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Kombucha meets circular economy: A microbiome and metabolite perspective on second fermentation with plant by-products

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ABSTRACT

Kombucha is a traditional fermented beverage produced through the fermentation of sugared tea by a symbiotic culture of bacteria and yeasts (SCOBY). In recent years, the valorisation of plant-based by-products as fermentation substrates has gained attention as a sustainable approach to improving both the nutritional and economic efficiency of fermented beverages. The present study investigated the production of kombuchas supplemented with pineapple, fennel, and carrot by-products during the secondary fermentation phase, aiming to evaluate their influence on fermentation dynamics, microbial ecology, and the chemical and aromatic profiles of the final products.

The experimental design integrated culture-dependent and culture-independent approaches, including amplicon sequencing, to characterize microbial community composition and evolution throughout fermentation. Chemical profiling was carried out using gas chromatography coupled with quadrupole mass spectrometry (GC-qMS) and high-performance liquid chromatography equipped with diode-array and refractive index detectors (HPLC-DAD/RI). The fermentation process was monitored during both the primary and secondary stages, and a shelf-life assessment was conducted over 14 days of refrigerated storage (4 °C) to evaluate product stability.

Microbiological results indicated a predominance of *Schizosaccharomyces* spp., while *Komagataeibacter* spp. was the only bacterial genus identified. A significant reduction in α-diversity was observed over time, suggesting selective adaptation of the microbial community to the fermentation environment. β-diversity analysis revealed clear differences among samples collected after 8 and 22 days, reflecting the combined influence of time and substrate composition on microbial succession. Chemical analyses demonstrated an increase in acetic acid concentration and a progressive decline in pH throughout fermentation, consistent with the metabolic activity of acetic acid bacteria. Among volatile organic compounds (VOCs), alcohols and organic acids were the most abundant chemical classes detected. Several VOCs were associated with minor yeast genera, including *Hannaella*, *Galactomyces*, *Aureobasidium*, and *Millerozyma*, whereas *Schizosaccharomyces* spp. showed a strong correlation with specific aroma-active compounds, highlighting its key role in defining the sensory characteristics of the beverage.

Overall, this study provides new evidence on how different vegetable by-products and microbial consortia influence the development of chemical and aromatic compounds in kombucha. The findings highlight the potential of using by-products as a sustainable, value-added strategy for producing fermented beverages, while also supporting the principles of the circular economy and resource-efficient food systems.

1. Introduction

Kombucha is a traditional fermented tea beverage obtained through the symbiotic metabolic activity of bacteria and yeasts within a cellulose matrix known as the SCOBY (Sanwal et al., 2023). Kombucha has recently gained increasing attention as a functional beverage due to its content of organic acids, bioactive compounds, and probiotic microorganisms (DuMez-Kornegay et al., 2024). The worldwide market for this beverage is estimated at approximately USD 4–5 billion in 2024. It is projected to grow at a compound annual growth rate (CAGR) of 13–16%

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over the next decade (Grand View Research, 2025). Kombucha fermentation proceeds in two distinct stages: a primary aerobic fermentation lasting 7–14 days, followed by a secondary anaerobic fermentation that occurs in sealed bottles, enhancing carbonation and flavour complexity (Sanwal et al., 2023). During the first fermentation, acetic acid bacteria (AAB), mainly belonging to the genera *Komagataeibacter*, *Acetobacter*, and *Gluconobacter*, oxidize ethanol into organic acids and synthesize cellulose, forming the characteristic surface biofilm (Phung et al., 2023; Yang et al., 2022). Yeasts such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Zygosaccharomyces* spp. hydrolyse sucrose and produce ethanol, which serves as a substrate for bacterial metabolism (Suffys et al., 2023). Lactic acid bacteria (LAB) are also occasionally detected but play a less dominant role in kombucha compared with other fermented foods (Suffys et al., 2023). The joint metabolism of bacteria and yeasts drives acidification, aroma formation, and the synthesis of bioactive compounds, conferring kombucha its characteristic sensory and potential health-promoting properties (Li et al., 2024; Teneva & Denev, 2023). Biologically, active compounds from microorganisms are not the only potential bio-preservatives; plant-based extracts can also serve this function (Teneva & Denev, 2023). Plant-based by-products, rich in polyphenols, carotenoids, fibres, and essential oils, represent promising ingredients for enhancing functional and sensory characteristics of fermented beverages (Chiarini et al., 2024). Their integration into food fermentations also aligns with circular economy principles, promoting the valorisation of agro-industrial residues and reducing environmental impact (Chaudhary & Singh, 2024; Santos et al., 2021).

Despite growing interest in sustainable fermentation practices, limited quantitative information is available regarding the use of vegetable by-products during the second fermentation of kombucha. This stage is particularly suitable for introducing such ingredients, as it directly affects carbonation, aromatic development, and the final microbial balance. According to recent reviews, up to 30–40% of processed fruits and vegetables may become by-products or waste streams (Salas-Millán & Aguayo, 2024) and losses in the sector can reach up to 60% overall (Marcelli et al., 2025).

Carrot (Kaur et al., 2022), pineapple (Roda & Lambri, 2019) and fennel (Santoro et al., 2024) have been reported to produce by-products in amounts of up to 30%, 50%, and 60%, respectively, highlighting their potential for valorisation within a circular economy framework. Fruit juice production generates large quantities of by-products, representing 20–80% of the processed fruit strategies (Kandemir et al., 2022). These residues pose environmental challenges but also offer significant opportunities for recovery and valorisation, driving growing research interest in sustainable exploitation strategies (Kandemir et al., 2022). By-products are particularly relevant due to their distinct chemical profiles. Pineapple residues are rich in pectin, polyphenols, and organic acids (Meena et al., 2022). Carrot contains carotenoids, flavonoids, and minerals (Chaudhary & Singh, 2024), while fennel essential oil is characterized by a high monoterpene content with recognized aromatic and antioxidant potential (Chiboub et al., 2019; Kausar et al., 2024). Incorporating even a portion of these residues into fermentation could therefore contribute meaningfully to waste valorisation and circular economy objectives. However, the microbial and chemical mechanisms underlying these transformations remain poorly understood. Therefore, the present study aims to elucidate the impact of pineapple, fennel, and carrot by-products from fruit juice production on kombucha fermentation during the secondary stage. Specifically, the objectives are to characterize microbial community dynamics using both culture-dependent and amplicon sequencing approaches. Additionally, volatile organic compounds (VOCs) and other chemical constituents were analysed using gas chromatography coupled with quadrupole mass spectrometry (GCq-MS) and high-performance liquid chromatography equipped with diode-array and refractive index detectors (HPLCDAD/RI). These analyses integrate microbial and metabolomic data to explore correlations between microbial taxa, metabolite production, and aroma development. By linking microbial ecology with sustainable ingredient valorisation, this research contributes to understanding how circular economy strategies can be effectively implemented in fermented beverage production, promoting both environmental sustainability and product innovation.

2. Materials and methods

2.1. Kombucha and by-products preparation

The preparation of kombucha involved the use of natural mineral water with a low chlorine content, characterized by the following physicochemical parameters: specific electrical conductivity at 20 °C of 414 μS/cm, fixed residue at 180 °C of 262 mg/l, oxidizability <0.5 mg/l O_2, free carbon dioxide <10 mg/l, silica 16.6 mg/l, bicarbonates 283 mg/l, calcium 47.1 mg/l, magnesium 26.9 mg/l, nitrates 9 mg/l, sodium 5.7 mg/l, sulphates 4.4 mg/l, chlorides 2.7 mg/l, potassium 0.94 mg/l, fluorides <0.1 mg/l, and sodium content <0.0006%. Black tea leaves were added at a concentration of 8.25 g/l (EPEL, Spain), together with sucrose at 50 g/l (Eridania, Italy). Black tea leaves were infused for five minutes according to the manufacturer's guidelines. Once the tea reached room temperature, the commercial SCOBY (KombuChic, France) was added, constituting approximately 4% (*w/v*) of the total volume. Finally, a portion (3–10% *v/v*) of a previous kombucha batch (six-day fermented kombucha; K6) was added as a starter to obtain an initial pH of 4 ± 0.1. The first fermentation of kombucha was conducted under aerobic conditions for six days at 24 °C (K6), followed by a second fermentation. The second fermentation was performed using fruit and vegetable by-products obtained after juicing and stored at −20 °C. The by-product matrices consisted separately of pineapple (*Ananas comosus*, KA), carrot (*Daucus carota*, KC), and fennel (*Foeniculum vulgare*, KF). Unflavoured kombucha was fermented as the control (KN). The end point of the first fermentation was determined by measuring the pH, which reached a value of approximately 3.5 (Mettler Toledo, FiveEasy).

The following procedure was carried out for the second fermentation. Twenty grams of by-product pulp and 100 ml of kombucha (six-day fermented kombucha; K6) were placed in food-grade plastic jars (Nalgene®, Rochester, NY, USA) on a mixer and left to stir for 1 h. The suspension was then centrifuged at 1200 rpm for 15 min (Heraeus Megafuge 11R, Thermo Electron Corporation, Waltham, MA, USA). The supernatant was transferred to a stomacher bag and pasteurised in a thermostatic bath at 75 °C for 20 min. At the end of pasteurization, 100 ml of the filtered flavoured liquid (VWR; 129–0734) was added to first-fermentation kombucha (200 ml) without SCOBY at room temperature. The mixture was then left to ferment in sealed bottles for 48 h at 24 °C (second fermentation; K8). The samples were collected at regular intervals throughout the fermentation process (K1, K4, K6, K8 days of fermentation at 24 °C). The K0 samples refer to kombucha at the initial stage of fermentation, collected immediately after the SCOBY was added to the tea. The shelf life of the beverage and microbial activity were evaluated through microbiological and chemical analyses after 7 and 14 days of storage at 4 °C (K15 and K22).

2.2. Microbial load evaluation

Microbiological analyses were performed by serial dilutions in Ringer's solution (Neogen, NCM0191K) followed by cultivation on selective media for the enumeration of lactic acid bacteria, yeasts, fungi, and acetic acid bacteria, as well as for verification of the effectiveness of by-product pulp pasteurization. The acetic acid bacteria were enumerated on Acetic Acid Medium (AAM), composed of 10 g/l glucose (Neogen, NCM0241B), 8 g/l yeast extract (Neogen, NCM0218B), 15 g/l peptone (Neogen, NCM0237A), 15 g/l agar (Neogen, NCM0238A), 5 ml/l ethanol 99% (Sigma-Aldrich, 02891), 3 ml/l acetic acid (Sigma-Aldrich, 338826) with Delvocid® Instant 200 mg/l (DSM, 192864) (Lisdiyanti et al., 2001). AAM plates were incubated at 30 °C for 96 h.

The absence of *Enterobacteriaceae* was confirmed through microbiological analysis using VRBG Violet Red Bile Glucose (VRBG) agar (Neogen, NCM0022B) incubated at 37 °C for 24 h. Yeasts were enumerated on Wallerstein Laboratory Nutrient Agar (WL) (Neogen, NCM0118A) incubated at 25 °C for 72 h. Fungi were enumerated using Malt Extract Agar (MEA) (Neogen, NCM0093B) with tetracycline (50 mg/l, Sigma-Aldrich, 87128), incubated at 30 °C for 72 h. The total aerobic microbial count was obtained on Plate Count Agar (PCA) (Neogen, NCM0010A) incubated at 30 °C for 72 h. Lactic acid bacteria have been enumerated on De Man Rogosa Sharpe (MRS) (Neogen, NCM0035A) supplemented with citric acid 10% *v*/v to a pH of 4.8 and Delvocid® Instant 100 mg/l (DSM, 192864). MRS plates were incubated for 96 h at 30 °C in anaerobiosis (Thermo Scientific™ Oxoid™ AnaeroGen 3.5 l) (Coton et al., 2017).

Three kombucha batches were sampled at 0, 1, 4, 6 (first fermentation), 8 (second fermentation), and 15, 22 (shelf life) days for microbiological analysis. At each sampling point, five millilitres of sample were stored at −20 °C for subsequent chemical and amplicon sequencing analyses.

2.3. Quantification of sugars, organic acids, and volatile organic compounds

The quantification of sugars and organic acids during fermentation was performed using HPLC-DAD/RI. For each sampling point, 1 ml of sample was collected and filtered using nylon membrane syringe filters (0.22 μm pore size; Lab Logistic Group, Meckenheim, DE), then transferred into 1.5 ml vials (Phenomenex, Torrance, CA, USA). The HPLC system (Thermo Electron Corporation, Waltham, MA, USA) was equipped with an SCM 100 degasser, a P2000 isocratic pump, an AS3000 autosampler, a UV6000LP photodiode array detector, and a RI-150 refractive index detector, connected in series for the identification of organic acids and sugars, respectively. The mobile phase consisted of water acidified with 0.013 N H_2SO_4, and isocratic elution was performed at a flow rate of 0.6 ml/min. Separation was achieved using a reverse-phase Aminex HPX-87H column (300 mm × 7.8 mm) coupled with a Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA) operated at 65 °C. Chromatographic data were collected using the ChromQuest 5.0 software (Thermo Electron Corporation, Waltham, MA, USA), and quantification was carried out using calibration curves based on analytical internal standards injected under the same conditions as the samples.

VOCs present in the kombucha samples were identified and quantified using headspace solid-phase microextraction (HS-SPME) followed by GC-qMS. For VOCs extraction, 0.5 g of the sample was weighed into 20 ml vials (Agilent Technologies, Santa Clara, CA, USA), to which 5 μl of 1,3,5-triisopropylbenzene (53.5 ppm) was added as an internal standard. The vials were immediately sealed with PTFE-silicone septum caps. Extraction was carried out using a COMBI PAL System autosampler for SPME (CT Analytics AG, Zwingen, Switzerland) equipped with an HS-SPME unit. Samples were conditioned at 60 °C for 15 min. An SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) was then exposed to the headspace for 30 min and subsequently desorbed in the injection port of the GC system in splitless mode at 260 °C. GC-qMS analyses were conducted using a Shimadzu GC-2010 gas chromatograph coupled to a quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). VOCs were separated using an Zebron Wax Plus capillary column (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness). The oven temperature program was as follows: initial temperature of 40 °C held for 5 min, increased to 200 °C at 5 °C/min, then to 240 °C at 10 °C/min with a final hold of 5 min. Helium was used as the carrier gas at a constant flow rate of 1 ml/min, with injection in splitless mode. Mass spectrometric detection was performed in electron impact (EI) mode at 70 eV. The ion source and quadrupole temperatures were set at 240 °C. Data acquisition was carried out in full-scan mode over a mass range of 33–300 *m/z* with a scan time of 0.30s (Barbosa-Pereira et al., 2019). All samples were analysed in technical duplicate for each biological replicate.

2.4. DNA extraction and amplicon sequencing

DNA extraction was performed from 1 ml of kombucha for each fermentation and shelf-life stage; samples were centrifuged at 15,000 ×*g* for 10 min and extracted using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre®, MC85200 and MC89010) after supernatant removal. After the addition of 25 μl of lysozyme (50 mg/ml), the samples were incubated at 37 °C for 30 min at 800 rpm. After the incubation, 300 μl of Tissue & Cell Lysis Solution and 1 μl of proteinase K (50 mg/ml; *w/v* in ultrapure water) were added, and samples were incubated at 65 °C for 15 min at 800 rpm, followed by placement on ice for 5 min. After the addition of 150 μl of Protein Precipitation Reagent (MPC), the samples were centrifuged at 15,000 ×*g* for 10 min at 4 °C, and the supernatant was transferred into new tubes. After the addition of an additional 25 μl of MPC, the samples were mixed and centrifuged at 15,000 ×*g* for 10 min at 4 °C. The supernatant was transferred again and, after the addition of 500 μl of isopropanol, centrifuged at 15,000 ×*g* for 10 min at 4 °C. After isopropanol removal, 100 μl of ethanol (70% *v*/v in ultrapure water) were added, followed by centrifugation at 15,000 ×*g* for 10 min at 4 °C. This procedure was performed twice. The nucleic acid pellet was left to dry for 30 min and suspended in 50 μl of ultrapure water (Thermo Fisher, J71786.AP). The samples were treated with RNAse A (Thermo Scientific, EN0531) for 30 min at 37 °C. DNA quality and quantity were assessed using a NanoDrop® Spectrophotometer ND-100 (Thermo Fisher).

Amplicons were generated from the 16S rDNA (bacteria) and 26S rDNA (fungi) regions. The V3–V4 amplicon library was constructed from the 16S rRNA gene region (Hautefeuille et al., 2024) using the primer pair forward (5′-TACGGGAGGCAGCAG-3′) (Turner et al., 1999) and reverse (5′-CCAGGGTATCTAATCC-3′) (Kisand et al., 2002). The 26S rDNA was amplified with LS2 (5′-GAGTCGAGTTGTTTGGGAAT-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (Mota-Gutierrez et al., 2019). The amplification was performed in a final volume of 25 μl using KAPA HiFi HotStart ReadyMix PCR Kit 2× (Roche, 07958935001) and each primer at a final concentration of 200 nM. The PCR amplification conditions were: 95 °C for 3 min followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and final extension at 72 °C for 10 min. The PCR products were prepared with the Hamilton automatic machine (Hamilton, Reno, Nevada, USA) following the Illumina sequencing protocol. Briefly, the products amplified from PCR were purified using the Agencourt AMPure kit (Beckman Coulter, Milan, Italy), and the resulting products were tagged with sequencing adapters using the Nextera XT library preparation kit (Illumina Inc., San Diego, CA, USA), according to the manufacturer's instructions. The sequencing was performed on the Illumina NovaseqX platform by Novogene company (Planegg/Martinsried, Germany).

The bioinformatics analysis was performed using QIIME 2 version 2022.2.0 (https://docs.qiime2.org/; accessed on 20 April 2025). The raw reads were imported into QIIME2 for quality and chimera filtering step by dada2 denoise-paired script. The sampling depth used was 10000 for bacteria and 34980 for fungi. The amplicon-sequence variants (ASVs) were used for taxonomic assignment by comparison with the Greengenes2 version 2022.10 (bacteria) and Unite version 8.1 (fungi) databases, obtaining taxa abundances. Sequences of specific taxa have been uploaded to nucleotide BLAST to identify taxa at the species level (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 25 August 2025). The raw sequence reads are available in the National Center for Biotechnology Information database (NCBI; https://www.ncbi.nlm.nih.gov/bioproject/; accessed on 25 August 2025) at the bioproject accession number PRJNA1236227.

2.5. Statistical analysis

Statistical analysis was performed using RStudio software version 4.2.2 (2022-10-31 ucrt). Shapiro-Wilk and Levene's W-tests were used respectively to check the normality and homogeneity of the data. The Kruskal-Wallis (K—W) test and analysis of variance (ANOVA) were used to assess overall differences and variations between multiple groups. These tests were used for non-parametric (K—W) and parametric (ANOVA) data. Dunn's test with FDR correction was used in the post-hoc analyses for non-parametric data, Tukey's test was used for parametric data unless differentially indicated. The R package microeco version 1.13.0 (Liu et al., 2021) was used to asses correlation between microbiome and kombucha characteristics and to evaluate the microbiome composition.

3. Results and discussion

3.1. Microbial dynamics and fermentation dynamics

Microbial counts showed no significant differences (*p-value* > 0.05) in total microbial, and yeast loads among sampling points, with an average load of 6.00 CFU/ml (Table S1). This stability suggests that the introduction of plant by-products during secondary fermentation did not adversely affect overall microbial dynamics. The maintenance of a consistent microbial community may contribute to predictable fermentation outcomes, supporting both reproducibility of the process and preservation of flavour characteristics. Moreover, a stable microbial load during the flavouring stage is advantageous for the controlled development of VOCs and aromatic profiles, ensuring the desired sensory attributes of the final product. This observation is consistent with previous findings in kombucha literature where a core microbiota persists over time (Ben Saad et al., 2025) and microbial stability was observed under different storage or processing conditions (Liao et al., 2024).

A higher AAB load was observed after 4 days of fermentation (average 6.33 CFU/ml, ± 0.38) compared with day 0 (average 5.04 CFU/ml, ± 0.62). This was followed by a decrease during the shelf life of samples flavoured with pineapple, carrot and fennel at day 22, when compared with the last day of first fermentation (day 6; average 6.42 CFU/ml, ± 0.09) (K-W/Dunn's test, *p-value* < *0.05*) (Table S1). This increase is consistent with the metabolic activity of AAB in acetic acid production, a hallmark of kombucha fermentation (Nyhan et al., 2022). The subsequent decline in AAB during the shelf life period, particularly in pineapple-, carrot-, and fennel-flavoured samples, suggests that bioactive compounds present in these plant by-products may exert antimicrobial effects, selectively suppressing AAB populations. A similar phenomenon has been reported in vinegar fermentation using pineapple by-products (Tanamool et al., 2020). Consistently, the unflavoured samples exhibited a stable microbial load of 6.11 CFU/ml (± 0.49), in contrast to the flavoured samples, which showed a significantly lower average load of 3.64 CFU/ml (± 1.56) (*p-value* < *0.05*). The pH decreased from an initial value of 4.0 (± 0.06) to 3.4 (± 0.13) by day 6. Moreover, pineapple-flavoured kombucha exhibited lower pH values compared with carrot-flavoured and time-zero kombucha, whereas fennel and unflavoured kombucha showed significantly lower pH values relative to the first fermentation (*p-value* < 0.05) (Fig. S1). This progressive acidification represents an additional selective factor influencing microbial populations in fermented foods (Botta et al., 2025). LAB and *Enterobacteriaceae* were not detected (< 1 CFU/ml). The absence of LAB is not surprising considering that frequently their presence in kombucha is not reported and AAB are the bacteria principally involved in its fermentation (Suffys et al., 2023). The metabolic activity of AAB, resulting in acetic acid accumulation and pH reduction, plays a pivotal role in maintaining the antimicrobial environment of kombucha (Sanwal et al., 2023), as reflected by the absence of *Enterobacteriaceae* in all samples.

The amplicon sequencing allowed the detection of bacterial ASVs related to *Komagataeibacter rhaeticus*, however most reads were not identified in specific taxa (> 98%) (Table S2 and Fig. S2A), suggesting a complex and under-characterized bacterial consortium. The AAB *Komagataeibacter* spp. is considered one of the main bacterial genera in kombucha and SCOBY (Suffys et al., 2023). The analysis of fungal ASVs demonstrated that *Schizosaccharomyces pombe* (BLAST confirmed) was the main species in all samples with a relative abundance above 70%, followed by not identified species of Ascomycota and Basidiomycota phyla and *Acremonium* spp. (Figs. 1A and S2B). *S. pombe* represents one of the most commonly isolated and identified species in kombucha (Teoh et al., 2004) representing a dominant yeast species (Bishop et al., 2022). Minor fungal taxa were detected in kombucha at different fermentation stages. Among these taxa, *Aureobasidium* spp. was already detected in other fermented foods and beverages, including olives (Georgalaki et al., 2025; Traina et al., 2024) and is considered interesting in vitivinicultural sector, demonstrating the ability to produce metabolites known for their influence on sensory profile (Bozoudi & Tsaltas, 2018). *Zygosaccharomyces* spp. was identified in kombucha even as dominant taxa (Arıkan et al., 2020) but present in the samples object of study at low abundances. Another minor taxon was *Galactomyces* spp., *Galactomyces geotrichum* was isolated from kombucha resulting a rare taxon (Tran et al., 2020). However, the detection of taxa at very low abundances within kombucha microbial communities should be interpreted cautiously, as these sequences may represent environmental contaminants or transient members rather than ecologically stable and functionally significant constituents of the fermentative consortium (Ojo & de Smidt, 2023; Reva et al., 2015).

The α-diversity analysis of fungal taxa demonstrates that Observed, Chao1, Shannon's and Fisher's index were different among fermentations (*p-value* < *0.05*) (Fig. S3). These indexes were higher at fermentation beginning compared to first fermentation at day 4 and kombucha flavoured with carrot at day 8. Moreover, Simpson, inverted Simpson, Shannon, ACE, Chao1, Fisher and observed indexes were negatively correlated with yeast microbial load on MEA. Similarly, ACE, chao1, Fisher and Observed were negatively correlated with average yeast loads (MEA/WL) and, excluding ACE, with WL (Table S3). Microbial diversity was low, in agreement with previous studies (Arıkan et al., 2020; Ben Saad et al., 2025), demonstrating that α-diversity indices significantly decreased over fermentation time and in flavoured samples. This aspect suggests a shift towards a specialized microbial community possibly driven by substrate depletion and antimicrobial effects of by-products. β-diversity (Bray–Curtis's distance) did not show significant differences among fermentations when considering fermentation time and ingredients. However, significant differences were observed between samples collected at day 8 and day 22 (permutational multivariate analysis of variance; PERMANOVA, *p-value* = *0.04*) (Fig. S4). Moreover, the comparison of Bray-Curtis's distances between sample groups indicates higher differences between first and second fermentation/shelf life samples than between second fermentations/shelf life samples (*p-value* < *0.05*) (Fig. S5). These microbial composition dynamics likely influenced kombucha's chemical and sensory properties (Li et al., 2024), emphasizing the role of fermentation duration and ingredient selection in shaping the microbial ecosystem. The mycobiota composition confirmed the higher compositional differences of samples at the beginning of fermentation, with an increase of *S. pombe* as fermentation progressed (Fig. 1B).

3.2. Chemical transformations during first and second fermentations

HPLC-DAD/RI analysis revealed the presence of glucose, fructose, glycerol, and ethanol, with significant differences observed between fermentations (*p-value* < *0.05*) (Table S1). The second fermentation led to a marked increase in glycerol concentration. Unflavoured, pineapple-, and fennel-flavoured kombucha after 8, 15, and 22 days exhibited higher glycerol levels (average 0.48 g/l, ± 0.12) compared with

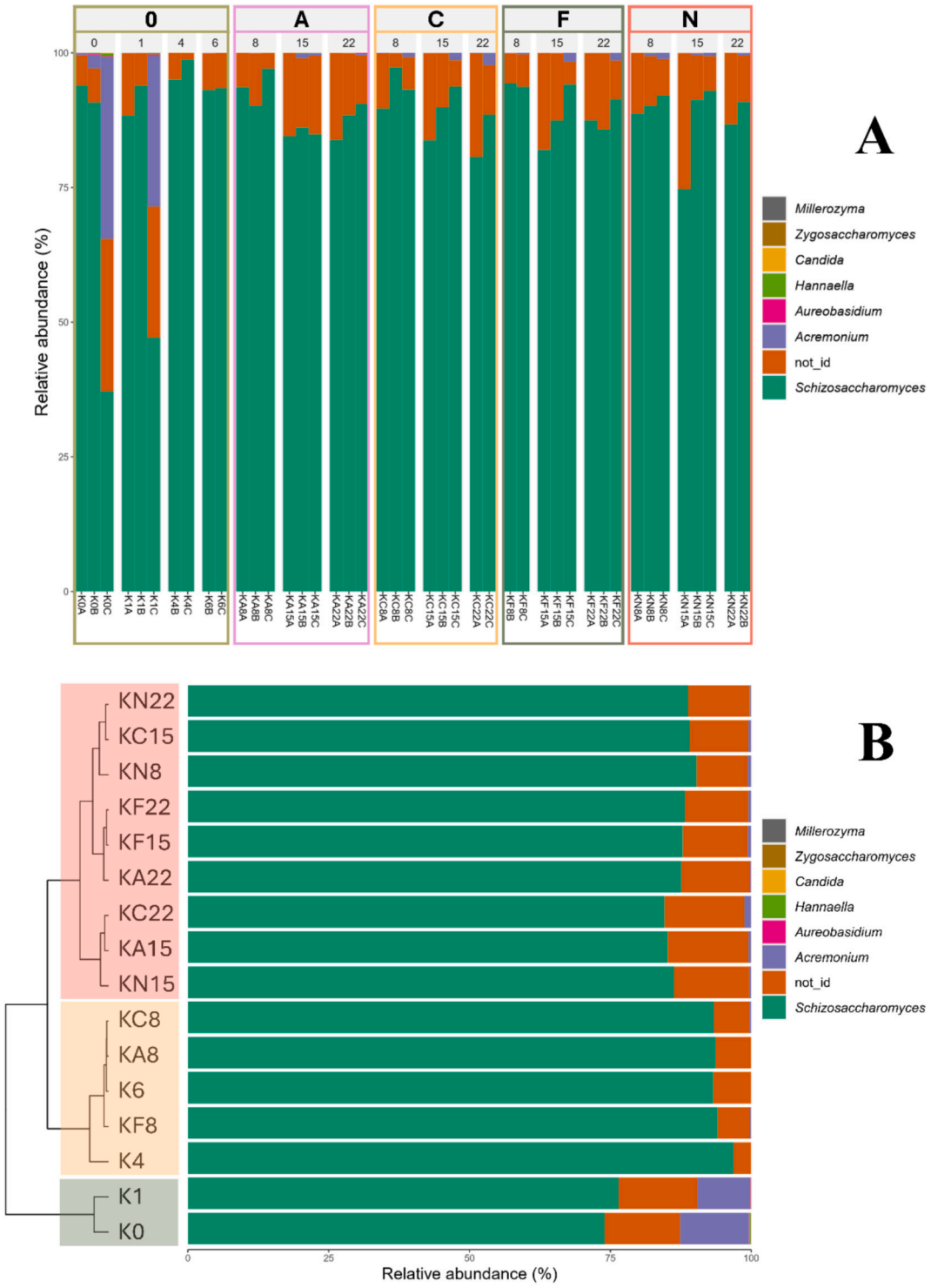

Fig. 1. Fungal taxa relative abundances separated by fermentation time and ingredients (A) and hierarchical clustering with dendrogram (B). Panel A shows the mycobiota composition of samples grouped by fermentation stage: first fermentation (0), second fermentation (8), and shelf life (15, 22), according to the by-products used. Panel B displays three distinct clusters in the dendrogram, highlighted by green (first fermentation, time 0 and 1), yellow (first fermentation and flavoured fermentation), and red boxes (flavoured fermentation and shelf-life samples). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kombucha at time zero (average 0.10 g/l, ± 0.10). Unflavoured samples showed lower glycerol concentrations than fennel-flavoured samples and lower ethanol levels than pineapple- and carrot-flavoured ones (*p-value* < *0.05*) (Fig. S6). Samples KA8, KA15, KA22, KC15, KC22, KF8, KF15, KF22, KN15, and KN22 exhibited a significant increase in ethanol levels compared with kombucha at time zero (average difference 13.86 g/l, ± 3.08). The K—W test indicated significant differences in fructose concentrations across fermentations (*p-value* = *0.043*), although pair-wise comparisons (K-W/Dunn's test, *p-value* > *0.05*) did not identify significant differences between individual groups. Overall, ethanol and glycerol were significantly more abundant in second fermentation and shelf life samples flavoured with pineapple, carrot, and fennel compared

with first fermentation samples (*p-value* < *0.05*) (Fig. S6). The accumulation of glycerol and ethanol during second fermentation reflects ongoing yeast fermentative metabolism and co-metabolism with acetic acid bacteria under low-oxygen conditions (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). The enhanced glycerol content may result from osmotic regulation pathways in yeasts under stress, while the increase in ethanol suggests efficient carbohydrate conversion, possibly stimulated by substrate by-products. Differences across flavours (higher glycerol in fennel, higher ethanol in pineapple and carrot) suggest that the chemical composition of the added by-products modulates yeast activity. The significant variability in fructose concentrations may indicate preferential utilization of glucose by yeasts, leaving residual fructose for other community members and thus contributing to microbial succession (Villarreal-Soto et al., 2018). Overall, these chemical trends support the view that secondary fermentation can modify metabolite profiles in interaction with microbial dynamics, with potential for fine-tuning via substrate choice within a circular-economy framework.

Several significant correlations between metabolites, microbial loads, and pH were observed (Pearson's correlation >0.6 or < −0.6, *p-value* < *0.05*), although not all variable combinations showed statistically significant associations (Fig. 2). Glycerol and ethanol were positively correlated with glucose in fermentations with pineapple and carrot. Glucose was positively correlated with pH decrease in unflavoured and carrot-flavoured kombucha, and with acetic acid in first fermentation samples. PCA microbial loads were positively correlated with glucose and fructose concentrations in the first fermentation. Fructose was positively correlated with acetic acid and MEA microbial loads in K0 samples, whereas MEA and average yeast loads (MEA + WL) were negatively correlated with fructose in KF and KN samples. The observed correlations between microbial loads and sugar concentrations suggest distinct microbial dynamics and associated variations in the chemical composition of kombucha, influencing both pH and metabolite profiles where statistically supported. Glycerol showed strong positive correlations with ethanol, pH decrease, and acetic acid in most conditions, with the exception of K0 samples. PCA and WL microbial loads were positively correlated with glycerol in fennel-flavoured kombucha. Ethanol was correlated with acetic acid and pH decrease in KC, KF, and KN samples and during the first fermentation. MEA microbial load was positively correlated with ethanol in pineapple-fermented samples. Acetic acid was strongly correlated (> 0.8) with pH decrease in all conditions and with PCA (K0, KA, KC), WL (KA, KF), MEA (K0, KA), and average yeast load in pineapple-flavoured kombucha. The significant correlations observed for selected metabolites suggest that microbial metabolism contributed to increased levels of compounds such as ethanol and acetic acid, concurrently driving a pH decrease. This effect is particularly relevant, since low pH together with elevated acetic acid concentration is well known to enhance the microbiological stability of beverages (e.g. in acid-preserved foods) (Yassunaka Hata et al., 2023). Moreover, the differing correlations between MEA, WL, and total fungal load imply that the choice of culture medium influences the growth, and hence detection, of different fungal populations (Cheng et al., 2023). Overall, multivariate statistical analysis indicated that ethanol and glycerol accumulation was associated with a progressive pH decrease (Fig. 3), contributing to the separation between first fermentation samples and second fermentation or shelf-life samples (*p-value* < *0.05*). This trend reflects the typical metabolic pathways of yeasts and acetic acid bacteria during kombucha maturation (Jayabalan et al., 2014;

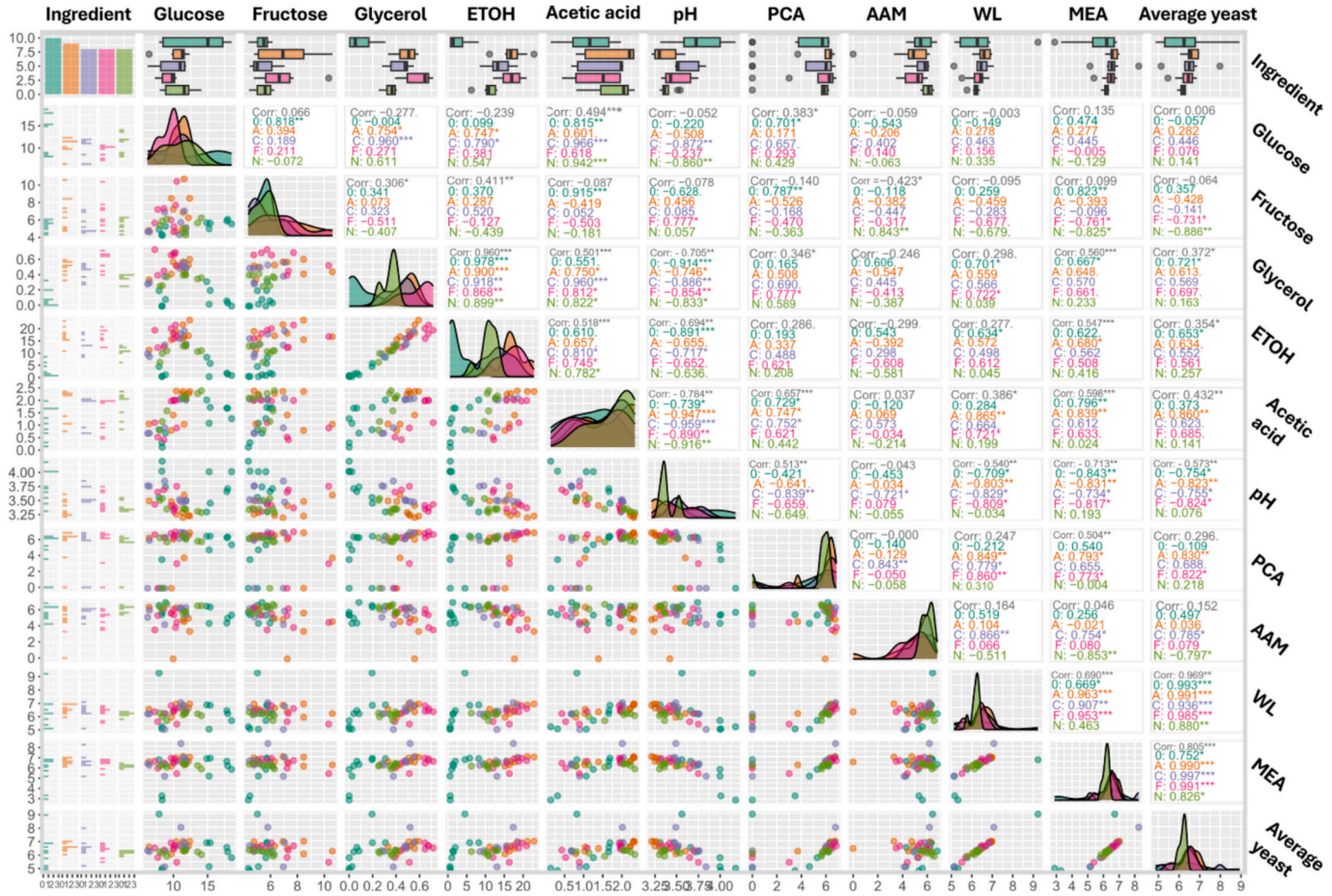

Fig. 2. Pearson's correlation between microbial loads, pH and HPLC-DAD/RI results. The significant correlations (*p-value* < *0.05*) are indicated by asterisk. The groups are indicated by 0 (first fermentation), N (unflavoured), A (pineapple), C (carrot) and F (fennel).

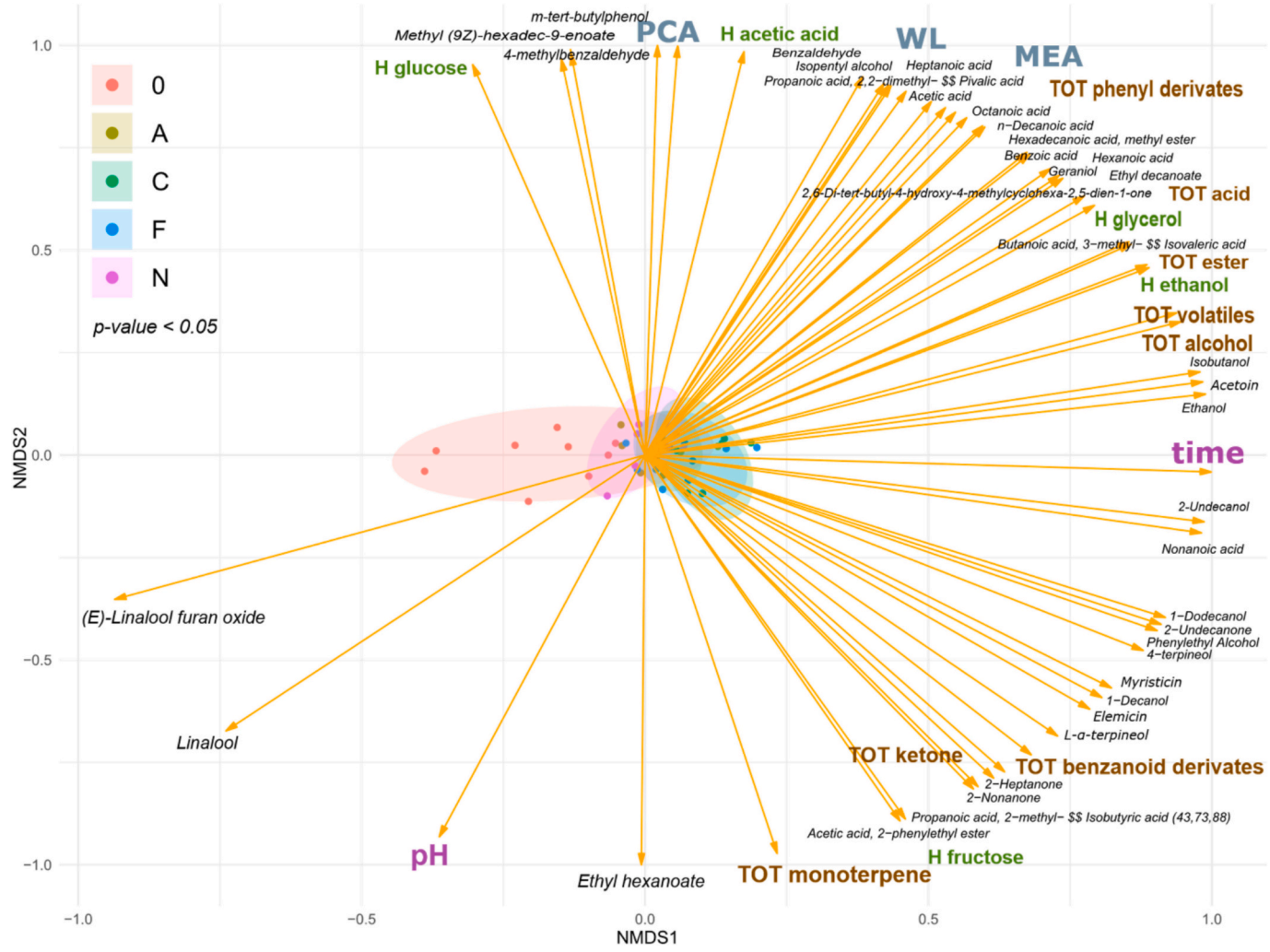

Fig. 3. Non-metric Multidimensional Scaling (NMDS) analysis of VOCs, HPLC-DAD/RI, microbial loads, and pH data. Fermentation groups are indicated in the legend as 0 (first fermentation), A (pineapple), C (carrot), F (fennel), and N (unflavoured). Fermentation time was included as a variable, and HPLC-DAD/RI data are indicated by the initial letter "h." Arrow lengths have been normalized to unit length to emphasize variable directions in NMDS space; consequently, all arrows have equal length.

Marsh et al., 2014) and highlights the close interplay between microbial activity and chemical evolution in flavoured systems.

3.3. Volatile organic compounds dynamics and sensory relevance

Alcohols and acids were the most abundant VOCs in kombucha samples, with average concentrations of 2873.30 and 1160.81 ppb, respectively (Table S1), in agreement with previous reports (Dartora et al., 2023; Zhao et al., 2018). A significant increase in total acids concentration was observed in samples KA8 and KA15 compared to K0 (p-*value* < 0.05) (Fig. S7A), while total ketones were significantly higher in samples KA15–22 and KF/C22 than in K0 (p-*value* < 0.05) (Fig. S7B). Overall, VOCs accumulation increased with fermentation progression (Dartora et al., 2023).

The effects of each plant by-product (pineapple, carrot, fennel) were evaluated relative to the unflavoured control (KN) and across ingredients over time, highlighting statistically significant differences while reporting non-significant comparisons. Although plant by-products modulated several VOCs, many compounds did not differ significantly from the control, indicating a selective rather than global effect on the volatilome.

Several VOCs were detected exclusively in specific fermentations. Myristicin (1,3-benzodioxole, 4-methoxy-6-(2-propenyl)-) was detected only in carrot- and fennel-flavoured kombucha, with average concentrations of 35.61 ± 2.67 and 6.05 ± 0.67 ppb, respectively. Elemicin was detected exclusively in carrot-flavoured samples, as was isobutanol. In addition, benzenoid derivatives were found only in carrot- and fennel-flavoured kombucha, highlighting the role of plant by-products in shaping distinctive aromatic profiles.

Several VOCs differed significantly across fermentations (p-*value* $<$ 0.05) (Table S1). For instance, pivalic acid was more abundant in KC15 and KF15 compared with K0 and K1, while 2-undecanone increased in KA15 and KC22 relative to first fermentation samples. Shelf-life samples flavoured with pineapple and fennel showed higher concentrations of ketones such as 2-nonanone and 2-heptanone, whereas α-terpineol increased in all flavoured kombuchas at day 22 compared with first fermentation.

Comparisons based solely on flavouring agents revealed significant differences in VOC classes regardless of fermentation time (Figs. 3–4, Table S5). Pineapple-flavoured kombucha showed higher total acids and aldehydes, whereas carrot-flavoured samples were enriched in benzenoid compounds. Fennel-flavoured kombucha showed increased concentrations of specific esters and terpenes, with variations dependent on fermentation time. Overall, plant by-product supplementation significantly modulated the aroma profile of kombucha, contributing to diverse sensory attributes including floral, fruity, green, fatty, and

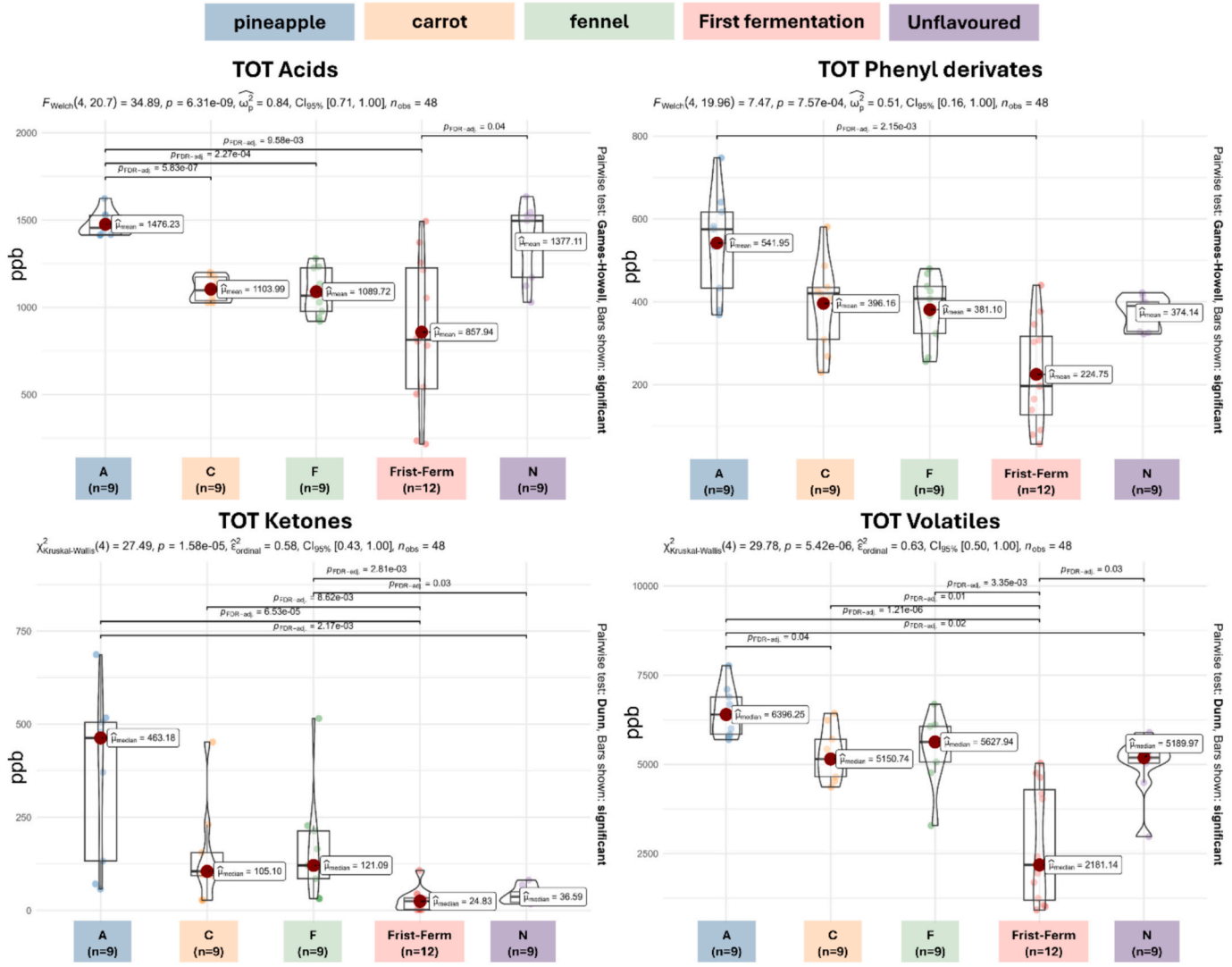

Fig. 4. Total VOCs divided per category that resulted differentially concentrated between fermentations expressed in part per billion (ppb), considering different ingredients (A = pineapple, C = carrot, F = fennel, first Ferm = first fermentation, N = unflavoured). The plots show statistical test results FDR corrected with *p*-values <0.05.

woody notes (Aprea, 2020). However, sensory studies with trained panels are required to confirm the perceptual relevance of these changes.

3.4. Associations between microbiome and chemical characteristics

Microbiome analysis revealed several significant correlations with chemical data (Pearson's correlation >0.8 or < −0.8, FDR-adjusted *p-value* < 0.05; Table S4). Correlations are reported at the highest reliably identifiable taxonomic level, and ingredient-related comparisons were performed by pooling all sampling times to increase statistical robustness.

Basidiomycota was positively correlated with 2-undecanone in K0, whereas Ascomycota was positively correlated in KF with ethyl hexanoate and nonanoic acid, and in KC with 2-heptanone, propanoic acid, 2-methyl- (isobutyric acid), and pH (i.e., associated with lower acidity). Conversely, a negative correlation was observed between Ascomycota and ethanol in unflavoured kombucha. In addition to representing key fungal taxa involved in the fermentation of various foods, these phyla have also shown an influence on volatile compound profiles. Basidiomycota have been reported to produce alcohols and esters (Buzzini et al., 2005), demonstrating, together with Ascomycota, a significant impact on the concentration of volatile compounds and, consequently, on the sensory characteristics of fermented products (Gao et al., 2024).

Tremellales was positively correlated with 2-undecanone in first fermentation samples. Sordariomycetes was positively correlated with 2-undecanone in K0, Hypocreales were positively correlated with acetic acid and 4-terpineol in fennel and pineapple flavoured kombucha, respectively. Saccharomycetales was positively correlated with ethyl hexanoate in fennel- and carrot-flavoured kombucha, while showing negative correlations with isobutanol and acetic acid. First fermentation demonstrated a positive correlation between *Saccharomycetaceae* and *Zygosaccharomyces* spp. with 2-undecanone. The unflavoured kombucha was characterized by a positive correlation of *Colacogloea* spp. with hexanoic acid and myristicin (1,3-benzodioxole, 4-methoxy-6-(2-propenyl)-) and of *Hannaella* spp. with ethyl hexanoate, 4-terpineol and α-terpineol. Kombucha fermented with pineapple showed correlation between benzoic acid and the genus *Hannaella*. *Acremonium* spp. was negatively correlated with hexanoic acid in unflavoured samples. *Galactomyces* spp. and *Aureobasidium* spp. were positively correlated with ethyl hexanoate in carrot flavoured kombucha. Moreover, *Aureobasidium* spp. was also correlated with ethyl hexanoate in fennel-flavoured kombucha, which additionally showed a positive correlation between *Millerozyma* spp. and 4-terpineol. Overall, VOC concentrations increased

during fermentation, with flavoured samples showing a more pronounced enhancement compared to the unflavoured control. This suggests that by-product fermentation enhances aromatic compounds, as reported for other fermented foods (Chiarini et al., 2024), likely driven by yeast metabolism in kombucha (Bishop et al., 2022). The positive association of Tremellales and Sordariomycetes with ketones such as 2-undecanone indicates a potential involvement in lipid or amino acid degradation pathways, as previously reported for certain basidiomycetous yeasts contributing to aroma complexity in fermented foods (Buzzini et al., 2005). The consistent correlations of Saccharomycetales, *Galactomyces*, and *Aureobasidium* with esters and terpenes (e.g., ethyl hexanoate, 4-terpineol, α-terpineol) point to their participation in esterification reactions and monoterpene biotransformation, processes known to influence fruity and floral notes in beverages such as wine (Padilla et al., 2016). Conversely, negative correlations between *Acremonium* and hexanoic acid or between Saccharomycetales and acetic acid may reflect inhibitory effects of organic acid accumulation or metabolic competition for carbon sources under acidic stress (Jayabalan et al., 2014; Tran et al., 2020; Wang et al., 2023). The correlation of the genera *Acremonium*, *Aureobasidium*, *Colacogloea*, *Galactomyces*, *Millerozyma* and *Hannaella* with several VOCs, indicates that minor taxa can influence fermentation dynamics and aroma development. However, the detection of taxa at very low abundances within kombucha microbial communities should be interpreted cautiously, as these sequences may represent environmental contaminants or transient members rather than ecologically stable and functionally significant constituents of the fermentative consortium (Ojo & de Smidt, 2023; Reva et al., 2015). Microbial diversity was low, consistent with previous studies (Arıkan et al., 2020; Ben Saad et al., 2025), with alpha diversity indices significantly decreasing over fermentation time and in flavoured samples. This aspect suggests a shift towards a specialized microbial community, possibly driven by substrate depletion and the antimicrobial effects of by-products. Collectively, these findings highlight that fungal community structure and plant by-product supplementation jointly shape the volatilome of kombucha, reinforcing the idea that specific yeasts and filamentous fungi can be selectively stimulated to modulate sensory attributes and stability of the beverage (Pak et al., 2025). This opens perspectives for the valorisation of agro-industrial by-products as targeted modulators of microbial activity and aroma development in functional fermented beverages (Villarreal-Soto et al., 2018).

The analysis of correlations between α-diversity indices, chemical data, and culture-dependent bacterial loads, considering different sampling times and ingredients, did not reveal statistically significant associations (*p-value* > 0.05). Differences were observed considering the whole dataset (Pearson's correlation < -0.5 and > 0.5, FDR-adjusted *p-value* < 0.05) (Table S4). The microbial loads of MEA demonstrated negative correlation with ACE, Simpson, observed, Chao1, Shannon, inverted Simpson and Fisher indexes. The microbial loads of WL were negatively correlated with Observed, Chao1 and Fisher. The average yeast loads (MEA and WL) were negatively correlated with ACE, observed, Chao1 and Fisher indexes. Shannon and inverted Simpson indexes were negatively correlated with total acids. The negative correlations between microbial loads, diversity indexes, and total acids suggest that increased yeast proliferation and acid accumulation contribute to a selective pressure reducing microbial diversity. This trend reflects a typical ecological shift during fermentation, where acid-tolerant taxa become dominant as the environment becomes progressively more acidic (Tran et al., 2020). This observation is consistent with the metabolic activity of AAB, which typically proliferate during fermentation, producing acetic acid and lowering the pH, a defining feature of kombucha fermentation (Nyhan et al., 2022). The RDA analysis demonstrated that VOCs concentration and acidity increase

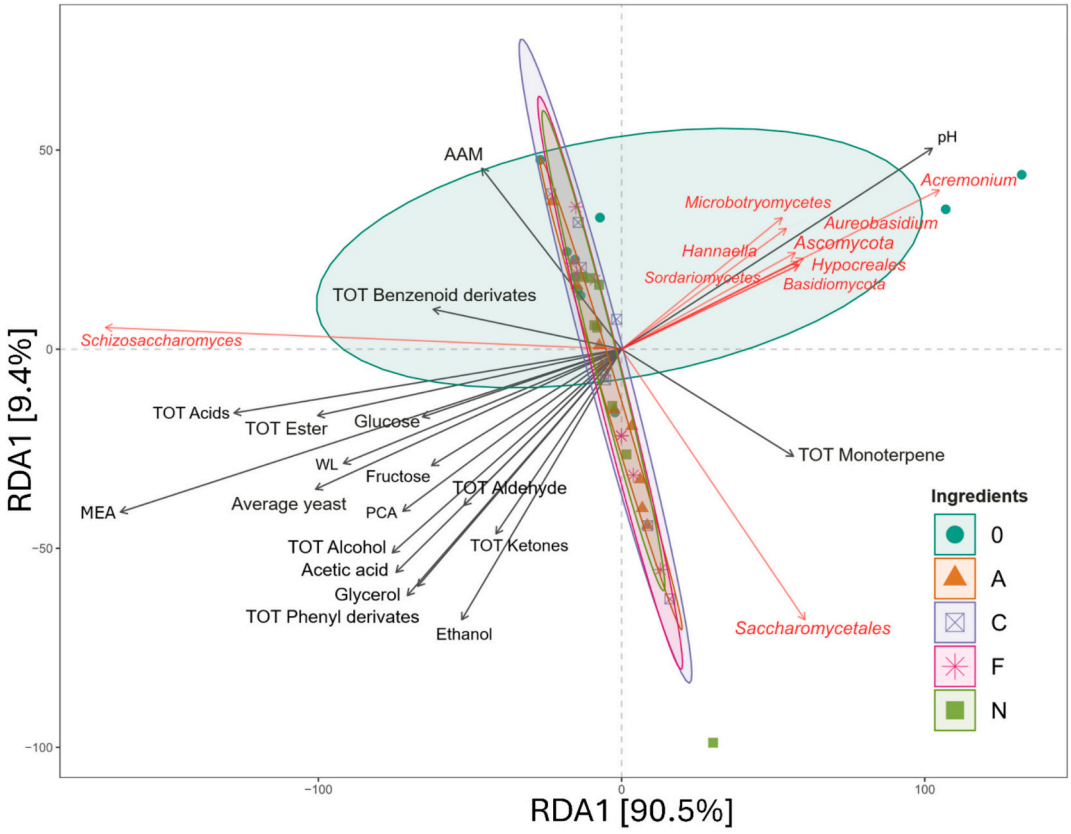

Fig. 5. Redundancy Analysis (RDA) including taxa composition (in red), HPLC, VOCs categories and pH of samples divided per ingredients (A = pineapple, C = carrot, F = fennel, 0 = first fermentation, N = unflavoured). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were associated with *Schizosaccharomyces*, and with consequent reduction in fermentation biodiversity observable by the taxa present in the opposite part of the plot (Fig. 5). *Schizosaccharomyces* further demonstrated its importance in kombucha fermentation (Ojo & de Smidt, 2023), representing a taxon relatively stable in abundance (Tran et al., 2020) and consistently associated with an overall increase in VOCs concentrations (Fig. 5).

4. Conclusions

The fermentation trials performed on kombucha produced with plant by-products confirmed the typical characteristics of the kombucha fermentation process. However, the use of pineapple, carrot, and fennel by-products led to a second fermentation with distinctive features, including specific VOCs and microbial communities that interacted and jointly shaped the overall fermentation dynamics. Microbial analysis revealed the presence of a single bacterial species, the acetic acid bacterium *K. rhaeticus*, which is commonly associated with kombucha fermentations. Further studies employing advanced sequencing techniques, such as shotgun metagenomics, will be required to elucidate the identity and functional roles of currently unclassified reads detected in the dataset. Fungal taxa exhibited greater biodiversity than bacterial taxa, with *S. pombe* as the dominant species. The observed correlations between microbial taxa and volatile compounds with known sensory relevance highlight the complex interactions between chemical and microbiological components during fermentation. Future sensory evaluations, involving both trained panels and consumers, will allow a better understanding of the impact of specific taxa and VOCs on the sensory properties and overall acceptability of the final beverage.

Importantly, the incorporation of plant by-products not only modulated the aromatic and sensory profiles of kombucha but also provided a valuable approach for reusing food industry residues, aligning with the principles of circular economy and sustainable production. These results demonstrate that the application of by-products in fermentation represents more than a waste recovery strategy, as it can actively enhance the sensory and potential economic value of both the by-products and the resulting kombucha. Increasing consumer awareness of sustainability in the food system may further boost the marketability of kombucha beverages, enriched with upcycled plant materials.

Finally, future research should also investigate the potential probiotic activities of microorganisms present in by-product-based kombucha, as well as their viability and stability over time. This will be necessary to comprehensively assess the beverage's functional and preservation properties. In summary, this study provides novel insights into the microbial-chemical interactions shaping kombucha fermentation, highlighting the potential of plant by-products not only to modulate the beverage's sensory and microbial profiles but also to promote sustainability within the food system.

Use of AI

Some textual elements in graphical abstract and minor English editing were performed with the assistance of ChatGPT-4o (omni), developed by OpenAI (2025).

CRediT authorship contribution statement

Elisabetta Chiarini: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Davide Buzzanca:** Writing – original draft, Visualization, Validation, Software, Methodology, Data curation. **Alessandra Devizia:** Writing – original draft, Investigation, Formal analysis. **Manuela Giordano:** Writing – review & editing, Validation, Methodology, Formal analysis. **Franco Dipietro:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Giuseppe Zeppa:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition. **Valentina Alessandria:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giuseppe Zeppa reports financial support was provided by Biova Project. Franco Dipietro reports a relationship with Biova Project that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.118597.

Data availability

Illumina sequences are available at bioproject PRJNA1236227 on NCBI. Other data are available in the supplemental tables.

References

Aprea, E. (2020). Special Issue "Volatile compounds and smell chemicals (odor and aroma) of food". *Molecules, 25*(17), 3811. https://doi.org/10.3390/molecules25173811

Arıkan, M., Mitchell, A. L., Finn, R. D., & Gürel, F. (2020). Microbial composition of kombucha determined using amplicon sequencing and shotgun metagenomics. *Journal of Food Science, 85*(2), 455–464. https://doi.org/10.1111/1750-3841.14992

Barbosa-Pereira, L., Rojo-Poveda, O., Ferrocino, I., Giordano, M., & Zeppa, G. (2019). Assessment of volatile fingerprint by HS-SPME/GC-qMS and E-nose for the classification of cocoa bean shells using chemometrics. *Food Research International, 123*(May), 684–696. https://doi.org/10.1016/j.foodres.2019.05.041

Ben Saad, E., Friedrich, A., Fischer, F., Courot, O., Schacherer, J., & Bleykasten, C. (2025). Comprehensive survey of kombucha microbial communities of diverse origins and fermentation practices. *FEMS Yeast Research, 25*(November 2024). https://doi.org/10.1093/femsyr/foaf005

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*(March), Article 100025. https://doi.org/10.1016/j.focha.2022.100025

Botta, C., Spada, D., Buzzanca, D., Zadeh, N. S., Tsoureki, D., Biolcati, F., … Dolci, P. (2025). Batch-dependent microbiota variation in mixed vegetable fermentations shapes the probiotic potential of autochthonous multifunctional cultures. *Food Research International, 220*(March), Article 117039. https://doi.org/10.1016/j.foodres.2025.117039

Bozoudi, D., & Tsaltas, D. (2018). The multiple and versatile roles of *Aureobasidium pullulans* in the vitivinicultural sector. *Fermentation, 4*(4), 85. https://doi.org/10.3390/fermentation4040085

Buzzini, P., Romano, S., Turchetti, B., Vaughan, A., Pagnoni, U. M., & Davoli, P. (2005). Production of volatile organic sulfur compounds (VOSCs) by basidiomycetous yeasts. *FEMS Yeast Research, 5*(4–5), 379–385. https://doi.org/10.1016/j.femsyr.2004.10.011

Chaudhary, S., & Singh, B. (2024). Pineapple by-products utilization: Progress towards the circular economy. *Food and Humanity, 2*(January), Article 100243. https://doi.org/10.1016/j.foohum.2024.100243

Cheng, C.-Y., Zhang, M.-Y., Niu, Y.-C., Zhang, M., Geng, Y.-H., & Deng, H. (2023). Comparison of fungal genera isolated from cucumber plants and rhizosphere soil by using various cultural media. *Journal of Fungi, 9*(9), 934. https://doi.org/10.3390/jof9090934

Chiarini, E., Alessandria, V., Buzzanca, D., Giordano, M., Seif Zadeh, N., Mancuso, F., & Zeppa, G. (2024). Valorization of fruit by-products through lactic acid fermentation for innovative beverage formulation: Microbiological and physiochemical effects. *Foods, 13*(23), 3715. https://doi.org/10.3390/foods13233715

Chiboub, W., Sassi, A. B., Amina, C. M., Souilem, F., El Ayeb, A., Djlassi, B., … Harzallah-Skhiri, F. (2019). Valorization of the green waste from two varieties of fennel and carrot cultivated in Tunisia by identification of the phytochemical profile and evaluation of the antimicrobial activities of their essentials oils. *Chemistry & Biodiversity, 16*(1). https://doi.org/10.1002/cbdv.201800546

Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., Fall, A., Daube, G., & Coton, E. (2017). Unraveling microbial ecology of industrial-scale kombucha fermentations by metabarcoding and culture-based methods. *FEMS Microbiology Ecology, 93*(5), 1–16. https://doi.org/10.1093/femsec/fix048

Dartora, B., Hickert, L. R., Fabricio, M. F., Ayub, M. A. Z., Furlan, J. M., Wagner, R., … Sant'Anna, V.. (2023). Understanding the effect of fermentation time on physicochemical characteristics, sensory attributes, and volatile compounds in green tea kombucha. *Food Research International, 174*(July), 1–11. https://doi.org/10.1016/j.foodres.2023.113569

DuMez-Kornegay, R. N., Baker, L. S., Morris, A. J., DeLoach, W. L. M., & Dowen, R. H. (2024). Kombucha tea-associated microbes remodel host metabolic pathways to suppress lipid accumulation. *PLoS Genetics, 20*(3), Article e1011003. https://doi.org/10.1371/journal.pgen.1011003

Gao, J., Geng, H., Chai, R., Wu, T., Huang, W., You, Y., & Zhan, J. (2024). Fungal community composition and its relationship with volatile compounds during spontaneous fermentation of cabernet sauvignon from two Chinese wine-growing regions. *Foods, 13*(1). https://doi.org/10.3390/foods13010106

Georgalaki, M., Ferrocino, I., Buzzanca, D., Anastasiou, R., Zoumpopoulou, G., Giabasakou, D., … Tsakalidou, E. (2025). The natural fermentation of Greek Tsounati olives: Microbiome analysis. *Foods, 14*(15), 2568. https://doi.org/10.3390/foods14152568

Grand View Research. (2025). Kombucha Market (2025 - 2030). GVR report. *Volume, GVR-1-68038-250-1*, 1–110..

Hautefeuille, S., Tareb, R., Bouju-Albert, A., Misery, B., Laroche, B., Guillou, S., & Haddad, N. (2024). Extensive multivariate dataset characterizing bacterial community diversity and *Campylobacter* contamination level in a large number of conventional broilers carcasses after air chilling and refrigerated storage. *Data in Brief, 56*, Article 110858. https://doi.org/10.1016/j.dib.2024.110858

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea—Microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 538–550. https://doi.org/10.1111/1541-4337.12073

Kandemir, K., Piskin, E., Xiao, J., Tomas, M., & Capanoglu, E. (2022). Fruit juice industry wastes as a source of bioactives. *Journal of Agricultural and Food Chemistry, 70*(23), 6805–6832. https://doi.org/10.1021/acs.jafc.2c00756

Kaur, P., Subramanian, J., & Singh, A. (2022). Green extraction of bioactive components from carrot industry waste and evaluation of spent residue as an energy source. *Scientific Reports, 12*(1), 16607. https://doi.org/10.1038/s41598-022-20971-5

Kausar, T., Laaraj, S., Hussain, A., Noutfia, Y., Bouhrim, M., Mothana, R. A., … Elfazazi, K. (2024). Use of dehydrated carrot (*Daucus carota*) pomace and almond (*Prunus dulcis*) powder for partial replacement of wheat flour in cake: Effect on product quality and acceptability. *Frontiers in Sustainable Food Systems, 8*(November), 1–15. https://doi.org/10.3389/fsufs.2024.1443841

Kisand, V., Cuadros, R., & Wikner, J. (2002). Phylogeny of culturable estuarine bacteria catabolizing riverine organic matter in the northern Baltic Sea. *Applied and Environmental Microbiology, 68*(1), 379–388. https://doi.org/10.1128/AEM.68.1.379-388.2002

Li, S., Wang, R., Liu, R., Wang, L., Wang, X., Wei, J., … Wang, Z. (2024). Exploring the dynamic characteristic of typical kombucha induced by symbiotic microbiota succession from four Chinese regions: A comprehensive analytical framework. *Food Research International, 198*(August), Article 115335. https://doi.org/10.1016/j.foodres.2024.115335

Liao, T., Li, X.-R., Fan, L., Zhang, B., Zheng, W.-M., Hua, J.-J., … Cheng, L.-H. (2024). Nature of back slopping kombucha fermentation process: Insights from the microbial succession, metabolites composition changes and their correlations. *Frontiers in Microbiology, 15*(August), 1–13. https://doi.org/10.3389/fmicb.2024.1433127

Lisdiyanti, P., Kawasaki, H., Seki, T., Yamada, Y., Uchimura, T., & Komagata, K. (2001). Identification of *Acetobacter* strains isolated from Indonesian sources, and proposals of *Acetobacter syzygii* sp. nov., *Acetobacter cibinongensis* sp. nov., and *Acetobacter orientalis* sp. nov. *The Journal of General and Applied Microbiology, 47*(3), 119–131. https://doi.org/10.2323/jgam.47.119

Liu, C., Cui, Y., Li, X., & Yao, M. (2021). Microeco: An R package for data mining in microbial community ecology. *FEMS Microbiology Ecology, 97*(2), 1–9. https://doi.org/10.1093/femsec/fiaa255

Marcelli, A., Osimani, A., & Aquilanti, L. (2025). Vegetable by-products from industrial processing: From waste to functional ingredient through fermentation. *Foods, 14*(15), 2704. https://doi.org/10.3390/foods14152704

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 Microbiology, 38*, 171–178. https://doi.org/10.1016/j.fm.2013.09.003

Meena, L., Sengar, A. S., Neog, R., & Sunil, C. K. (2022). Pineapple processing waste (PPW): Bioactive compounds, their extraction, and utilisation: A review. *Journal of Food Science and Technology, 59*(11), 4152–4164. https://doi.org/10.1007/s13197-021-05271-6

Mota-Gutierrez, J., Ferrocino, I., Rantsiou, K., & Cocolin, L. (2019). Metataxonomic comparison between internal transcribed spacer and 26S ribosomal large subunit (LSU) rDNA gene. *International Journal of Food Microbiology, 290*(July 2018), 132–140. https://doi.org/10.1016/j.ijfoodmicro.2018.10.010

Nyhan, L. M., Lynch, K. M., Sahin, A. W., & Arendt, E. K. (2022). Advances in kombucha tea fermentation: A review. *Applied Microbiology, 2*(1), 73–103. https://doi.org/10.3390/applmicrobiol2010005

Ojo, A. O., & de Smidt, O. (2023). Microbial composition, bioactive compounds, potential benefits and risks associated with kombucha: A concise review. *Fermentation, 9*(5), 472. https://doi.org/10.3390/fermentation9050472

Padilla, B., García-Fernández, D., González, B., Izidoro, I., Esteve-Zarzoso, B., Beltran, G., & Mas, A. (2016). Yeast biodiversity from DOQ priorat uninoculated fermentations. *Frontiers in Microbiology, 7*(JUN), 1–11. https://doi.org/10.3389/fmicb.2016.00930

Pak, J., Park, H., Baek, K., Son, H.-S., & Kwak, S. (2025). Yeast-based precision fermentation for the biosynthesis of terpenoids. *Future Foods, 12*(August), Article 100752. https://doi.org/10.1016/j.fufo.2025.100752

Phung, L. T., Kitwetcharoen, H., Chamnipa, N., Boonchot, N., Thanonkeo, S., Tippayawat, P., … Thanonkeo, P. (2023). Changes in the chemical compositions and biological properties of kombucha beverages made from black teas and pineapple peels and cores. *Scientific Reports, 13*(1), 7859. https://doi.org/10.1038/s41598-023-34954-7

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), 35. https://doi.org/10.1186/s13568-015-0124-5

Roda, A., & Lambri, M. (2019). Food uses of pineapple waste and by-products: A review. *International Journal of Food Science & Technology, 54*(4), 1009–1017. https://doi.org/10.1111/ijfs.14128

Salas-Millán, J.Á., & Aguayo, E. (2024). Fermentation for revalorisation of fruit and vegetable by-products: A sustainable approach towards minimising food loss and waste. *Foods, 13*(22), 3680. https://doi.org/10.3390/foods13223680

Santoro, V., Rosa, E., Donadio, G., Polito, F., De Feo, V., & De Tommasi, N. (2024). Foeniculum vulgare miller bracts, revalorization of a local food waste. *Scientific Reports, 14*(1), 31287. https://doi.org/10.1038/s41598-024-82779-9

Santos, D. I., Martins, C. F., Amaral, R. A., Brito, L., Saraiva, J. A., Vicente, A. A., & Moldão-Martins, M. (2021). Pineapple (*Ananas comosus* L.) by-products valorization: Novel bio ingredients for functional foods. *Molecules, 26*(11), 3216. https://doi.org/10.3390/molecules26113216

Sanwal, N., Gupta, A., Bareen, M. A., Sharma, N., & Sahu, J. K. (2023). Kombucha fermentation: Recent trends in process dynamics, functional bioactivities, toxicity management, and potential applications. *Food Chemistry Advances, 3*(July), Article 100421. https://doi.org/10.1016/j.focha.2023.100421

Suffys, S., Richard, G., Burgeon, C., Werrie, P.-Y., Haubruge, E., Fauconnier, M.-L., & Goffin, D. (2023). Characterization of aroma active compound production during kombucha fermentation: Towards the control of sensory profiles. *Foods, 12*(8), 1657. https://doi.org/10.3390/foods12081657

Tanamool, V., Chantarangsee, M., & Soemphol, W. (2020). Simultaneous vinegar fermentation from a pineapple by-product using the co-inoculation of yeast and thermotolerant acetic acid bacteria and their physiochemical properties. *3 Biotech, 10*(3), 115. https://doi.org/10.1007/s13205-020-2119-4

Teneva, D., & Denev, P. (2023). Biologically active compounds from probiotic microorganisms and plant extracts used as biopreservatives. *Microorganisms, 11*(8), 1896. https://doi.org/10.3390/microorganisms11081896

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of kombucha fermentation. *International Journal of Food Microbiology, 95*(2), 119–126. https://doi.org/10.1016/j.ijfoodmicro.2003.12.020

Traina, C., Ferrocino, I., Bonciolini, A., Cardenia, V., Lin, X., Rantsiou, K., & Cocolin, L. (2024). Monitoring the yeasts ecology and volatiles profile throughout the spontaneous fermentation of Taggiasca cv. Table olives through culture-dependent and independent methods. *International Journal of Food Microbiology, 417*(March), Article 110688. https://doi.org/10.1016/j.ijfoodmicro.2024.110688

Tran, T., Grandvalet, C., Verdier, F., Martin, A., Alexandre, H., & Tourdot-Maréchal, R. (2020). Microbial dynamics between yeasts and acetic acid bacteria in kombucha: Impacts on the chemical composition of the beverage. *Foods, 9*(7), 963. https://doi.org/10.3390/foods9070963

Turner, S., Pryer, K. M., Miao, V. P. W., & Palmer, J. D. (1999). Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal of Eukaryotic Microbiology, 46*(4), 327–338. https://doi.org/10.1111/j.1550-7408.1999.tb04612.x

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J., & Taillandier, P. (2018). Understanding kombucha tea fermentation: A review. *Journal of Food Science, 83*(3), 580–588. https://doi.org/10.1111/1750-3841.14068

Wang, W., Zhang, F., Dai, X., Liu, Y., Mu, J., Wang, J., Ma, Q., & Sun, J. (2023). Changes in vinegar quality and microbial dynamics during fermentation using a self-designed drum-type bioreactor. *Frontiers in Nutrition, 10*(February), 1–12. https://doi.org/10.3389/fnut.2023.1126562

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), 670. https://doi.org/10.3390/nu14030670

Yassunaka Hata, N. N., Surek, M., Sartori, D., Serrato, R. V., & Spinosa, W. A. (2023). Role of acetic acid bacteria in food and beverages. *Food Technology and Biotechnology, 61*(1), 85–103. https://doi.org/10.17113/ftb.61.01.23.7811

Zhao, Z. J., Sui, Y. C., Wu, H. W., Zhou, C. B., Hu, X. C., & Zhang, J. (2018). Flavour chemical dynamics during fermentation of kombucha tea. *Emirates Journal of Food and Agriculture, 30*(9), 732–741. https://doi.org/10.9755/ejfa.2018.v30.i9.1794