al., 2021). Thus, though the existing literature on kombucha are important, most of these studies assessed the microbiome associated with only the finished products bottled and shipped to supermarkets (Andreson et al., 2022; Kaashyap et al., 2021; Wang et al., 2022a). This might be a limitation, as different producers could have various processes that could impact the microbial composition of their final products (Kim and Adhikari, 2020). Others that studied the fermentation process only assessed a few time points (Arikan et al., 2020; Barbosa et al., 2021). To date, none of these studies compared the microbiomes of kombucha produced in a commercial setting with those brewed at home. This we believe is important to qualify from a quality assurance and indeed functional food viewpoint, especially with the increase in the popularity of self-made kombucha. Finally, the underlying mechanisms that influence the microbial communities associated with a typical kombucha fermentation remains underexplored. To this end, we built a comprehensive picture of temporal shifts in the microbial community during kombucha fermentation under a series of small scale and commercial conditions to investigate how (1) process of fermentation and (2) style of fermentation influences kombucha microbial community. We also analysed some sugars and organic acids found in both kombuchas for further comparison of the traits that may vary between the facilities, and to evaluate any major trends between microbial composition and beverage content.

2. Materials and methods

2.1. Kombucha fermentation and sampling

"Home" kombucha brew was produced in the sanitary conditions (food grade) of the food laboratory of Lincoln University using SCOBY sourced from Get Cultured Limited, New Zealand. This was delivered in a pouch containing a suitable volume of fermented liquid tea. Firstly, this was used to brew 1 L (L) volumes to produce enough kombucha tea needed to set up larger experimental brews using the back slopping process. For experimental set up, glass jars of 5 L were used. For each batch, we boiled 3 L of tap water and added 255 g of sucrose i. e 85 g per liter. To this, we added 6 bags of commercially available ("Bell original" brand) black tea (ca. 2 g per bag). This was steeped for 5 min and removed. After cooling down thoroughly, 450 ml of kombucha tea (pH = 2.55) which we prepared previously was added. We included one circular pellicle and covered it with a breathable fabric firmly secured with a rubber band. Each batch was allowed to ferment for eight days, and temperature was fixed at 25 °C. From this, 200 ml duplicate samples of the tea phase were taken daily. Each experiment was set up independently (biological duplicates), for two batches i. e a total of 4 kombucha sets. For SCOBY, samples from the newly formed pellicle were collected using a sterile scalpel. Care was taken to ensure proper sampling of microbial communities by dissecting a concentric circle from the middle that includes both the top and bottom layer as outlined by Harrison and Curtin (2021). Using the Mettler Toledo SevenEasy S20 pH Meter (Mettler Toledo, Giessen, Germany), we recorded the pH of all home brew kombucha samples at the time of collection. Measurements were done in triplicates and the average value was taken.

Commercial kombucha analysis was carried out in collaboration with a SME kombucha producer situated in New Zealand. From large fermentation vessels, kombucha samples were obtained daily from the faucet below and from the top for a period of eight days. This was done for adequate representation of microorganisms. On the last day, samples were collected from the newly formed SCOBY using the same method as utilized for the home brew kombucha. Sampling was done for three consecutive batches that also utilized a back slopping approach. Due to circumstances beyond our control, replicates and pH measurements were not possible for the commercial set of kombucha samples. Note that for the SCOBY in both the commercial and home kombucha brew, only samples from the end of fermentation were analysed. "Starting" SCOBYs were not taken from the beginning to avoid any sort of contamination issues. In addition, few samples were missing or not taken as at the time of sampling. These are all shown in Supplementary Table 1.

2.2. DNA extraction

Samples from each batch were stored at −80 °C until all sampling procedures were completed and ready for further analysis. For DNA extraction from the liquid phase, samples were allowed to completely defrost overnight at 4 °C. We centrifuged (Heraeus Multifuge X3R, Thermo Scientific) at 4700 g for 10 min at 4 °C to generate a pellet. This was ready for DNA extraction and was stored at −20 °C. For the biofilm processing, the method applied by Marsh et al. (2014) was utilized with slight modification. Briefly, 250 mg of the pellicle was collected using a sterile blade. We made sure the sampled portion consisted of the upper and lower SCOBY layer as described by Harrison and Curtin (2021), so as to correctly infer the community composition. This was washed thrice with a suitable volume of UltraPure distilled water (invitrogen). This was diced into small pieces with the aid of a sterile blade and transferred into a 2 ml microcentrifuge tube. To this tube we added 300 mg of sterile glass beads (Sigma-Aldrich) and 750 μl of cellulase (Sigma-Aldrich). Using a TissueLyser II (QIAGEN) for 10 min, we lysed the SCOBY samples. After the lysis, the lysate was incubated at 40 °C for 60 min. Finally, we generated a pellet by centrifugation at 3000*g* for 5 min using a benchtop microcentrifuge (Eppendorf – Germany) which was used for DNA extraction.

DNA extraction was achieved using the Mag-Bind Environmental DNA 96 kit (OMEGA). The manufacturer's recommended soil protocol was largely followed only modifying at the cell lysing step where we used the TissueLyser II (QIAGEN) for 5 min. The quality of our extracted DNA was assessed using 1.5% agarose gel electrophoresis. Finally, we estimated the total DNA concentration and purity using a DeNovix DS-11 spectrophotometer.

2.3. Metabarcoding

The two step amplification method applied by Ohwofasa et al. (2023) was utilized. For bacteria, the 515 F/806 R primer pair was used to target the V4 region of the 16 S ribosomal DNA for the first step. Fungal analysis targeted the large subunit (LSU) ribosomal DNA region using LSU200(A)-F/LSU481(A)-R and LSU200-F/LSU481-R as described by Asemaninejad et al. (2016). We used the KAPA 3G plant PCR kit scaled down to a reaction volume of 15 μl following the manufacturer's

recommended protocol. We utilized the following thermal cycling conditions. Denaturation at 95 °C for 120 s, 95 °C for 20 s (35 cycles), 52.5 °C (Bacteria)/55 °C (fungi) for 20 s (35 cycles - annealing), and 72 °C for 30 s (35 cycles - extension). A final extension was carried out for 10 min at 72 °C.

PCR products generated from the first step PCR served as the template DNA for the second step PCR. Using barcoded primers, we carried out the second step PCR using the following thermal cycling conditions. Initial denaturation at 95 °C for 2 min, 5 cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 30 s, and final extension at 72 °C for 2 min. Purification of PCR products was done using SeraMag Magnetic Speed-Beads (Rohland and Reich, 2012) so as to normalize concentration and remove primer dimers. Qubit (dsDNA HS Assay Kit, Invitrogen, Carlsbad, United States) was then used to accurately determine our DNA concentration. Based on amplicon length and the number of samples contained in each library, we pooled all libraries in an equimolar manner. We assessed the quality of the final pooled library using LabChip GX Touch Nucleic Acid Analyzer (PerkinElmer, Waltham, United States). The Illumina MiSeq platform at Auckland Genomics Facility (University of Auckland) was used for sequencing (phiX spike 10 %, 250 × 2 cycles).

2.4. Amplicon sequence variant (ASV) cluster and taxonomic assignment

For the commercial kombucha brew, raw sequence reads downloaded from Illumina in BCL files were converted to fastq format using the bcl2fastq Illumina software (Illumina 2017). Demultiplexing was done using Claident (Tanabe and Toju, 2013). We evaluated and merged all paired end reads using PEAR (Zhang et al., 2014). The amplicon-based DADA2 pipeline (Callahan et al., 2016) was utilized for further analysis of these sequences. Here, key steps such as quality filtering and chimera removal are carried out. Taxonomic assignment for prokaryotic organisms was done using the SILVA v132 16 S rRNA database (Quast et al., 2012) while fungal taxonomic assignment utilized the UNITE taxonomic reference (Abarenkov et al., 2021). Occasionally, we relied on the Basic local alignment search tool (BLAST) (Altschul et al., 1990) to achieve a better taxonomic resolution. All analyses were done on the New Zealand eScience Infrastructure (NeSI) HPC environment.

2.5. Determination of sugar and organic acids using HPLC

2.5.1. Organic acids

The method employed by Shi et al. (2011) using the High Performance Liquid Chromatography (HPLC) (Shimadzu Corporation, Kyoto. Japan) was modified as follows. All chemicals used except otherwise stated were sourced from Supelco Bellefonte, PA, USA, through Sigma-Aldrich, Australia. The Rezex ROA-Organic Acid H+ (8%) column (3000 × 7.8 mm, Phenomenex) which had a Guard column (Carbo-H 4 × 3.0; Phenomenex) was utilized to separate and analyse organic acids. The mobile phase was 5 mM H_3SO_4, and this was filtered through a 0.45 μm membrane. The flow rate was at 0.5 ml/min with the column temperature maintained at 55 °C. 20 μl sample was injected and the UV detector (SPD-20 A) was set at a wavelength of 210 nm. A mixture of the standard stock solution using analytical grade D-gluconic acid, citric acid, oxalic acid, L-lactic acid, succinic acid, L-malic acid, acetic acid, and formic acid were prepared, and standard curve solutions were prepared from the standard stock solution. The standard curve concentrations were 0, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 120.0, 150.0, 300.0, 500.0 ppm. For sample preparation, 5 ml of kombucha samples were filtered using a 0.2 μm Nylon membrane. These filtered samples were diluted 10 times using deionized (DI) water before injection. All organic acids in samples were identified by comparing retention time of organic acid standards. Using the external calibration standard curve, sample quantification was determined with the peak area of the chromatograms. The Lab solution software (Version 5.87 SP1) was used to process all data.

2.5.2. Sugars

The same High Performance Liquid Chromatography (HPLC) system as above was used to analyse sugars. D-fructose, D-glucose, and sucrose were sourced from Sigma- Aldrich. The Prevail™ Carbohydrate ES Columns (250 × 4.6 mm) fitted with a guard column (Prevail Carbohydrate guard column (7.5 × 4.6 mm)) was employed (The GRACE Davison). The gradient mobile phase consisted of A acetonitrile, B DI water (0–5 min, 20%B; 5–10 min 20%-50%B; 10–11 min 50%-20%B; 11–15 min 20%B). The flow rate was 1 ml/min with the column temperature kept at 20 °C. The injection volume was 4 μl. The 3300-ELSD detector had a flow rate of 1.4 L/min at a temperature of 38 °C with a gain of 4. A mixture of the standard stock solution was prepared by dissolving D-fructose, D-glucose and sucrose in DI water. The concentration of D-fructose and D-glucose was 2500 ppm while the concentration of sucrose was 5000 ppm. Standard curve concentrations for D-fructose and D-glucose were 0, 25.0, 50.0, 100.0,200.0, 300.0, 400.0, 450 ppm and the concentration of sucrose were 0, 50.0, 100.0, 200.0, 400.0, 600.0, 800.0, 900.0 ppm. 5 ml of kombucha samples were filtered using a 0.2 μm Nylon membrane. These filtered samples were diluted 80 times with DI water before injection. Identification of sugars was done as described above.

2.6. Statistical analysis

Analysis of microbial diversity was carried out using the open-source R programming language (v4.1.0). All packages utilized are listed in Supplementary File 1. Briefly, using the phyloseq (v1.38.0) package (McMurdie and Holmes, 2013), we generated a phyloseq object for bacteria and fungi respectively. To account for any dissimilarities in library sizes i. e normalizing for sampling depth, the ASV abundances were transformed to relative abundance. We used these transformed data for subsequent analysis. All commercial and home brew samples were processed separately at this point. We estimated alpha diversity within each kombucha batch using the Shannon diversity index. For testing differences among the groups, we implemented the non-parametric permutation based MANOVA using ADONIS function in vegan (Dixon, 2003).

To compare the microbiome of the commercial kombucha versus that of the home brew kombucha, two batches of both kombucha types were taken and processed together using phyloseq. For this, we selected batch 2 and batch 3 of the commercial brew and both replicates of batch 2 for the home brew. These were selected with the assumption that since a back slopping approach was used, the microbiome of the fermentation process must have stabilized in these batches as compared to earlier batches. This was visualized through the non-metric multidimensional scaling (NMDS) that applied unweighted UniFrac as a distance matrix. We further applied DESeq2 (Love et al., 2014) to identify specific ASVs that were differentially expressed in each kombucha type.

Using the lmer function of lme4 package (Bates et al., 2014), we employed linear models to investigate if pH or sugar (glucose, fructose and sucrose) concentration (independent variable) could predict the relative abundance of a given bacteria or fungi (dependent variable). For presentation of models, we utilized the stargazer package (Hlavac, 2018). To be sure that all assumptions of a linear model were met, we performed the Shapiro-Wilk (Shapiro and Wilk, 1965) tests so as to establish if residuals were normally distributed. We also checked for homoscedasticity using the Bartlett test (Bartlett, 1937).

3. Results

3.1. Abundance of bacterial and fungal communities associated with the commercial kombucha brew

For bacteria, a total of 272 amplicon sequence variants (ASVs) were realized upon taxonomic assignment. This resulted in 149 genera after taxonomic assignment. Though many of these were in limited

abundance, the bacterial community detected in all three batches of the commercial kombucha brew showed a high diversity. All three batches had 28 genera which were common and detected in all brews. Of these, the most abundant genera, which together made up 60% of the bacterial community included *Komagataeibacter*, *Gluconobacter*, *Sphingomonas, Bradyrhizobium*, *Methylobacterium*, *Caulobacter*, *Anoxybacillus,* and *Ammoniphilus*. This is shown in Fig. 1 (A, B and C) and Supplementary Table 2. Alpha diversity estimated using the Shannon index revealed no significant differences in the number of bacterial taxa found in all three batches (Supplementary Fig. 1). However, significant compositional differences (i.e. in the identity of taxa recognized) between the samples were reported by PERMANOVA ($F = 2.68$, $R^2 = 0.23$, $p = 00.001$).

The fungal communities had fewer ASVs with a total of 99. Taxonomic assignment then gave rise to 31 fungal genera. In all three batches, about 95% of the fungi detected consisted of *Saccharomyces*, *Hanseniaspora, Pichia*, *Brettanomyces, Zygosaccharomyces*, *Aureobasidium,* and *Metschnikowia.* The first batch had the highest abundance of *Pichia* (29.6%), while the second and third batch was mainly dominated by *Hanseniaspora* (52.7% and 55.7% respectively). Fig. 1 (D, E and F) and Supplementary Table 3 outlines this information. Like the bacterial community, the Shannon index revealed no differences in the alpha diversity (Supplementary Fig. 2). On the other hand, PERMANOVA uncovered significant ($F = 3.18$, $R^2 = 0.26$, $p = 00.006$) differences between the fungal species composition of the three kombucha batches.

3.2. Dominant bacterial and fungal communities detected in the home made kombucha brew

417 amplicon sequence variants (ASVs) were detected upon the analysis of the bacterial sequences in the home kombucha brew. Using this to carry out taxonomic assignment generated 124 bacterial genera. 17 of these genera were detected in all batches and replicates (Supplementary Table 4). However, as can be seen in Fig. 2 (A, B), populations were dominated by *Komagataeibacter,* which made up more than 60% of the bacterial community in all home brewed kombucha. Other bacterial organisms present in all batches include *Ammoniphilus*, *Paenibacillus*, *Bradyrhizobium*, *Sphingomonas*, *Rhodococcus,* and *Methylobacterium.* Both the Shannon diversity (Supplementary Fig. 3) and PERMANOVA ($p = 00.2797$) revealed no significant differences between the batches.

Fungal sequences of the home brewed kombucha revealed 175 ASVs, which were assigned to 30 genera (Supplementary Table 5). All batches and replicates had 9 genera in common. These were *Saccharomyces*, *Hanseniaspora, Brettanomyces*, *Zygosaccharomyces*, *Metschnikowia*, *Aureobasidium, Penicillium*, *Lachancea* and *Rhodotorula.* Like the commercial brew, the top six genera constituted more than 90% of the total fungal community detected (Fig. 2 C, D). Thus, both batches revealed no significant alpha (Supplementary Fig. 4) and beta diversity (PERMANOVA; $p = 00.0659$).

3.3. Dominant microbial communities in the newly formed SCOBY in both kombucha types were similar

The bacterial and fungi communities found in the SCOBY of the home brewed and the commercial kombucha shared similarities. Supplementary Table 6 shows the abundance of all organisms detected. As can be seen, we detected 40 bacterial genera in the SCOBY of the home brewed kombucha. For the commercial brew, we identified only 18 genera. However, most of these genera were found in limited abundances; the genus *Komagataeibacter* accounted for over 78% of the bacterial community in both the commercial and home brewed kombucha SCOBY (Fig. 3A and B).

For fungi, the commercial brew had 15 genera, as compared to the home brewed kombucha which had 9. Variations included the genus

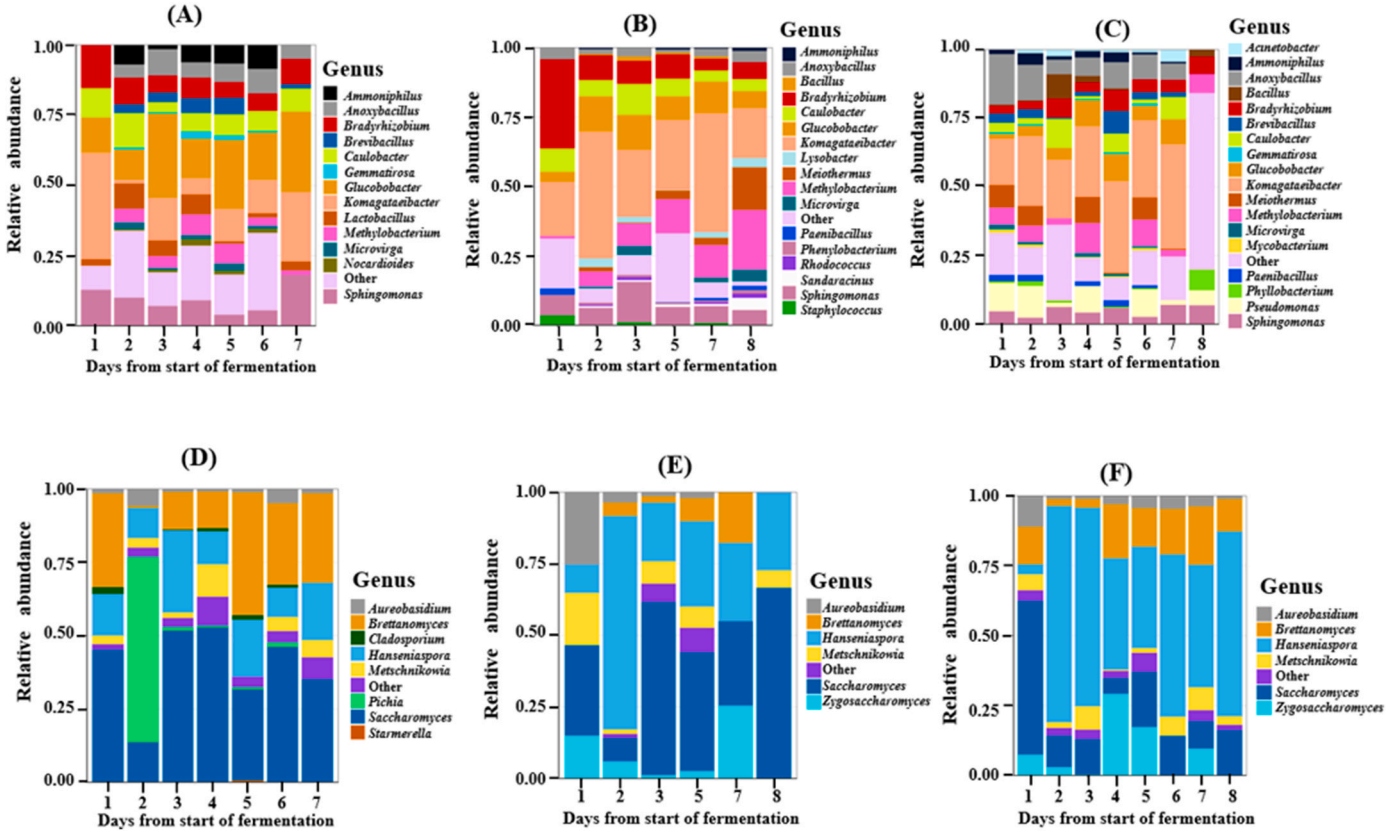

Fig. 1. Relative abundances of bacteria (A, B, C) and fungi (D, E, F) at the genus level in Batch 1 (A, D), Batch 2 (B, E) and Batch 3 (C, F) of commercial kombucha brews.

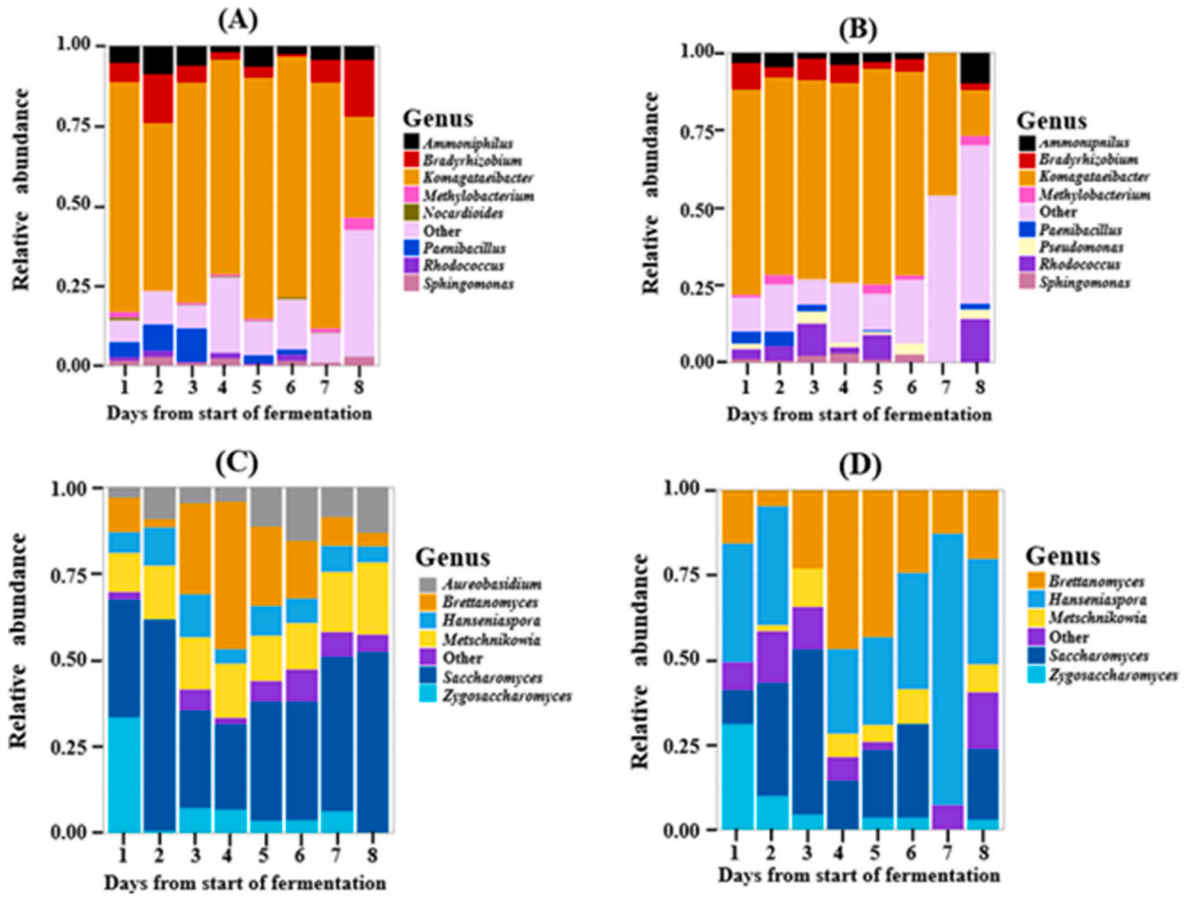

Fig. 2. Relative abundances of bacteria (A, B) and fungi (C, D) at the genus level in Batch 1 Replicate A (A, C), Batch 1 Replicate B (B, D) of the home made kombucha brew. Supplementary Fig. 5 depicts Batch 2 (Replicate A and B).

Hanseniaspora that accounted for 23% of the fungi detected in the SCOBY of the home kombucha brew, whereas the commercial brew had 8.4%. Similarly, 13.7% of the commercial brew was *Brettanomyces,* while the home brew had 5%. Despite these differences, *Zygosaccharomyces* and *Saccharomyces* accounted for over 50% of fungi detected in the SCOBY of the commercial and home kombucha. The relative abundance plots are shown in Fig. 3.

3.4. Major bacterial and fungal differences between the commercial and home-brewed kombucha products

The relative abundance of some bacterial genera appeared to be more prevalent in one product compared to the other. The home brewed kombucha had higher proportions of *Ammoniphilus* (Fig. 4A) and *Komagataeibacter* (Fig. 4D) whereas the commercial product contained high numbers of the genera *Anoxybacillus* (Fig. 4B), *Methylobacterium* (Fig. 4E) and *Sphingomonas* (Fig. 4F). Alpha diversity (Observed, Shannon, InvSimpson and Chao 1) analyses indicated that the commercial kombucha brew had a higher bacterial richness and diversity, than the home brewed kombucha (Supplementary Fig. 6A). This was supported by the output of DESeq2 which identified 23 differentially expressed ASVs in the commercial kombucha brew. In contrast, the home brewed kombucha had three ASVs which were differentially expressed, and all were identified as *Komagataeibacter* at the genus level (Supplementary Table 7).

For fungal communities, the dominant genera were similar in both kombucha types (Fig. 4 H, I, J, K and L; Fig. 5B). Thus, the alpha diversity measure revealed no significant differences between the fungal genera of the home and commercial kombucha brew (Supplementary Fig. 6B). Nonetheless, analysis of DESeq2 output revealed 5 ASVs to be abundant in the home brewed kombucha, while 14 ASVs were in the commercial product (Supplementary Table 8).

3.5. pH as a selection factor in kombucha: the case of komagataeibacter

The pH of home brew kombucha decreased continuously as fermentation progressed (Supplementary Fig. 7). One way ANOVA reported no significant difference ($p > 00.05$) across the pH values of all homemade kombucha. With the significantly higher relative abundance of *Komagataeibacter* in the home made kombucha, we fitted a linear model using its abundance as a dependent variable and the concentrations of the organic acids, sugars, and the pH as the explanatory variable. Table 1 shows that pH had the most significant interaction with the relative abundance of *Komagataeibacter.* As pH decreases, its relative abundance increases ($F = 7.12$, Adj. $R^2 = 0.44$, $p = 00.0002$). With the absence of pH data from the commercial samples, we predicted their pH values using the concentration of gluconic acid in each sample. A linear model (Supplementary Table 9A) has shown this acid to be a good predictor of pH values. To ascertain if this model could accurately estimate the pH, we used it to predict the pH of the home made kombucha brew. These values were compared with the experimental pH of the home kombucha brew which we measured earlier. No significant differences were reported. When the pH of the home made kombucha was compared with that of the commercial brew, a relatively higher pH which was statistically significant was associated with the commercial brew. These are shown in Supplementary Table 10.

4. Discussion

The increase in popularity of kombucha as a healthy drink has been

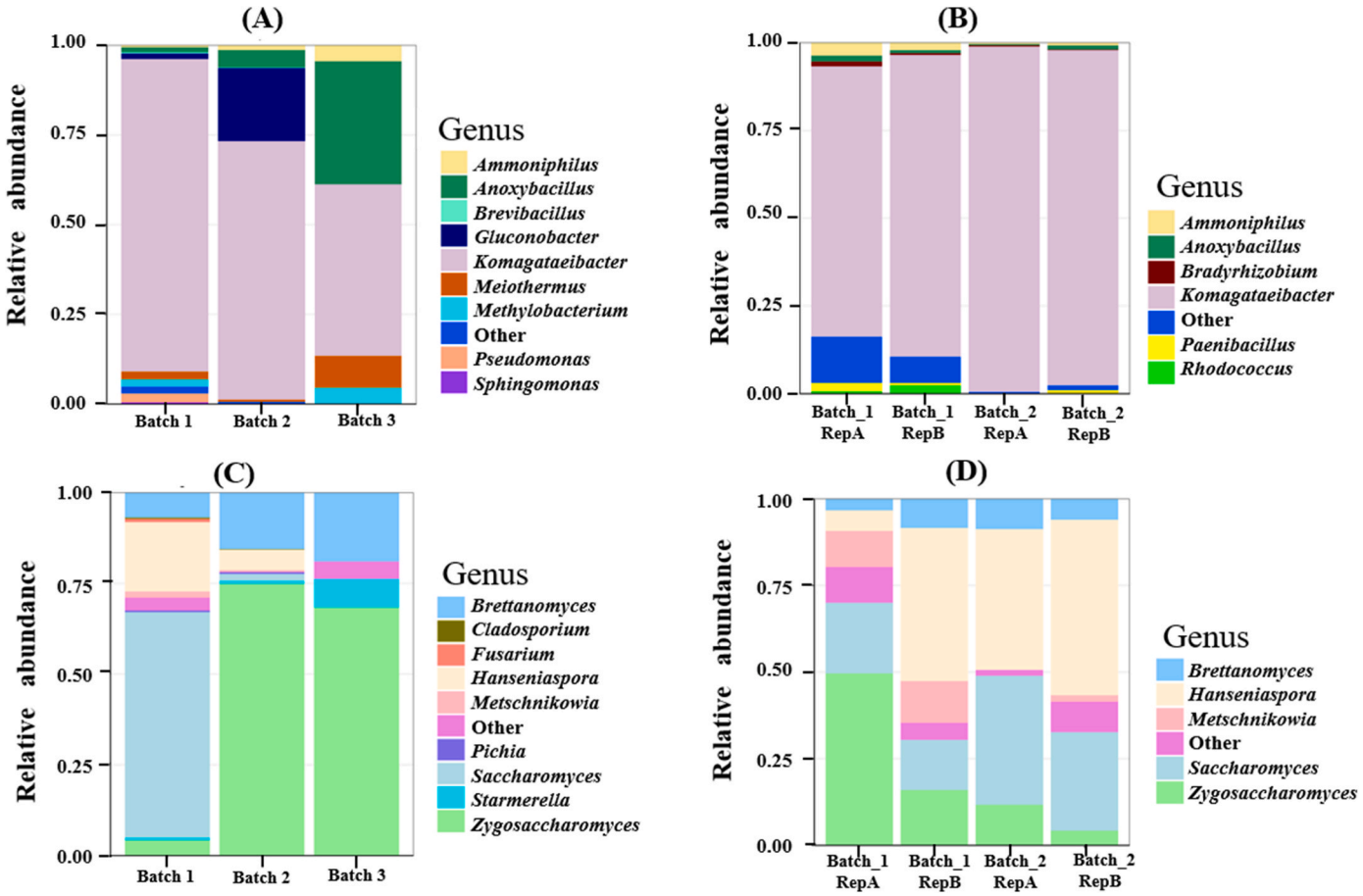

Fig. 3. Relative abundances of bacteria (A, B) and fungi (C, D) at the genus level in the newly formed SCOBY at the end of Kombucha fermentation in commercial (A, C), and home kombucha brew (B, D).

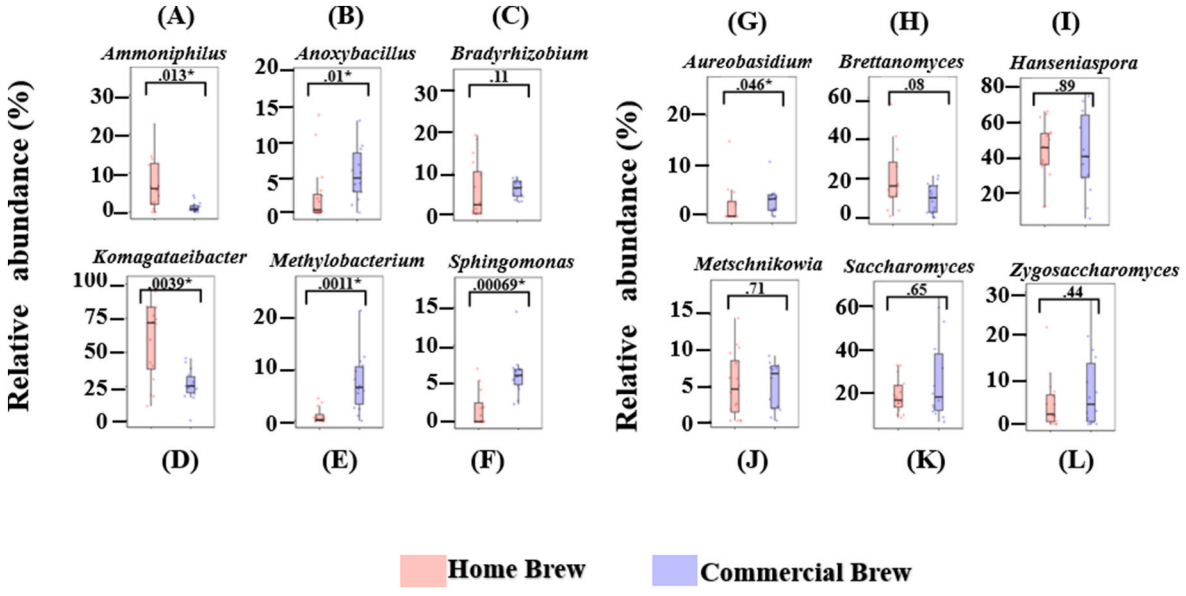

Fig. 4. Relative abundance of the top six most dominant bacterial and fungal in the commercial and home kombucha brew.

attributed to its potential health benefits (de Miranda et al., 2022). This has thus been matched with a corresponding increase in numerous research works in this area (Andreson et al., 2022; Arikan et al., 2020; Bishop et al., 2022; Wang et al., 2022b; Yang et al., 2022). However, to the best of our knowledge, none of these studies have compared an entire commercial kombucha fermentation process to that produced on a small scale (homemade). The major aim of this research was to assess the microbial diversity associated with kombucha fermentation from a commercial producer and compare with that produced on a small scale. We also analysed the sugars and some organic acids present, in a bid to

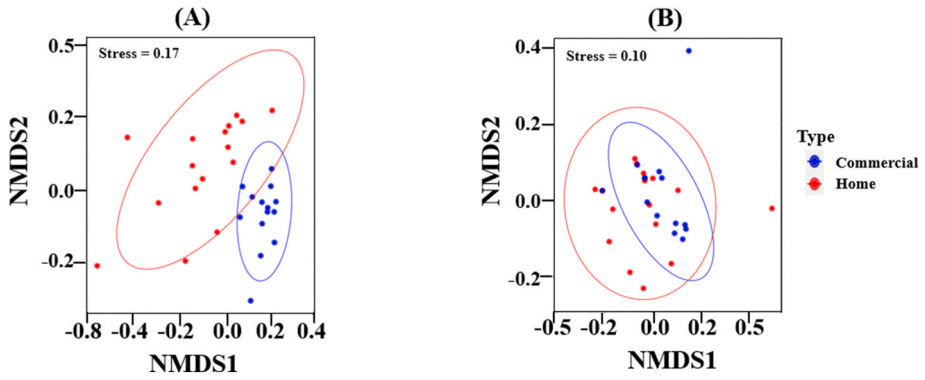

Fig. 5. NMDS ordination for (A) Bacterial communities; (B) Fungal communities in Kombucha fermentations. Samples in blue and red depict the commercial and home brewed kombucha respectively. Statistically significant ($p < 0.05$) groupings are represented with ellipses at 95% confidence intervals. A better separation can be observed with the bacterial communities. The dominant fungal communities of the home and commercial kombucha brew were similar.

Table 1
Result from the linear model showing the estimated effect of all independent variables on *Komagataeibacter* relative abundance.

	Dependent Variable: Rel. Abundance of *Komagataeibacter*
Constant	6.39
	t = 4.83
	$p = 0.00005$***
pH	−1.74
	t = −4.46
	$p = 0.0002$***
Fructose	−0.17
	t = −4.41
	$p = 0.0002$***
Glucose	0.16
	t = 4.19
	$p = 0.0003$***
Acetic_acid	−0.33
	t = −3.84
	$p = 0.0007$***
Observations	32
R^2	0.51
Adjusted R^2	0.44
Residual Std. Error (df = 27)	0.18
F Statistic (df = 4; 27)	7.12***

Note: *p < 0.1; **p < 0.05; ***p < 0.01.

uncover mechanisms that influence the microbial community associated with a standard kombucha fermentation.

Overall, in both the commercial and home brew kombucha, we detected more bacterial taxa as compared to fungal. This is supported by previous research groups (Arikan et al., 2020; Coton et al., 2017; Villarreal-Soto et al., 2020). In terms of general microbial abundance, more complex bacterial and fungal communities were associated with the commercial kombucha brew than the home-made product. This could probably be due to the fact that being a commercial facility, it experiences a high turnover of microbial diversity on a continuous basis as compared to a home setting where it was just a one-off fermentation process. Note that all batches and replicates (home/lab brew) were set up using the same approach. However, with a backslopping step, it is possible that the first batch may not be a stable microbial community. This was the key reason why the microbiome comparison (commercial versus home brew) was done using the second batch onwards. For specific bacterial abundance, the home made kombucha was dominated by *Komagataeibacter,* accounting for over 60% in all batches and replicates (Fig. 2A and B). The dominance of this genus in kombucha is established in the literature (Barbosa et al., 2021; Gaggia et al., 2018; Yang et al., 2022). Though it was also detected in the commercial kombucha brew, it was not as dominant as seen in the home brew (Fig. 4D). The reason for this might be connected to the well-known priority effect phenomenon which affects species diversity in many ecological communities (Dhami et al., 2016). Briefly, with a one-time fermentation as observed in the home made kombucha, *Komagataeibacter* was able to establish dominance easily. However, with a complex microbial community as seen in the commercial brew, the possibility of other species capable of competing and thereby limiting *Komagataeibacter* influence becomes enhanced. This may also be related to the strong interaction between pH and *Komagataeibacter* relative abundance which we mentioned. This hypothesis is supported by the estimated pH values of the commercial kombucha brew. This brew which had a lower *Komagataeibacter* abundance showed higher pH values. The home brew kombucha on the other hand, with lower pH ranges had an abundance of *Komagataeibacter*. More research will be required to verify this. Also, there is a possibility that the dominant *Komagataeibacter* species or strain might differ from one batch of fermentation to another. This was inferred from our DESeq2 output as 2 ASVs identified as *Komagataeibacter* were differentially expressed in the commercial brew while 3 other ASVs were highly expressed in the home kombucha brew. However, these could not be identified with any certainty beyond the genus level (Supplementary Table 7). *Komagataeibacter* are known to synthesize gluconic acids (Li et al., 2021). With its higher relative abundance in the home made kombucha, this might possibly explain why we obtained higher concentrations of gluconic acid (Supplementary Table 11) in the home brew kombucha as compared to the commercial brew. Li et al. (2022) have previously suggested that a higher gluconic acid content is associated with an improved sensory quality of kombucha.

The difference in the abundance of other bacterial genera in the commercial brew and home kombucha brew was pronounced (Fig. 4). These include *Ammoniphilus,* which was more evident in the home brew and *Anoxybacillus, Methylobacterium* and *Sphingomonas* whose relative abundance was higher in the commercial brew. *Ammoniphilus* has been described as halotolerant after it was detected in highly saline environments (Mukhopadhyay et al., 2021). It has also been isolated from the root rhizosphere and it was suggested that their abundance might suggest nitrogen metabolism and amino acid biosynthesis (Ramadan et al., 2021). It remains to be seen if the same applies to its detection in kombucha fermentation. *Anoxybacillus* was scant in the home brew (less than 2%), while it was relatively high in the commercial brew. The same can be said of its abundance in the SCOBY found at the end of fermentation in the commercial brew (Fig. 3A). *Anoxybacillus* have frequently been linked with hot springs beds (McClure, 2006), however, their isolation from foods such as gelatin and milk powders have been reported (De Clerck et al., 2004; Goh et al., 2013). It has also been described to grow preferentially in the presence of ethanol (Dai et al., 2011). *Methylobacterium* has been detected in a variety of sources such as soil, plants, fermenters, drinking water systems and cocoa bean fermentation (Kato et al., 2008; Serra et al., 2019). Its ability to colonize

various environments have been attributed to its flexibility in terms of carbon sources (Dourado et al., 2015). Besides *Sphingomonas* which have been detected in kombucha fermentation (Barbosa et al., 2021), as well as homemade yoghurts (Orhan et al., 2021), we believe ours is the first study to report the presence of *Ammoniphilus*, *Anoxybacillus* and *Methylobacterium* in kombucha. The role these taxa play in kombucha fermentation is thus currently unknown.

In contrast to the bacterial communities, yeast diversity in both kombucha types was less pronounced (Fig. 5; Supplementary Fig. 6B). All batches and replicates had either *Hanseniaspora* or *Saccharomyces* as the most abundant taxa. Nonetheless, several notable differences were observed. For example, the genus *Pichia* which was quite substantial (29%) in batch 1 of the commercial brew (Fig. 1D), but less evident in the home brew. van Wyk et al. (2023) recently demonstrated that *P. kluyveri* had direct positive implications on the aroma profile of Kombucha. Thus, the sensory characteristics of the resulting kombucha from that batch might have been sensorially better compared to the others. However, no sensory analysis was done to verify this at this time.

The relative abundance of *Komagataeibacter* which was formerly known as *Gluconacetobacter* (Laureys et al., 2020; Vargas et al., 2021) can be seen to correlate with glucose concentration. High relative abundance corresponds with elevated glucose concentration (Table 1 and Supplementary Fig. 9C). Our model also indicates that a high *Komagataeibacter* relative abundance is consistent with low fructose concentration (Table 1 and Supplementary Fig. 9B). This might suggest that in Kombucha, fructose is indeed preferentially utilized before glucose. This is supported by earlier reports (Sievers et al., 1995; Wang et al., 2022b). For pH as earlier mentioned, a high relative abundance of *Komagataeibacter* was associated with lower pH ranges (Table 1 and Supplementary Fig. 9A). The ability of this genus to tolerate low pH ranges have been reported (Zhang et al., 2017). With the significant influence of pH as inferred from the linear model (Table 1), this could mean that pH shapes microbial community in kombucha and that this is primarily driven by the most dominant acetic acid bacteria (AAB); *Komagataeibacter* in this case. Likewise, an increase in its relative abundance is consistent with decreased acetic acid concentration (Table 1 and Supplementary Fig. 9D). This is particularly interesting given that *Komagataeibacter* is a well-known member of the AAB (Valera et al., 2016). However, this could possibly be accounted for by the fact that *Komagataeibacter* is noted to also metabolize acetic acid (Nascimento et al., 2021).

High relative abundance of *Saccharomyces* was seen to be associated with low glucose concentration. This phenomenon was noted in the home brew (Supplementary Table 9B) and the commercial brew (Supplementary Table 9C), though not statistically significant in the latter. This observation might be related to the preference of *Komagataeibacter* in utilizing fructose as outlined previously. With the accumulation of glucose in the medium due to its preference for fructose, this might have an impact on the growth of *Saccharomyces*. Various research groups have shown that the effect of high glucose concentrations on *Saccharomyces* might be detrimental (D'Amato et al., 2006; New et al., 2014). The mechanism by which high glucose levels lead to reduced *Saccharomyces* relative abundance in kombucha could be related to Gomar-Alba et al. (2015). They suggested that high glucose concentrations might increase the sensitivity of *Saccharomyces* to ethanol. Another possibility may be related to the oxidation of glucose. Acetic acid bacteria (AAB) could oxidize glucose into glucuronic acid, gluconic acid and 2-ketogluconic acid (Laureys et al., 2020). These organic acids are known to inhibit the growth of *Saccharomyces* (Kawahata et al., 2006). This could possibly explain why *Saccharomyces,* which is known to thrive in other harsh environments such as wine fermentations for example, is rarely seen as a major player in kombucha fermentations. More studies will be required to verify all of these.

5. Conclusions

Most kombucha microbial studies to date have only analysed a few time points or samples at their point of sale. Thus, to the best of our knowledge, our study is the first to analyse the entire kombucha fermentation process across multiple batches. Furthermore, we compared the microbiome associated with the entire fermentation process between a commercial producer and that produced on a small scale. We show that the microbial community of the commercial kombucha fermentation process was richer as compared to that produced in the home. Key differences include the relative abundance of *Anoxybacillus*,

Methylobacterium and *Sphingomonas* which were higher in the commercial brew, while *Ammoniphilus* was in the home brew. *Ammoniphilus*, *Anoxybacillus*, and *Methylobacterium* have rarely been associated with kombucha fermentation. Thus, in the future, more research will be needed to understand their role in the kombucha fermentation process. For fungi, we show that the dominant fungal community present in the tea phase and SCOBY were similar in both kombucha types. Furthermore, using linear modelling, we report that a high relative abundance of *Komagataeibacter* is associated with low pH, high glucose concentration and low fructose concentration. All of these contribute towards improved understanding of the role that diverse microorganisms have in the development of this increasingly important beverage, and potentially to targeted interventions to enable product diversification.

CRediT authorship contribution statement

Aghogho Ohwofasa: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, and, Writing – review & editing. **Manpreet Dhami:** Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Writing – review & editing. **Christopher Winefield:** Formal analysis, Investigation, Supervision, Writing – review & editing. **Stephen L.W. On:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Access to data is given in embedded links.

Acknowledgements

We are most grateful to the Lincoln University Centre of Excellence – Food for Future Consumers for funding the doctoral studies of A.O. We also thank Joy Jiao, Ana Podolyan and Panimalar Vijayan for their technical support. We are indebted to the commercial kombucha producer who allowed us access to their facilities and to samples of their product. We acknowledge the use of the high-performance computing (HPC) facilities for the bioinformatics analysis undertaken in this study. This is facilitated by the Ministry for Business Innovation and Employment (MBIE).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100694.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2021. UNITE General FASTA Release for Fungi 2. UNITE Community. https://doi.org/10.15156/BIO/1280089.

Alderson, H., Liu, C., Mehta, A., Gala, H.S., Mazive, N.R., Chen, Y., Serventi, L., 2021. Sensory profile of kombucha brewed with New Zealand ingredients by focus group and word clouds. Fermentation 7 (3), 100. https://www.mdpi.com/2311-5637/7/3/100.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/s0022-2836(05)80360-2.

Andreson, M., Kazantseva, J., Kuldjarv, R., Malv, E., Vaikma, H., Kaleda, A., Vilu, R., 2022. Characterisation of chemical, microbial and sensory profiles of commercial kombuchas. Int. J. Food Microbiol. 373, 109715 https://doi.org/10.1016/j.ijfoodmicro.2022.109715.

Arikan, M., Mitchell, A.L., Finn, R.D., Gurel, F., 2020. Microbial composition of Kombucha determined using amplicon sequencing and shotgun metagenomics. J. Food Sci. 85 (2), 455–464. https://doi.org/10.1111/1750-3841.14992.

Asemaninejad, A., Weerasuriya, N., Gloor, G.B., Lindo, Z., Thorn, R.G., 2016. New primers for discovering fungal diversity using nuclear large ribosomal DNA. PLoS One 11 (7), e0159043. https://doi.org/10.1371/journal.pone.0159043.

Barbosa, C.D., Trovatti Uetanabaro, A.P., Rodrigues Santos, W.C., Caetano, R.G., Albano, H., Kato, R., Alves Lacerda, I.C., 2021. Microbial–physicochemical integrated analysis of kombucha fermentation. Lwt 148, 111788. https://doi.org/10.1016/j.lwt.2021.111788.

Bartlett, M.S., 1937. Properties of sufficiency and statistical tests. Proc. Roy. Soc. Lond. Math. Phys. Sci. 160 (901), 268–282.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting Linear Mixed-Effects Models Using Lme4. *arXiv preprint arXiv:1406.5823*.

Batista, P., Penas, M.R., Pintado, M., Oliveira-Silva, P., 2022. Kombucha: perceptions and future prospects. Foods 11 (13), 1977. https://www.mdpi.com/2304-8158/11/13/1977.

Bishop, P., Pitts, E.R., Budner, D., Thompson-Witrick, K.A., 2022. Kombucha: biochemical and microbiological impacts on the chemical and flavor profile. Food Chemistry Advances 1. https://doi.org/10.1016/j.focha.2022.100025.

Brewer, S.S., Lowe, C.A., Beuchat, L.R., Ortega, Y.R., 2021. Survival of Salmonella and shiga toxin–producing Escherichia coli and changes in indigenous microbiota during fermentation of home-brewed kombucha. J. Food Protect. 84 (8), 1366–1373.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.

Cardoso, R.R., Neto, R.O., dos Santos D'Almeida, C.T., do Nascimento, T.P., Pressete, C.G., Azevedo, L., de Barros, F.A.R., 2020. Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. Food Res. Int. 128, 108782.

Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., Coton, E., 2017. Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. FEMS Microbiol. Ecol. 93 (5) https://doi.org/10.1093/femsec/fix048.

D'Amato, D., Corbo, M.R., Nobile, M.A.D., Sinigaglia, M., 2006. Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system. Int. J. Food Sci. Technol. 41 (10), 1152–1157.

Dai, J., Liu, Y., Lei, Y., Gao, Y., Han, F., Xiao, Y., Peng, H., 2011. A new subspecies of Anoxybacillus flavithermus ssp. yunnanensis ssp. nov. with very high ethanol tolerance. FEMS Microbiol. Lett. 320 (1), 72–78. https://doi.org/10.1111/j.1574-6968.2011.02294.x.

De Clerck, E., Vanhoutte, T., Hebb, T., Geerinck, J., Devos, J., De Vos, P., 2004. Isolation, characterization, and identification of bacterial contaminants in semifinal gelatin extracts. Appl. Environ. Microbiol. 70 (6), 3664–3672.

de Miranda, J.F., Ruiz, L.F., Silva, C.B., Uekane, T.M., Silva, K.A., Gonzalez, A.G.M., Lima, A.R., 2022. Kombucha: a review of substrates, regulations, composition, and biological properties. J. Food Sci. 87 (2), 503–527. https://doi.org/10.1111/1750-3841.16029.

Dhami, M.K., Hartwig, T., Fukami, T., 2016. Genetic basis of priority effects: insights from nectar yeast. Proc. Biol. Sci. 283 (1840) https://doi.org/10.1098/rspb.2016.1455.

Diez-Ozaeta, I., Astiazaran, O.J., 2022. Recent advances in Kombucha tea: microbial consortium, chemical parameters, health implications and biocellulose production. Int. J. Food Microbiol., 109783

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14 (6), 927–930.

Dourado, M.N., Camargo Neves, A.A., Santos, D.S., Araújo, W.L., 2015. Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic Methylobacterium spp. BioMed Res. Int., 909016 https://doi.org/10.1155/2015/909016, 2015.

Gaggia, F., Baffoni, L., Galiano, M., Nielsen, D.S., Jakobsen, R.R., Castro-Mejia, J.L., Di Gioia, D., 2018. Kombucha beverage from green, black and rooibos teas: a comparative study looking at microbiology, chemistry and antioxidant activity. Nutrients 11 (1). https://doi.org/10.3390/nu11010001.

Goh, K.M., Kahar, U.M., Chai, Y.Y., Chong, C.S., Chai, K.P., Ranjani, V., Chan, K.-G., 2013. Recent discoveries and applications of Anoxybacillus. Appl. Microbiol. Biotechnol. 97, 1475–1488.

Gomar-Alba, M., Morcillo-Parra, M.Á., Olmo, M.l. d., 2015. Response of yeast cells to high glucose involves molecular and physiological differences when compared to other osmostress conditions. FEMS Yeast Res. 15 (5), fov039.

Grassi, A., Cristani, C., Palla, M., Di Giorgi, R., Giovannetti, M., Agnolucci, M., 2022. Storage time and temperature affect microbial dynamics of yeasts and acetic acid bacteria in a kombucha beverage. Int. J. Food Microbiol. 382, 109934 https://doi.org/10.1016/j.ijfoodmicro.2022.109934.

Harrison, K., Curtin, C., 2021. Microbial composition of SCOBY starter cultures used by commercial kombucha brewers in north America. Microorganisms 9 (5). https://doi.org/10.3390/microorganisms9051060.

Hlavac, M., 2018. Stargazer: well-formatted regression and summary statistics tables. R package version 5 (2), 2.

Illumina. https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html, 2017, 20th October 2023.

Kaashyap, M., Cohen, M., Mantri, N., 2021. Microbial diversity and characteristics of kombucha as revealed by metagenomic and physicochemical analysis. Nutrients 13 (12). https://doi.org/10.3390/nu13124446.

Kapp, J.M., Sumner, W., 2019. Kombucha: a systematic review of the empirical evidence of human health benefit. Ann. Epidemiol. 30, 66–70.

Kato, Y., Asahara, M., Goto, K., Kasai, H., Yokota, A., 2008. Methylobacterium persicinum sp. nov., Methylobacterium komagatae sp. nov., Methylobacterium brachiatum sp. nov., Methylobacterium tardum sp. nov. and Methylobacterium gregans sp. nov., isolated from freshwater. Int. J. Syst. Evol. Microbiol. 58 (5), 1134–1141.

Kawahata, M., Masaki, K., Fujii, T., Iefuji, H., 2006. Yeast genes involved in response to lactic acid and acetic acid: acidic conditions caused by the organic acids in Saccharomyces cerevisiae cultures induce expression of intracellular metal metabolism genes regulated by Aft1p. FEMS Yeast Res. 6 (6), 924–936. https://doi.org/10.1111/j.1567-1364.2006.00089.x.

Kim, J., Adhikari, K., 2020. Current trends in kombucha: marketing perspectives and the need for improved sensory research. Beverages 6 (1), 15.

Laavanya, D., Shirkole, S., Balasubramanian, P., 2021. Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation. J. Clean. Prod. 295, 126454 https://doi.org/10.1016/j.jclepro.2021.126454.

Laureys, D., Britton, S.J., De Clippeleer, J., 2020. Kombucha tea fermentation: a review. J. Am. Soc. Brew. Chem. 78 (3), 165–174. https://doi.org/10.1080/03610470.2020.1734150.

Li, R., Xu, Y., Chen, J., Wang, F., Zou, C., Yin, J., 2022. Enhancing the proportion of gluconic acid with a microbial community reconstruction method to improve the taste quality of Kombucha. Lwt 155, 112937.

Li, Z., Chen, S.-Q., Cao, X., Li, L., Zhu, J., Yu, H., 2021. Effect of pH Buffer and Carbon Metabolism on the Yield and Mechanical Properties of Bacterial Cellulose Produced by Komagataeibacter Hansenii ATCC 53582.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15 (12), 550. https://doi.org/10.1186/s13059-014-0550-8.

Marsh, A.J., O'Sullivan, O., Hill, C., Ross, R.P., Cotter, P.D., 2014. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. Food Microbiol. 38, 171–178.

McClure, P.J., 2006. 21 - spore-forming bacteria. In: Blackburn, C.d.W. (Ed.), Food Spoilage Microorganisms. Woodhead Publishing, pp. 579–623. https://doi.org/10.1533/9781845691417.5.579.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4), e61217.

Mukhopadhyay, R., Sarkar, B., Jat, H.S., Sharma, P.C., Bolan, N.S., 2021. Soil salinity under climate change: challenges for sustainable agriculture and food security. J. Environ. Manag. 280, 111736 https://doi.org/10.1016/j.jenvman.2020.111736.

Nascimento, F.X., Torres, C.A., Freitas, F., Reis, M.A., Crespo, M.T., 2021. Functional and genomic characterization of Komagataeibacter uvaceti FXV3, a multiple stress resistant bacterium producing increased levels of cellulose. Biotechnology Reports 30, e00606.

New, A.M., Cerulus, B., Govers, S.K., Perez-Samper, G., Zhu, B., Boogmans, S., Verstrepen, K.J., 2014. Different levels of catabolite repression optimize growth in stable and variable environments. PLoS Biol. 12 (1), e1001764 https://doi.org/10.1371/journal.pbio.1001764.

Ohwofasa, A., Dhami, M., Tian, B., Winefield, C., On, S.L.W., 2023. Environmental influences on microbial community development during organic pinot noir wine production in outdoor and indoor fermentation conditions. Heliyon 9 (5), e15658. https://doi.org/10.1016/j.heliyon.2023.e15658.

Orhan, F., Demirci, A., Gormez, A., 2021. Carbonate and silicate dissolving bacteria isolated from home-made yogurt samples. An Acad. Bras Ciências 93.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.

Ramadan, A.M., Nazar, M.A., Gadallah, N.O., 2021. Metagenomic analysis of rhizosphere bacteria in desert plant Calotropis procera. Geomicrobiol. J. 38 (5), 375–383.

Reva, O.N., Zaets, I.E., Ovcharenko, L.P., Kukharenko, O.E., Shpylova, S.P., Podolich, O.V., Kozyrovska, N.O., 2015. Metabarcoding of the kombucha microbial community grown in different microenvironments. Amb. Express 5 (1), 1–8.

Rohland, N., Reich, D., 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Res. 22 (5), 939–946. https://doi.org/10.1101/gr.128124.111.

Serra, J.L., Moura, F.G., Pereira, G.V.d.M., Soccol, C.R., Rogez, H., Darnet, S., 2019. Determination of the microbial community in Amazonian cocoa bean fermentation by Illumina-based metagenomic sequencing. Lwt 106, 229–239. https://doi.org/10.1016/j.lwt.2019.02.038.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52 (3/4), 591–611.

Shi, S., Condron, L., Larsen, S., Richardson, A.E., Jones, E., Jiao, J., Stewart, A., 2011. In situ sampling of low molecular weight organic anions from rhizosphere of radiata pine (Pinus radiata) grown in a rhizotron system. Environ. Exp. Bot. 70 (2–3), 131–142.

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., Teuber, M., 1995. Microbiology and fermentation balance in a kombucha beverage obtained from a tea fungus fermentation. Syst. Appl. Microbiol. 18 (4), 590–594. https://doi.org/10.1016/S0723-2020(11)80420-0.

Sun, T.-Y., Li, J.-S., Chen, C., 2015. Effects of blending wheatgrass juice on enhancing phenolic compounds and antioxidant activities of traditional kombucha beverage. J. Food Drug Anal. 23 (4), 709–718.

Tanabe, A.S., Toju, H., 2013. Two new computational methods for universal DNA barcoding: a benchmark using barcode sequences of bacteria, archaea, animals, fungi, and land plants. PLoS One 8 (10), e76910. https://doi.org/10.1371/journal.pone.0076910.

Teoh, A.L., Heard, G., Cox, J., 2004. Yeast ecology of Kombucha fermentation. Int. J. Food Microbiol. 95 (2), 119–126.

The GRACE Davison, 2023. PrevailTM: Carbohydrate ES HPLC Columns and ELSD, Applications CHROM10498. *Brochure #467A*.

Torán-Pereg, P., del Noval, B., Valenzuela, S., Martinez, J., Prado, D., Perisé, R., Arboleya, J.C., 2021. Microbiological and sensory characterization of kombucha SCOBY for culinary applications. Int. J. Gastron. Food Sci. 23, 100314 https://doi.org/10.1016/j.ijgfs.2021.100314.

Valera, M.J., Mas, A., Streit, W.R., Mateo, E., 2016. GqqA, a novel protein in Komagataeibacter europaeus involved in bacterial quorum quenching and cellulose formation. Microb. Cell Factories 15 (1), 88. https://doi.org/10.1186/s12934-016-0482-y.

van Wyk, N., Binder, J., Ludszuweit, M., Köhler, S., Brezina, S., Semmler, H., von Wallbrunn, C., 2023. The influence of Pichia kluyveri addition on the aroma profile of a kombucha tea fermentation. Foods 12 (10), 1938.

Vargas, B.K., Fabricio, M.F., Záchia Ayub, M.A., 2021. Health effects and probiotic and prebiotic potential of Kombucha: a bibliometric and systematic review. Food Biosci. 44, 101332 https://doi.org/10.1016/j.fbio.2021.101332.

Villarreal-Soto, S.A., Bouajila, J., Pace, M., Leech, J., Cotter, P.D., Souchard, J.-P., Beaufort, S., 2020. Metabolome-microbiome signatures in the fermented beverage, Kombucha. Int. J. Food Microbiol. 333, 108778.

Wang, B., Rutherfurd-Markwick, K., Zhang, X.X., Mutukumira, A.N., 2022a. Isolation and characterisation of dominant acetic acid bacteria and yeast isolated from Kombucha samples at point of sale in New Zealand. Curr. Res. Food Sci. 5, 835–844. https://doi.org/10.1016/j.crfs.2022.04.013.

Wang, B., Rutherfurd-Markwick, K., Zhang, X.X., Mutukumira, A.N., 2022b. Kombucha: production and microbiological research. Foods 11 (21). https://doi.org/10.3390/foods11213456.

Yang, J., Lagishetty, V., Kurnia, P., Henning, S.M., Ahdoot, A.I., Jacobs, J.P., 2022. Microbial and chemical profiles of commercial kombucha products. Nutrients 14 (3). https://doi.org/10.3390/nu14030670.

Zhang, H., Xu, X., Chen, X., Yuan, F., Sun, B., Xu, Y., Sun, D., 2017. Complete genome sequence of the cellulose-producing strain Komagataeibacter nataicola RZS01. Sci. Rep. 7 (1), 4431. https://doi.org/10.1038/s41598-017-04589-6.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30 (5), 614–620.