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Utilizing kombucha culture for coffee fermentation and biochemical characteristic analysis

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ABSTRACT

Coffee, an important global commodity, is grown on 10.2 million hectares in over 80 countries. Arabica coffee (*Coffea arabica*) is popular worldwide due to its superior flavour. As consumer interest in coffee flavour and quality continues to grow, this study aims to enhance the bioactive compounds, functionality, and sensory properties of Arabica coffee (*Coffea arabica*) by fermentation with black tea kombucha (K-coffee) and coffee kombucha (CK-coffee). Coffee showed a decrease in pH and an increase in titratable acidity during fermentation. The total phenolic and flavonoid content increased 1.77- and 1.95-fold in K-coffee and 2.07- and 2.60-fold in CK-coffee, respectively, after 24 h of fermentation compared to the coffee control, in which the kombucha broth was not inoculated, with an increased in trigonelline, chlorogenic acid and caffeic acid content. The caffeine content in CK-coffee increased 1.33-fold after 12 h of fermentation and remained relatively stable in K-coffee up to 24 h of fermentation compared to the coffee control. The antioxidant activity and inhibitory effect against α-glucosidase of fermented coffee increased to 2.24- and 2.40-fold after 24 h of fermentation, respectively, compared to the coffee control. Sensory evaluation highlighted noticeable differences in flavour among unfermented coffee, K-coffee, and CK-coffee with changes in volatile aroma compounds. These results support the feasibility of kombucha fermentation as a novel approach to develop specialty coffees with enhanced flavour and nutritional profiles.

1. Introduction

Coffee is a complex beverage containing various bioactive compounds, including caffeine, chlorogenic acid, and trigonelline, which is produced by processing and extracting the seeds of coffee cherries. Consumption of coffee has been associated with numerous health benefits, including the prevention of cardiovascular diseases and a reduction in the risk of certain cancers (Pourshahidi et al., 2016). As the global coffee market expands, consumers increasingly seek personalised experiences, emphasising health benefits, and unique flavour profiles. This growing demand underscores the need for innovative approaches to enhance the functional properties of coffee while preserving its sensory properties. The development of coffee flavour can be broadly categorised into two main phases. First, fermentation occurs naturally during the harvesting, drying, or washing coffee cherries, with inherent microbial strains playing a key role. This phase is critical for producing

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the initial flavour components in green beans. Second, during high-temperature roasting, various flavours emerge through the Maillard reaction (Cardoso et al., 2024). The use of bacteria or yeasts as starter cultures for coffee bean fermentation has been shown to enhance aroma, flavour, phenolic content and antioxidant activity (Haile and Kang, 2019; Kwak et al., 2018). Kwak et al. (2018) reported that the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of green coffee were increased by yeast fermentation for 24 h. In another study, Pereira et al. (2015) showed that aroma compounds in coffee beans were enhanced by fermentation using *Pichia fermentans*. In addition, co-inoculation of *Saccharomyces cerevisiae* and *Bacillus amyloliquefaciens* in coffee fermentation enhanced the sensory properties of coffee (Ferreira et al., 2024). However, the effects of microbiota from fermented beverages on the bioactive compounds, functionality, and sensory properties of coffee remain unclear.

Kombucha, a fermented beverage produced by fermenting sweetened black tea with a symbiotic culture of bacteria and yeast (SCOBY), has a number of health benefits, including promotion of gut health, antibacterial properties, and potential weight loss effects, as well as acting as a source of probiotics (Kim et al., 2023; Permatasari et al., 2021; Vázquez-Cabral et al., 2017; Vitas et al., 2018). Several factors affect the fermentation of kombucha, including fermentation temperature, sugar concentration, tea type, fermentation time, geometry, and complex microbial community (Kim et al., 2023; Laavanya et al., 2021). Barbosa et al. (2021) reported that the complex microbial community could determine the microbial interactions and quality of kombucha by producing metabolites during fermentation. Watawana et al. (2015) inoculated kombucha tea fungus into coffee sugar infusions and observed increases in the contents of total phenolics, chlorogenic acid, caffeic acid, and caffeine during fermentation. In another study, Blaszak et al. (2024) used fresh coffee infusions for coffee kombucha production and found that the total phenolic content (TPC) and antioxidant activities increased by 1.31–1.68-fold and 2.13–4.37-fold, respectively, compared to the control, while the caffeine concentration decreased by up to 1.54-fold. However, the use of kombucha broth for fermenting coffee beans has been limited. This study investigated the effects of fermenting coffee with two different types of kombucha—black tea kombucha and coffee kombucha—on the bioactive compounds, sensory profile, and biological functions of coffee. The microorganism communities of black tea kombucha and coffee kombucha used for coffee fermentation were analysed. For the fermented coffee, we also characterised the biochemical properties, including pH, titratable acidity (TA), total phenolic content (TPC), total flavonoid content (TFC) and main bioactive compounds (trigonelline, caffeine, chlorogenic acid, and caffeic acid); determined the functional properties, including antioxidant activities and α-glucosidase inhibitory activity; and performed sensory analysis via volatile compounds and sensory evaluation using an electronic tongue sensory system.

2. Materials and methods

2.1. Materials

Ethiopian Yirgacheffe G4 coffee beans, which were harvested at an altitude of 1875–1900 m in Yirgacheffe, Gedeo, Ethiopia, and black tea were purchased from W. Beans Company (Seoul, Korea) and Ahmad Tea (London, UK), respectively. Caffeine, trigonelline, chlorogenic acid, caffeic acid, gallic acid, acetic acid, Trolox, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), Folin-Ciocalteu reagent, quercetin, dimethyl sulfoxide, α-glucosidase from *S. cerevisiae*, and *p*-nitrophenyl α-D-glucopyranoside (pNPG) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Kombucha SCOBY and commercial kombucha were obtained from a local market in Pyeongchang (Gangwon-do, Korea). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Acros Organics (Geel, Belgium), while fluorescein was supplied by Alfa-Aesar (Ward Hill, MA, USA). Other chemicals of analytical grade were purchased from Duksan (Gyeonggi-do, Korea).

2.2. Kombucha preparation

Black tea and coffee kombucha were prepared by adding 10% (v/v) kombucha from previous fermentation and 0.5 g of SCOBY to a jar containing 8% (w/v) sucrose in a black tea or coffee infusion, covering with gauze, and incubating at 23 °C for 14 days (Blaszak et al., 2024).

2.3. Analysis of microbial community composition of kombucha

The microbial community of kombucha was analysed via next-generation sequencing of the 16S rRNA V3–V4 region and the rDNA internal transcribed spacer (ITS) region to identify bacteria and yeast, respectively (Macrogen, Seoul, Korea).

2.4. Fermentation of green coffee beans using kombucha

Samples of 1 kg of green coffee beans were autoclaved at 121 °C for 15 min and cooled to room temperature. Green coffee beans were inoculated with 750 mL of black tea or coffee kombucha and incubated at 30 °C for 0–24 h. The coffee beans were then dried at 60 °C until moisture content reached 4–5%. Hereafter, the coffee fermented with black tea kombucha is called K-coffee, and coffee fermented with coffee kombucha is called CK-coffee.

2.5. Coffee roasting

After fermentation, coffee beans were medium roasted at 225 °C–230 °C for 13 min using a coffee roaster (CBR-101; Gene Cafe, Plymouth, MA, USA). The degree of roasting of green coffee and fermented green coffee beans was adjusted to between 53 and 57 according to the Specialty Coffee Association of America using a coffee roaster analysis device (TRA-3000; Coffee Tonya, Yokohama, Japan).

2.6. Coffee extraction

After roasting, the coffee and fermented coffee were ground in a grinder. Samples of 2 g of ground coffee powder were mixed with 20 mL of boiled water, then steeped in a water bath at 80 °C for 5 min. The coffee supernatant was obtained via centrifugation at 8000×*g* for 20 min and lyophilised at −45 °C and 10 Pa (FD-550; Eyela, Tokyo, Japan) for further analysis.

2.7. Biochemical characterisation of fermented coffee

2.7.1. Analysis of pH and titratable acidity

For pH and TA analysis, 2 g of ground coffee powder was added to 20 mL of boiled water and steeped in a water bath at 80 °C for 5 min. The coffee supernatant obtained via centrifugation at 8000×*g* for 10 min was used for pH measurement using a pH meter (SP-2100; SUNTEX, Taipei, Taiwan). The TA was analysed by dropwise addition of 0.1 M NaOH to a 20-mL coffee sample with stirring until the pH reached 8.2; therefore, the TA was expressed as mL of 0.1M NaOH/g coffee.

2.7.2. Analysis of total phenolic and total flavonoid contents

The TPC and TFC of coffee and fermented coffee were determined using the Folin-Ciocalteu method with gallic acid as the standard and the aluminium chloride colorimetric method with quercetin as the standard, respectively, according to our previous report (Kim et al., 2023). The TPC was presented as mg gallic acid equivalent/g coffee (mg GAE/g coffee), while TPC was expressed as mg quercetin equivalent/g coffee (mg QE/g coffee).

2.7.3. High-performance liquid chromatography analysis of caffeine, trigonelline, chlorogenic acid and caffeic acid

Caffeine, trigonelline, chlorogenic acid, and caffeic acid in coffee and fermented coffee were analysed using high-performance liquid chromatography (HPLC) as reported previously (Jeon et al., 2017) with slight modifications. Coffee samples were injected into the HPLC system (Waters 2998 HPLX; Waters Corp., Milford, MA, USA) and analysed using a reverse-phase Sunfire™ C_{18} column (4.6 mm × 100 mm, 5 μm; Waters Corp.) with 50 mM phosphoric acid as solvent A and acetonitrile as solvent B as mobile phase at a flow rate of 0.8 mL/min. Elution was followed by 65% solvent A for 10 min and 10% solvent A for 10 min. Using a UV detector, caffeine, caffeic acid, and trigonelline were detected at 272 nm, while chlorogenic acid was detected at 325 nm.

2.7.4. Analysis of volatile compounds

The volatile compounds of coffee and fermented coffee were isolated by solid-phase microextraction (50/30 μM) and analysed using a gas chromatograph (7890N; Agilent, Santa Clara, CA, USA) equipped with a headspace sampler and a mass detector (5975C MSD; Agilent) at a range of 33–500 m/z as in our previous study (Ryu et al., 2024). The temperature gradient was set as 50 °C for 3 min and then increased to 150 °C at a rate of 5 °C/min.

2.8. Biological characterisation of fermented coffee

2.8.1. Antioxidant activity

The antioxidant activities of coffee and fermented coffee were evaluated using oxygen radical absorbance capacity (ORAC), ferric-reducing antioxidant powder (FRAP) and 1,1-diphenyl-2-picrylhydrazil (DPPH) assays, as described in our previous report (Kim et al., 2023) with modifications. For the ORAC assay, 10 μL of coffee, fermented coffee or Trolox (0–500 μM) was added to black 96-well plates. Then, 10 μL of 25 nM fluorescein in 75 mM sodium phosphate buffer (pH 7.4) and 100 μL of AAPH in 75 mM sodium phosphate buffer (pH 7.4) were added to the plates and allowed to react at 37 °C. The plates were read at an excitation wavelength of 485 nm and emission wavelength of 538 nm using a SpectraMax M3 microplate reader (Molecular Devices, San Jose, CA, USA). ORAC was presented as mM Trolox equivalent/g coffee (TE/g coffee). The FRAP assay measures the ability to reduce ferric (Fe^{3+}) ions into ferrous (Fe^{2+}) ions, a process that involves the donation of electrons (Benzie and Strain, 1996). For the assay, 6 μL of coffee, fermented coffee or ferrous sulphate heptahydrate (0–2500 μM) was mixed with 194 μL of FRAP working solution composed of 0.02 M ferric chloride hexahydrate and added to 96-well plates containing a working solution consisting of 0.02 M ferric chloride hexahydrate, 0.04 M HCl and 0.01 M TPTZ in 0.3 M sodium acetate buffer (pH 3.6) in a ratio of 1:1:10 (v/v/v). The plates were incubated at 37 °C for 30 min and read at 593 nm using a SpectraMax M3 microplate reader. FRAP is presented as mM Fe^{2+}/g coffee. For DPPH radical scavenging assay, 20 μL of coffee or fermented coffee was mixed with 100 μM DPPH in 96-well plates, followed by shaking for 1 min and incubation in the dark for 30 min. The plates were read at 517 nm using a SpectraMax M3 microplate reader and calculated relative to the control. The half-maximal scavenging activity (SC_{50}) was determined as the sample concentration required to reduce the absorbance by 50%.

2.8.2. α-Glucosidase inhibition assay

The α-glucosidase inhibitory effects of coffee and fermented coffee were examined as described in our previous report (Kwak et al., 2023). Briefly, 20-μL coffee samples were mixed with 0.1 U of α-glucosidase/mL in 50 mM phosphate buffer (pH 6.8) and incubated at 37 °C for 5 min. Then, 5 mM pNPG was added to start the reaction at 37 °C. After 8 min, 300 μL of 250 mM sodium carbonate was added to the reaction mixture and read at 405 nm. The α-glucosidase inhibitory effects of coffee and fermented coffee were calculated relative to a blank control mixture without coffee sample.

2.9. Sensory evaluation of fermentation coffee

The TS-5000Z taste sensing system (Insent Inc., Atsugi, Kanagawa, Japan) was used to measure the flavour profiles of the coffee samples. For each test, a 40-mL coffee sample was placed into the taste analyser. The taste attributes were measured three times, and the averages of these values were recorded. The parameters analysed included sourness, astringency, bitterness, aftertaste-B, umami, aftertaste-A, richness and saltiness.

2.10. Statistical analysis

Data are presented as the mean ± standard deviation (SD) of three replicates. GraphPad Prism 9.5.1 (GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analysis. Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests was used to compare the effects of time on pH, TA, TPC, TFC, and bioactive compounds such as trigonelline, caffeine, chlorogenic acid, and caffeic acid, as well as antioxidant activities, α-glucosidase inhibition, and sensory evaluation of K-coffee and CK-coffee. One-way ANOVA with Tukey's multiple comparisons was used to compare the volatile compounds of K-coffee and CK-coffee. Results with p-values less than 0.05 were considered to indicate significant differences. ANOVA with Dunnett's multiple comparisons test was used to distinguish levels of significance based on the probabilities of $p < 0.05$(*), 0.01(**), 0.001(***), and <0.0001(****) for fermented coffee compared to the control (coffee). OriginPro 2022b (OriginLab, Northampton, MA, USA) was used for principal component analysis (PCA) and Pearson correlations.

3. Results and discussion

3.1. Microbial community of kombucha SCOBY

Kombucha is a fermented tea beverage in which a symbiotic culture of bacteria and yeast (SCOBY) is added to sugared tea and fermented under natural conditions (Cardoso et al., 2020; Kim et al., 2023). Several factors affect the fermentation of kombucha, including fermentation temperature, sugar concentration, tea type, fermentation time and the complex microbial community (Kim et al., 2023; Laavanya et al., 2021). Barbosa et al. (2021) found that the complex microbial community could determine the microbial interactions and production quality of kombucha by producing metabolites during fermentation. Therefore, two types of kombucha, black tea kombucha and coffee kombucha, were selected for this study. The microbial community of kombucha, including bacteria and yeast, was analysed by sequencing of the 16S rRNA V3–V4 region and rDNA ITS region for identification of bacteria and yeast, respectively; the results are presented in Fig. 1. *Dekkera bruxellensis* was identified as the dominant yeast species in black tea kombucha, comprising 99.89% of the yeast population, with *Dekkera anomala* making up the remaining 0.11% (Fig. 1A). Our results were consistent with a study showing that *D. bruxellensis* was the dominant yeast species in black tea Kombucha (Coton et al., 2017). *D. bruxellensis* is responsible for acetic acid production during kombucha fermentation and contributes to the characteristic flavours in beer, cheese and sourdough fermentation (Gerós et al., 2000; Schifferdecker et al., 2014). The predominant bacterial species in black tea kombucha was identified as *Komagataeibacter nataicola*, which accounted for 74.37% of the bacterial community, followed by *Gluconobacter oxydans*, accounting for 25.55%, and seven other species each accounting for less than 0.1% (Fig. 1B). *Komagataeibacter* spp. (family *Acetobacteraceae*) is crucial for cellulose synthesis (Semjonovs et al., 2017), whereas *G. oxydans* is noted for its ability to oxidise sugars and alcohols into valuable by-products, such as acids, aldehydes and ketones (da Silva et al., 2022).

Analysis of the yeast community in coffee kombucha showed that *Starmerella davenportii* was the dominant yeast species, accounting for 82.74%, followed by *Pichia membranifaciens*, *Pichia occidentalis*,

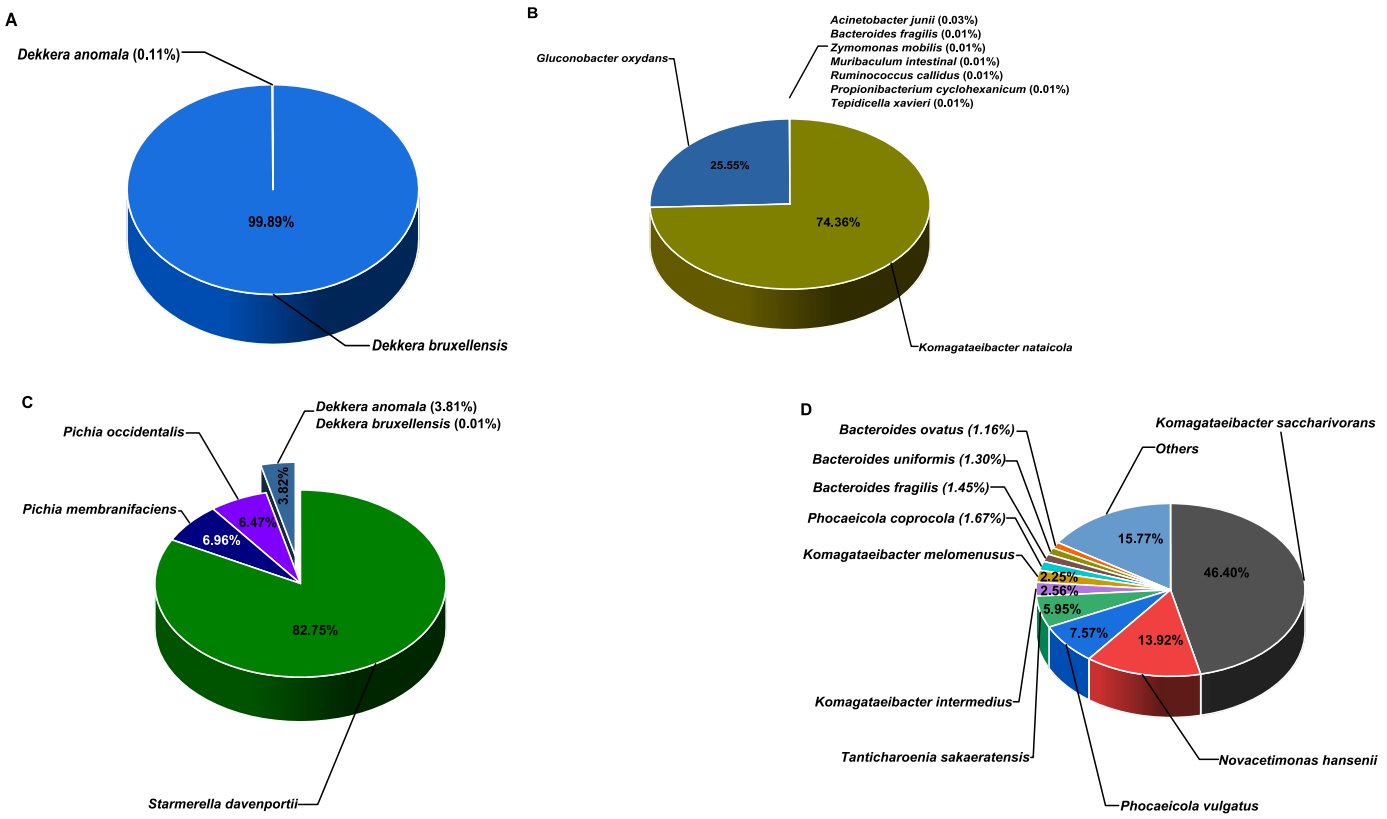

Fig. 1. Analysis of microbial composition of yeast in black tea kombucha (A), bacteria in black tea kombucha (B), yeast in coffee kombucha (C), and bacteria in coffee kombucha (D) using next-generation sequencing of the 16S rRNA V3–V4 region and the rDNA internal transcribed spacer (ITS) region.

D. anomala and *D. bruxellensis*, which comprised 17.25% (Fig. 1C). *S. davenportii* is a non-*Saccharomyces* yeast and is associated with increases in polyphenol content and antioxidant activity in fermented tea beverages (Xiao et al., 2025). It also contributes to the generation of organic acids and aroma components that enhance the flavour and functional quality of fermented tea beverages (Tu et al., 2020). *Komagataeibacter saccharivorans* (46.40%) was the dominant bacterial species in coffee kombucha, followed by *Novacetimonas hansenii* (13.92%), *Phocaeicola vulgatus* (7.57%), *Tanticharoenia sakaeratensis* (5.95%), *Komagataeibacter melomenusus* (2.56%), *Komagataeibacter intermedius* (2.25%), *Phocaeicola coprocola* (1.67%), *Bacteroides fragilis* (1.45%), *Bacteroides uniformis* (1.30%), *Bacteroides ovatus* (1.16%), and other species (15.77%) (Fig. 1D), which are listed in Table S1. Meng et al. (2024) reported that differences in bacterial and yeast communities could lead to the production of different volatile flavour compounds during fermentation. Therefore, both black tea kombucha and coffee kombucha were used for fermentation of coffee in this study.

3.2. Biochemical characterisation of fermented coffee

3.2.1. pH and titratable acidity of fermented coffee

Coffee without kombucha inoculation had an initial pH of 5.75, which decreased to 5.45 and 5.32 after inoculation with black tea kombucha and coffee kombucha broth, respectively (Fig. 2A). After 24 h of fermentation, the pH decreased to 4.94 for K-coffee and 4.96 for CK-coffee. There were no significant differences in pH between K-coffee and CK-coffee after 6, 12, 18, and 24 h of fermentation (Fig. 2A). Although the initial pH after kombucha inoculation was slightly higher for K-coffee than CK-coffee, there was no significant difference between them after 24 h of fermentation. The organic acids produced during kombucha fermentation affected the microbial activity. They reduced the pH (Kim et al., 2023), which contributed to the reduction in the pH of coffee after inoculation with kombucha broth, whereas the buffer capacity of fermented coffee prevented further changes in pH (Wang et al., 2022).

The TA of coffee was 9.37 ± 0.32 mL 0.1 M NaOH/g coffee, which increased to 14.47 ± 0.03 mL 0.1 M NaOH/g coffee and 16.30 ± 0.09 mL 0.1 M NaOH/g coffee after inoculation with black tea kombucha and coffee kombucha broth, respectively (Fig. 2B). Although there was no significant difference in pH between K-coffee and CK-coffee after 6, 12, 18 and 24 h of fermentation, the TA of K-coffee and CK-coffee increased with fermentation time. The TA of K-coffee was increased by 1.76-fold after 24 h of fermentation. There was no significant difference in the TA of K-coffee among 12, 18, and 24 h of fermentation (Fig. 2B). The TA of CK-coffee was increased by 1.64-fold after 24 h of fermentation. The acids in coffee can be categorised into two types: organic acids and chlorogenic acids (Yeager et al., 2023). Therefore, the increase in TA in fermented coffee was possibly caused by the increasing acid content after fermentation.

3.2.2. Total phenolic and total flavonoid contents in fermented coffee

The TPC of coffee was increased by 1.34- and 1.44-fold after inoculation with black tea kombucha and coffee kombucha broth, respectively (Fig. 3A). After 24 h of fermentation, the TPC of K-coffee and CK-coffee showed increases of 1.32- and 1.36-fold, respectively, compared to 0 h of fermentation (Fig. 3A). The TPC of K-coffee (6.94 ± 0.06 mg GAE/g coffee) was highest after 18 h of fermentation, while CK-coffee showed the highest TPC after 24 h of fermentation (7.54 ± 0.13 mg GAE/g coffee). K-coffee and CK-coffee showed increases of 1.91- and 2.01-fold in TPC, respectively, compared to coffee control. However, there were no significant differences in the TPC after 6, 12, and 18 h of fermentation for K-coffee and after 12, 18, and 24 h of fermentation for CK-coffee (Fig. 3A). Although there was no significant difference in the TPC between K-coffee and CK-coffee after inoculation with kombucha, CK-coffee had a 1.17-fold higher TPC after 24 h fermentation (Fig. 3A).

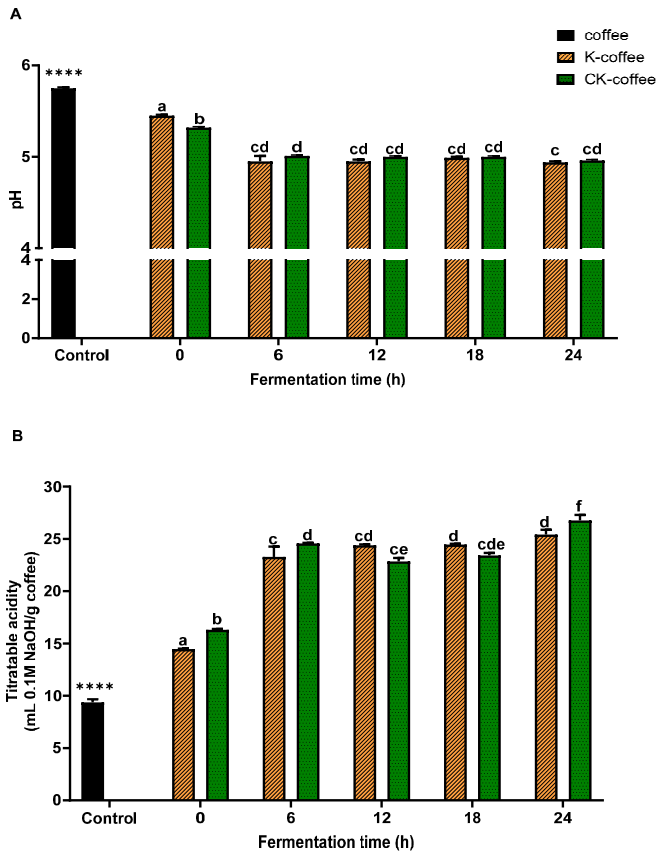

Fig. 2. pH value (A) and titratable acidity (B) of fermented coffee using black tea kombucha and coffee kombucha. Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests was used to compare the effect of time on pH and TA, whereas ANOVA with Dunnett's multiple comparisons test was used to distinguish the level of significance based on probability of $p < 0.05$(*), 0.01(**), 0.001(***), and <0.0001(****) of fermented coffee compared to coffee control. Different superscripts represent the significant difference with a confidence level of 0.05 ($p < 0.05$).

The TFC of coffee increased by 1.42- and 1.58-fold after inoculation with black tea kombucha and coffee kombucha broth, respectively (Fig. 3B). After 24 h of fermentation, the TFC of K-coffee and CK-coffee showed increases of 1.37- and 1.64-fold, respectively, compared to 0 h of fermentation (Fig. 3B). Both K-coffee and CK-coffee showed the highest TFC after 24 h of fermentation. Compared to coffee control, the TFC of K-coffee and CK-coffee increased by 1.95- and 2.60-fold, respectively, after 24 h of fermentation. There were no significant differences in the TFC after 0, 6, 12, and 18 h of fermentation for K-coffee and 0, 6, and 12 h of fermentation for CK-coffee (Fig. 3B). Although there was no significant difference in TFC between K-coffee and CK-coffee after 0, 6, and 12 h fermentation, CK-coffee had a 1.36- and 1.34-fold higher TFC than K-coffee after 18- and 24 h fermentation. Studies have shown that the metabolic activities of microorganisms can affect the TPC and TFC during fermentation (Kim et al., 2023; Wang et al., 2020). Similar results were reported for coffee fermented with *Saccharomyces* spp., in which the TPC and TFC were increased by 1.29 – 1.51-fold and 1.14 – 1.36-fold, respectively (Kwak et al., 2018). In another study, Rochín-Medina et al. (2018) reported that the TPC and TFC of spent coffee grounds were increased by solid-state fermentation using *Bacillus clausii*. These results suggested that the increases in TPC and TFC of coffee after fermentation were due to the release of bound or conjugated forms of phenolic compounds in coffee by enzymes producing during fermentation (Kwak et al., 2018; Rochín-Medina et al., 2018).

3.2.3. Analysis of trigonelline, caffeine, chlorogenic acid and caffeic acid in fermented coffee

Coffee is characterised by caffeine, trigonelline, chlorogenic and caffeic acid. Trigonelline is an alkaloid that comprises about 1% of the dry weight of the beans, and has been linked to hypoglycaemic effects, neuroprotection and anti-cancer properties (Ashihara et al., 2015). The trigonelline content in coffee was increased by 1.31- and 1.45-fold after inoculation with black tea kombucha and coffee kombucha broth, respectively, and increased with fermentation time (Fig. 3C). The trigonelline contents of K-coffee increased after 6 h of fermentation, remained steady until 12 h of fermentation, and ultimately reached 10.11 ± 0.37 mg/g coffee after 24 h of fermentation. However, there was no significant difference in the trigonelline content of K-coffee between 18- and 24 h of fermentation (Fig. 3C). The trigonelline contents of CK-coffee increased after 6 h of fermentation, remained steady until 18 h of fermentation, and ultimately reached 10.37 ± 0.27 mg/g coffee after 24 h of fermentation. However, there was no significant difference in trigonelline content of CK-coffee between 18 and 24 h of fermentation (Fig. 3C).

The caffeic acid content in coffee was increased by 1.69- and 1.81-fold after inoculation with black tea kombucha and coffee kombucha broth, respectively. The caffeic acid level in K-coffee increased after 6 h of fermentation and remained stable until 24 h of fermentation (Fig. 3D). The caffeic acid level in CK-coffee also increased after 6 h of fermentation, remained stable until 12 h of fermentation, and then increased to 1.91 ± 0.01 μg/g coffee after 24 h of fermentation (Fig. 3D).

The bioactive compound caffeine (1,3,7-trimethylxanthine) is a natural alkaloid found in the seeds and leaves of various plants. As caffeine is heat resistant, roasting coffee beans does not cause its degradation. It acts as a competitive inhibitor of adenosine receptors, blocking drowsiness and promoting brain arousal by facilitating dopamine secretion (Costenla et al., 2010). However, excessive caffeine intake can lead to adverse effects, including sleep disruption, nervousness, dizziness and palpitations. Korea's Ministry of Food and Drug Safety has set a daily caffeine intake limit of 400 mg for adults. In this study, we found that the caffeine content in K-coffee remained relatively stable throughout the fermentation process, fluctuating minimally from the initial concentration of 14.66 ± 0.29 mg/g to 14.82 ± 0.23 mg/g after 24 h of fermentation (Fig. 3E). This stability suggested that either the microbial community in K-coffee did not significantly metabolise caffeine or that the fermentation conditions used were not conducive to significant caffeine transformation. In contrast, CK-coffee peaked in caffeine concentration with a 1.33-fold increase after 12 h of fermentation before declining slightly, indicating a dynamic interaction between the coffee components and the microbial enzymes that may be involved in transient caffeine conversion processes. Here, the comparison between the two types of fermented coffee exhibited distinct patterns of phenolic transformation influenced by their respective microbial communities. Chlorogenic acid, a phenolic compound accounting for 4–9% of coffee, decomposes during roasting, breaking down into caffeic and quinic acids. As a potent antioxidant, chlorogenic acid has anti-inflammatory effects and blood pressure-lowering properties (Naveed et al., 2018). The level of chlorogenic acid in coffee was increased by 1.66- and 1.68-fold after inoculation with black tea kombucha and coffee kombucha broth, respectively, and then increased with fermentation time. The highest chlorogenic acid content was 24.91 ± 0.25 mg/g coffee for K-coffee and 27.09 ± 0.36 mg/g coffee for CK-coffee after 24 h of fermentation, representing increases of 2.82- and 3.15-fold, respectively, relative to coffee control. There were no significant differences in chlorogenic acid content between 18 and 24 h of fermentation for either K-coffee or CK-coffee (Fig. 3F). The concentration of chlorogenic acid and ferulic acid in coffee was increased after fermentation could be due to yeast, acetic acid bacteria and lactic acid bacteria in kombucha could produce β-glucosidase and cinnamoyl esterase during fermentation (Campos et al., 2009; Kheir et al., 2013; Kuo et al., 2018), which particularly contributed to release phenolic

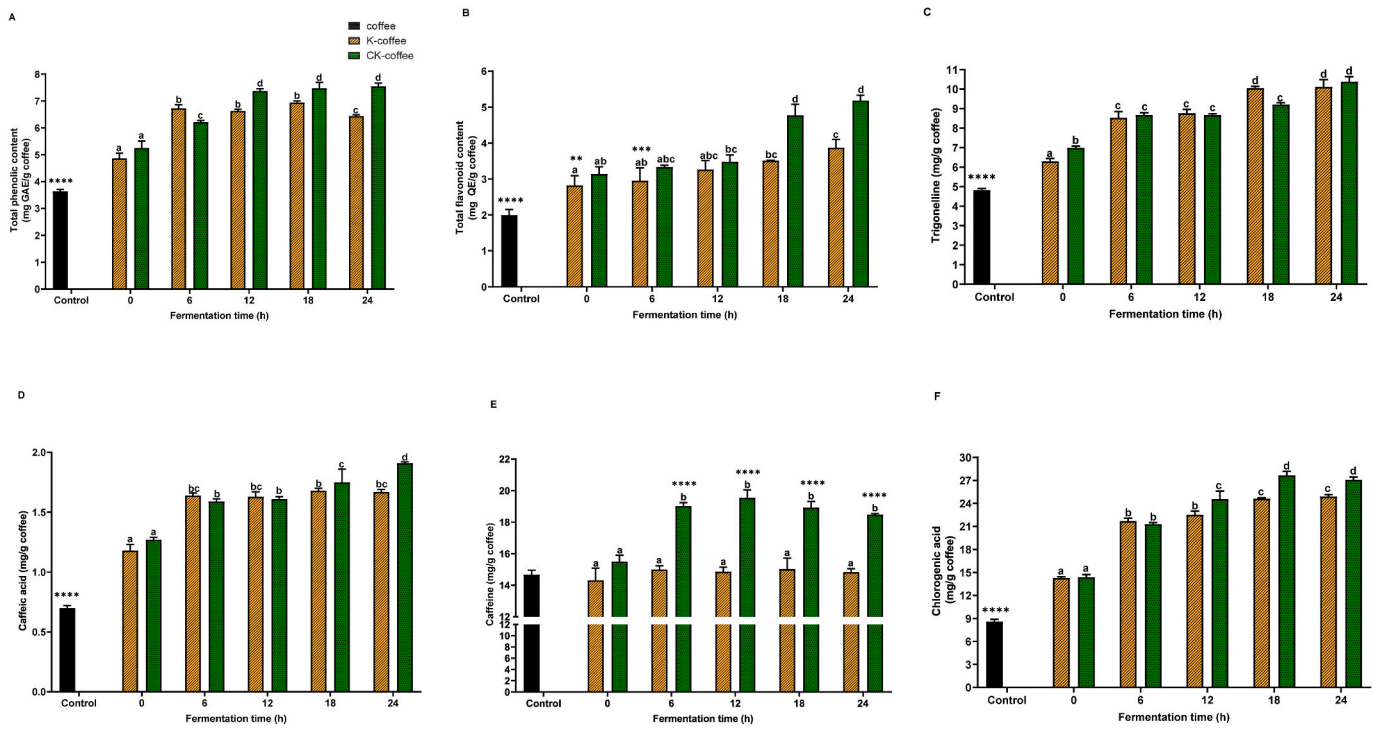

Fig. 3. Total phenolic content (A), total flavonoid content (B), trigonelline (C), caffeic acid (D) and caffeine (E), and chlorogenic acid (F) contents of coffee, K-coffee, and CK-coffee. Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests was used to compare the effect of time on TPC, TFC, trigonelline, caffeic acid, caffeine, and chlorogenic acid, whereas ANOVA with Dunnett's multiple comparisons test was used to distinguish the level of significance based on probability of $p < 0.05$(*), 0.01(**), 0.001(***), and <0.0001(****) of fermented coffee compared to coffee control. Different superscripts represent the significant difference with a confidence level 0.05 ($p < 0.05$).

compounds bound in the cell wall, raising their concentration after fermentation (Arancibia-Díaz et al., 2023)

3.2.4. *Antioxidant ability of fermented coffee*

The effects of microorganisms in kombucha on the antioxidant activities of coffee determined via ORAC, FRAP and DPPH scavenging activities are shown in Fig. 4. In ORAC tests measuring the antioxidant capacity based on single electron transfer, K-coffee significantly increased over fermentation time. Starting from 353.81 ± 10.28 mM TE/g coffee, the values rose steadily, reaching 762.02 ± 34.26 mM TE/g coffee after 24 h, representing an increase of 2.15-fold. However, there was no significant difference in ORAC values of K-coffee after 12, 18 and 24 h of fermentation. The ORAC value of CK-coffee increased with increasing fermentation, it showed a 2.24-fold increase, with values increasing from 504.38 ± 19.72 mM TE/g coffee to 791.68 ± 28.47 mM TE/g coffee at 24 h (Fig. 4A). This trend suggested that the antioxidant components in the coffee are much more effective at neutralising free radicals post-fermentation.

The FRAP assays, which assess the ability of antioxidants to donate a hydrogen atom, showed that K-coffee reached its peak antioxidant capacity of 1009.64 ± 5.35 mM Fe^{2+}/g coffee after 18 h of fermentation, representing an increase of 2.07- and 1.54-fold compared to coffee and K-coffee at 0 h fermentation (Fig. 4B). However, there was no significant difference in FRAP values of K-coffee after 18 and 24 h of fermentation. CK-coffee also showed an increase in antioxidant capacity after 6 h of fermentation, remained steady until 18 h of fermentation, and ultimately reached 1001.11 ± 0.97 mM Fe^{2+}/g coffee at 24 h (Fig. 4B). The changes in FRAP values over the fermentation period underscored the increased capacity of the coffee to reduce oxidants, enhancing its potential health benefits.

The results of DPPH assays were particularly intriguing, as they measured the reduction of DPPH radicals by both single electron and hydrogen atom transfer mechanisms. The results showed a general decline in SC_{50} over time for both coffee types, indicating improved free radical scavenging efficiency. The values for K-coffee and CK-coffee decreased to 137.67 ± 0.35 μg/mL and 130.89 ± 2.20 μg/mL, respectively, at 18 h, then it slightly increased to 158.69 ± 2.08 μg/mL and 137.53 ± 0.89 μg/mL, respectively, at 24 h (Fig. 4C). The SC_{50} of K-coffee and CK-coffee expressed 1.52- and 1.60-fold higher than that of coffee. This reduction in SC_{50} highlighted the enhanced antioxidant capacity, likely due to increased concentrations of polyphenols and the interaction of their hydroxy groups with reactive oxygen species. These results corroborated the findings reported previously that there are appreciable post-fermentation increases in the total polyphenol and flavonoid contents, primarily responsible for the observed increases in antioxidant capacity (Yan et al., 2023).

3.3. *α-Glucosidase inhibition activity of fermented coffee*

α-Glucosidases play a crucial role in the final step of carbohydrate digestion. Inhibition of α-glucosidases prevents the decomposition of disaccharides into monosaccharides, thus reducing postprandial blood sugar levels. A previous study showed that coffee extract suppressed α-glucosidase activity (Alongi and Anese, 2018). Therefore, the α-glucosidase inhibitory effects of coffee, K-coffee and CK-coffee were analysed and are shown as the percentage inhibition relative to the control in Fig. 4D. The α-glucosidase inhibitory activity of coffee was increased from 32.93 ± 0.74% to 36.71 ± 1.68% and 40.09 ± 2.21% by inoculation with black tea kombucha and coffee kombucha broth, respectively (Fig. 4D). The α-glucosidase inhibitory activity of K-coffee was increased by 1.96-fold and 1.76-fold compared to the α-glucosidase inhibitory activity of coffee and K-coffee at 0 h of fermentation and then decreased slightly after 24 h of fermentation (Fig. 4D). The highest α-glucosidase inhibitory activity of K-coffee was observed after 18 h of fermentation. There were no significant differences in the α-glucosidase inhibitory activity of K-coffee after 6, 12, 18, and 24 h of fermentation

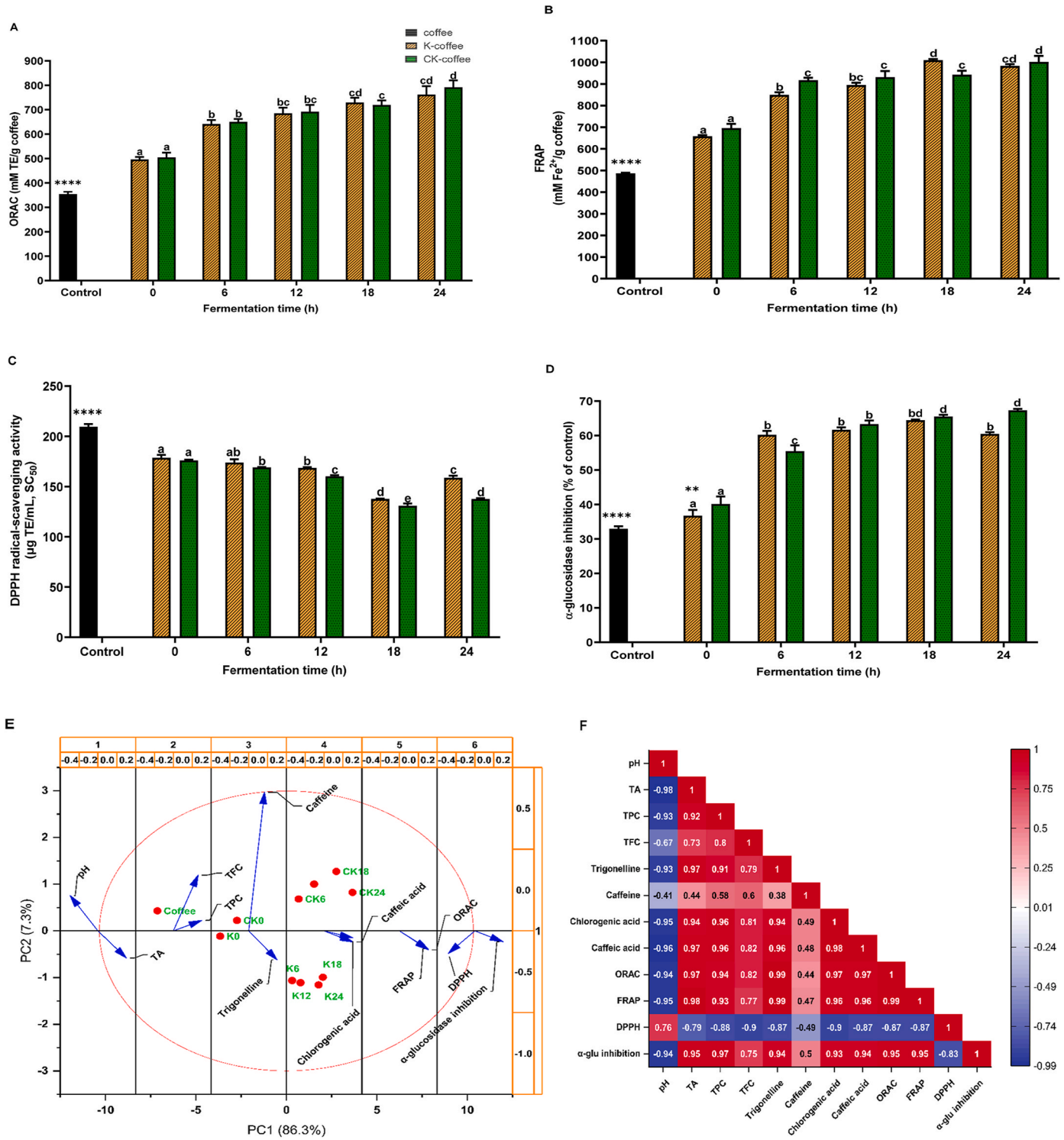

Fig. 4. Antioxidant properties by ORAC (A), FRAP (B), DPPH (C), α-glucosidase inhibitory activity (D), and principal component analysis (PCA) (E) and triangle heat map of Pearson correlation coefficient (F) of physicochemical compounds, antioxidant, and α-glucosidase inhibitory activity of coffee, K-coffee, and CK-coffee. Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests was used to compare the effect of time on antioxidant activities such as ORAC, FRAP, DPPH, and α-glucosidase, whereas ANOVA with Dunnett's multiple comparisons test was used to distinguish the level of significance based on probability of $p < 0.05$(*), 0.01(**), 0.001(***), and <0.0001(****) of fermented coffee compared to coffee control. Different superscripts represent the significant difference with a confidence level 0.05 ($p < 0.05$).

(Fig. 4D). The α-glucosidase inhibitory activity of CK-coffee was increased by 2.04-fold and 1.68-fold compared to the α-glucosidase inhibitory activity of coffee and CK-coffee at 0 h of fermentation. Although the highest α-glucosidase inhibitory activity of CK-coffee was observed after 24 h of fermentation, there was no significant difference in the α-glucosidase inhibitory activity of CK-coffee between 18 and 24 h of fermentation (Fig. 4D). Moreover, the α-glucosidase inhibitory activity of CK-coffee was 1.11-fold higher than that of K-coffee. Studies have shown that phenolic compounds, such as caffeic acid, trigonelline, chlorogenic acid and catechins, can inhibit α-glucosidase activity

(Hamden et al., 2013; Oboh et al., 2015). Therefore, the increases in TPC, TFC and bioactive compound contents of coffee, including trigonelline, caffeic acid and chlorogenic acid in fermented coffee, were related to increases in the α-glucosidase inhibitory activity of coffee, K-coffee and CK-coffee.

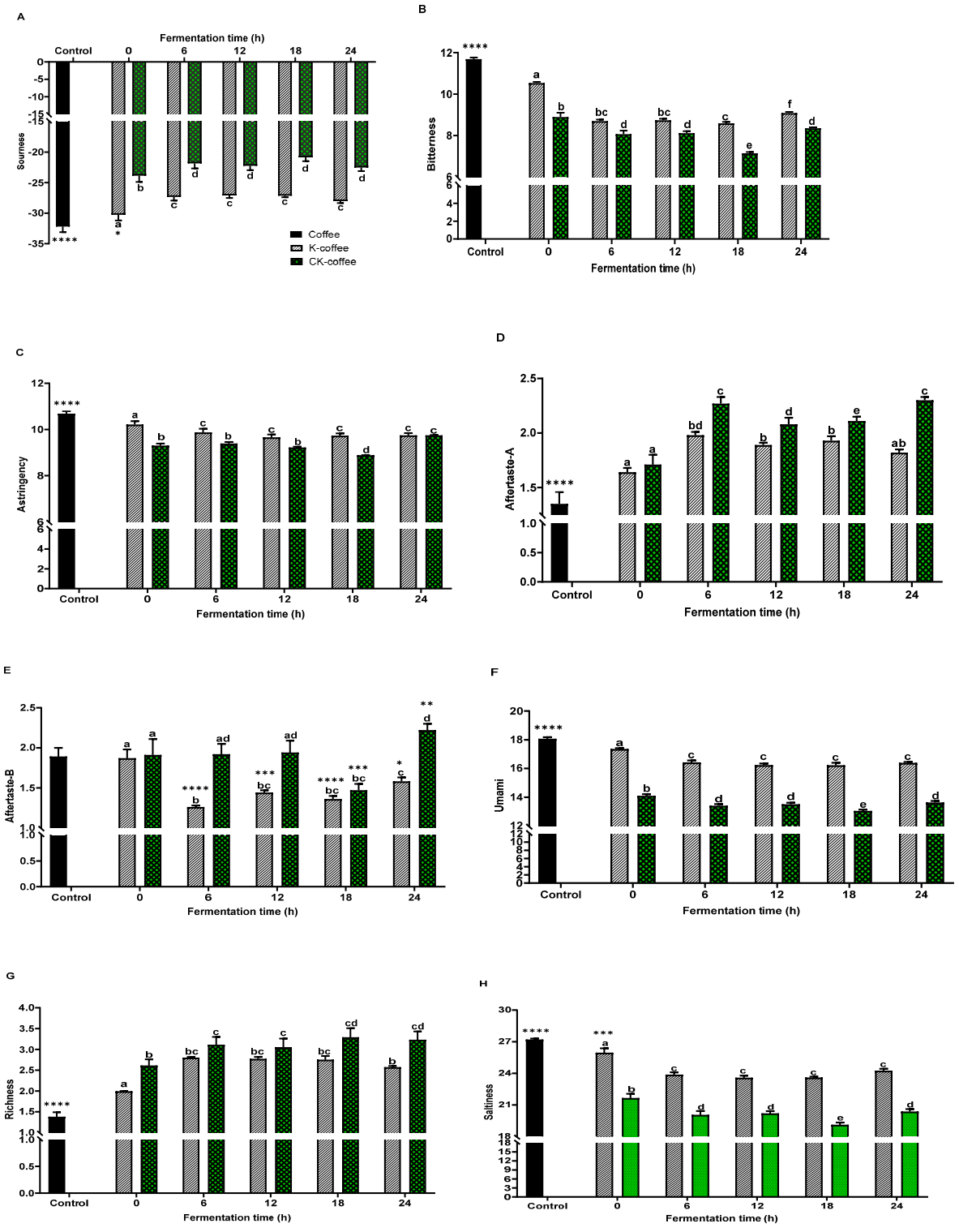

Fig. 5. Taste sensory evaluation of fermented coffee using kombucha by TS-5000Z taste sensing system, including sourness (A), bitterness (B), astringency (C), aftertaste-A (D), aftertaste-B (E), umami (F), richness (G), and saltiness (H). Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests was used to compare the effect of time on sensory taste, whereas ANOVA with Dunnett's multiple comparisons test was used to distinguish the level of significance based on probability of $p < 0.05$(*), 0.01(**), 0.001(***), and <0.0001(****) of fermented coffee compared to coffee control. Different superscripts represent the significant difference with a confidence level 0.05 ($p < 0.05$).

3.4. The relationship among fermentation time, phytochemical components, antioxidant properties, and inhibitory activity against α-glucosidase of fermented coffee

The effects of fermentation time of K-coffee and CK-coffee on pH, TA, TPC, TFC, trigonelline, chlorogenic acid, caffeic acid, caffeine, antioxidant properties via ORAC, FRAP, and DPPH radical scavenging activity, and α-glucosidase inhibitory activity were investigated by PCA analysis. Principal component 1 (PC1) and PC2 explained 93.6% of the total variance (Fig. 4E). PC1 explained 86.3% of the variance mainly by TA, TPC, trigonelline, chlorogenic acid, caffeic acid, ORAC, FRAP, and α-glucosidase inhibitory activity with correlation factor ($r > 0.30$) and pH and DPPH with correlation factor ($r > -0.29$) (Fig. 4E). PC2 explained 7.3% of the variance, mainly contributed by TFC and caffeine with the correlation's factor ($r > 0.34$) (Fig. 4E). In addition, a Pearson correlation test was performed to evaluate the relationship between these factors, and the results are shown in Fig. 4F. The pH of fermented coffee is strongly correlated with TA, TPC, trigonelline, chlorogenic acid, and caffeic acid ($r > -0.9$). The TPC of fermented coffee is strongly correlated with trigonelline, chlorogenic acid, and caffeic acid ($r > 0.9$). The antioxidant properties and inhibitory activity against α-glucosidase of fermented coffee by ORAC and FRAP assay showed a high correlation ($r > 0.93$) with TPC and the main components of coffee, including trigonelline, chlorogenic acid, and caffeic acid (Fig. 4F), while the antioxidant activities of fermented coffee by DPPH radical scavenging activity correlated strongly ($r = 0.9$) with TFC and chlorogenic acid (Fig. 4F).

3.5. Sensory evaluation of fermented coffee

To evaluate the taste characteristics of coffee, K-coffee and CK-coffee, we used an electronic tongue sensor system (TS-5000Z; Insent Inc.) (Fig. 5). The most pronounced change for K-coffee was observed in its sourness, which increased noticeably, shifting from -32.18 ± 0.94 to -27.32 ± 0.42 within 12 h (Fig. 5A). However, there was no significant difference in sourness of K-coffee after 6, 12, 18, and 24 h of fermentation. This increase in sourness was likely due to the accumulation of organic acids produced via microbial activity during fermentation. Conversely, bitterness and astringency in K-coffee decreased, reaching values of 8.59 ± 0.07 and 9.66 ± 0.12, after 18 h of fermentation (Fig. 5B and C). These decreases may have been attributable to the enzymatic breakdown of bitter and astringent compounds, such as chlorogenic acids and tannins, during fermentation. In addition, the aftertaste profiles of K-coffee evolved, with aftertaste-A peaking at 1.98 ± 0.03 at 6 h and aftertaste-B decreasing to 1.36 ± 0.04 at 18 h of fermentation (Fig. 5D and E). These shifts suggested a transformation in the persistence of flavour compounds, potentially enhancing the overall sensory appeal of the coffee. However, there was no significant difference in aftertaste of K-coffee after 6, 12, 18, and 24 h of fermentation. K-coffee showed a significant reduction in the umami intensity, registering a value of 16.22 ± 0.18 by 18 h, possibly due to the degradation of glutamate-like compounds. However, there were no significant differences in the umami intensity values of K-coffee after 6, 12, 18, and 24 h of fermentation (Fig. 5F). In contrast, richness increased, reaching 2.80 ± 0.02 at 6 h, indicating enhancement of flavour complexity. Saltiness declined substantially, aligning with the reduced perception of sodium or other minerals (Fig. 5G).

CK-coffee exhibited different patterns, with sourness peaking later at -20.85 ± 0.63 at 18 h of fermentation, suggesting a slower rate of acid production (Fig. 5A). Bitterness and astringency decreased similarly, showing minimum values of 7.15 ± 0.06 and 8.88 ± 0.01 after 18 h of fermentation, respectively, indicating the development of a milder flavour profile over time (Fig. 5B and C). The aftertaste A metrics for bitterness and astringency in CK-coffee increased, peaking at 2.22 ± 0.08 at 24 h, reflecting delayed release or sustained presence of these flavour components (Fig. 5D), while the aftertaste-B was reduced to 1.47 ± 0.08 after 18 h of fermentation (Fig. 5E). The diminished intensity of umami in CK-coffee to 13.04 ± 0.10 by 18 h of fermentation further suggested alterations in umami-related compounds (Fig. 5F), while an increase in richness to 3.29 ± 0.22 highlighted an enhancement of the depth of flavour (Fig. 5G). The substantial decrease in saltiness to 19.20 ± 0.23 at 18 h of fermentation was consistent with the overall trends observed in K-coffee, albeit at different rates and intensities (Fig. 5H). Kobayashi et al. (2010) compared sensory taste and human sensory scores; they found that a sample with a difference of 1 unit could be distinguished by human senses. The results indicated that the taste-sensory properties of coffee were modified by fermentation using kombucha microbiota. The distinct sensory changes in K-coffee and CK-coffee after fermentation underscored the impact of specific microbial communities and fermentation conditions on the flavour attributes of coffee. This enhanced understanding of flavour profile transformation can guide the optimisation of fermentation processes to produce coffee products with tailored sensory qualities. Based on the sensory results, volatile compounds were analysed in K-coffee and CK-coffee after 18 h of fermentation.

3.6. Volatile compounds in fermented coffee

This study was performed to investigate the effects of fermentation using kombucha on the aroma compounds of coffee. Twenty-six compounds belonging to nine groups (acetates, $n = 2$; alcohols, $n = 1$; ethers, $n = 1$; aldehydes, $n = 4$; ketones, $n = 4$; furans, $n = 2$; pyrazines, $n = 7$; pyridine, $n = 1$; terpenes, $n = 3$) were detected in coffee control, while twenty-five compounds belonging to nine groups (acetates, $n = 1$; alcohols, $n = 2$; ethers, $n = 1$; acids, $n = 1$; aldehydes, $n = 5$; ketones, $n = 4$; furans, $n = 2$; pyrazines, $n = 7$; terpenes, $n = 2$) were detected in K-coffee, and twenty-three compounds belonging to ten groups (acetates, $n = 1$; alcohols, $n = 2$; ethers, $n = 1$; acids, $n = 1$; aldehydes, $n = 5$; ketones, $n = 3$; furans, $n = 2$; pyrazines, $n = 4$; pyridine, $n = 1$; terpenes, $n = 3$) in CK-coffee (Table 1). The concentrations of volatile compounds in coffee, K-coffee and CK-coffee were calculated as percentages of total volatile compounds. Pyrazine were the most abundant compounds (37.04%), followed by ketone (25.73%) and acetate (15.32%) in coffee control (Table 1). In K-coffee, aldehydes (31.97%) were the most abundant compounds, followed by pyrazine (22.38%) and alcohol (10.65%), whereas aldehydes (34.83%) were the most abundant compounds, followed by alcohol (11.94%) and pyrazine (10.99%) (Table 1).

In the alcohol group, 2-furanmethanol, characterised by a sweet, creamy, and vanilla aroma, was increased from $6.94 \pm 0.06\%$ in coffee control to $8.73 \pm 0.01\%$ in K-coffee and $9.87 \pm 0.05\%$ in CK-coffee (Liu et al., 2021). Ethylene oxide, characterised by a sweet, ether-like odor, was decreased from $2.57 \pm 0.02\%$ in coffee control to $1.69 \pm 0.44\%$ and $1.20 \pm 0.08\%$ in K-coffee and CK-coffe respectively (Lin et al., 2007). Acetic acid, responsible for the sour, salty, and vinegar aroma of coffee, was not detected in coffee control but was present in K-coffee and CK-coffee at $9.94 \pm 0.50\%$ and $11.90 \pm 0.70\%$, respectively (Yeager et al., 2023). Aldehydes, characterised by chocolate and malty aromas, were detected in K and CK-coffee at relatively high concentrations (Zhai et al., 2022). The total aldehyde content in coffee control was 4.44% in contrast to 31.97% in K-coffee and 34.83% in CK-coffee, with 2-furancarboxaldehyde being the most dominant volatile compound at $13.79 \pm 1.34\%$ in K-coffee and $16.56 \pm 0.95\%$ in CK-coffee. These increases in aldehyde contents emphasised their important roles in shaping the aroma profile of coffee fermented using kombucha. In the ketone group, 2,3-pentanedione, characterised by buttery flavour, was increased 3.42-fold in K-coffee and 3.25-fold in CK-coffee (Das and Smid, 2019) (Table 1). In the furan group, the content of 2-methyltetrahydrofuran-3-one, characterised by a sweet aroma, was increased from $2.35 \pm 0.34\%$ in coffee control to $4.64 \pm 0.2\%$ in K-coffee and $5.14 \pm 0.21\%$ in CK-coffee (Table 1) (Haile et al., 2020). Pyrazines, nitrogen-containing heterocyclic molecules, are key flavour components in developing

Table 1
Analysis of volatile compounds of coffee after 18 h of fermentation using kombucha.

No	Group	Compound	RT	Volatile compound (of total %)		
				Control	K-coffee (18 h)*	CK-coffee (18 h)**
1	**Acetate**	Methyl acetate	1.984	5.24 ± 0.12[a]		
2		Acetol acetate	5.395	5.61 ± 0.64[a]	8.02 ± 0.28[b]	7.86 ± 0.95[b]
3	**Alcohol**	2-Furanmethanol	5.271	6.94 ± 0.06[a]	8.73 ± 0.01[b]	9.87 ± 0.05[c]
4		1-(2-furanyl)-ethanol	6.058	–	1.92 ± 0.01[a]	2.07 ± 0.06[b]
5	**Ester**	Acetic acid, methyl ester	1.984	4.47 ± 0.12	–	–
6	**Ether**	Ethylene oxide	1.722	2.57 ± 0.02[a]	1.69 ± 0.44[b]	1.20 ± 0.08[b]
7	**Acid**	Acetic acid	2.281	–	9.94 ± 0.50[a]	11.90 ± 0.70[b]
8	**Aldehyde**	3-Mehylbutanal	2.522	1.13 ± 0.04[a]	3.11 ± 0.05[b]	3.26 ± 0.09[b]
9		2- Methylbutanal	2.621	1.96 ± 0.03[a]	5.49 ± 0.24[b]	6.41 ± 0.28[b]
10		5-Methyl-2-furancarboxaldehyde	6.899	0.71 ± 0.06[a]	9.08 ± 0.22[b]	8.21 ± 0.30[c]
11		2-Furancarboxaldehyde	4.91	–	13.79 ± 1.34[a]	16.56 ± 0.95[b]
12		Sorbic aldehyde	8.276	0.64 ± 0.04[a]	0.50 ± 0.03[b]	0.39 ± 0.03[c]
13	**Ketone**	Acetone	1.913	5.62 ± 0.08[a]	3.22 ± 0.14[a]	5.03 ± 0.17[b]
14		2,3-Pentanedione	2.908	0.84 ± 0.08[a]	2.87 ± 0.35[b]	2.73 ± 0.11[b]
15		3,3-Dimethyl-2-butanone	6.731	3.80 ± 0.04[a]	0.61 ± 0.04[b]	
16		Azine	3.519	15.47 ± 0.78[a]	2.52 ± 0.22[b]	2.02 ± 0.10[b]
17	**Furan**	2-Methyltetrahydrofuran-3-one	4.424	2.35 ± 0.34[a]	4.64 ± 0.21[b]	5.14 ± 0.21[b]
18		2,5-Dimethyl-3(2H)-furanone	6.646	0.35 ± 0.06[a]	0.49 ± 0.06[b]	0.49 ± 0.02b
19	**Pyrazine**	2-Methyl pyrazine	4.685	7.32 ± 0.34[a]	3.56 ± 0.01[b]	3.47 ± 0.17[b]
20		2-Ethylpyrazine	6.061	3.86 ± 0.06[a]	9.53 ± 0.34[b]	
21		2,5-Dimethylpyrazine	6.018	3.65 ± 0.31[a]	4.89 ± 0.89[b]	4.91 ± 0.35[b]
22		2,6-Dimethylpyrazine	6.011	13.36 ± 0.13	–	–
23		2-Ethyl-6-methyl pyrazine	7.188	2.83 ± 0.02[a]	1.68 ± 0.1[b]	1.69 ± 0.20[b]
24		2-Ethyl-5-methyl pyrazine	7.229	4.98 ± 0.05[a]	0.97 ± 0.11[b]	0.92 ± 0.01[b]
25		2-Ethyl-3methyl parazine	7.265	–	1.35 ± 0.15	–
26		3-Ethyl-2,5-dimethylparazine	8.174	1.04 ± 0.02[a]	0.40 ± 0.01[b]	
27	**Pyridine**	3-Ethylpyridine	6.702	3.56 ± 0.31[a]		3.57 ± 0.08[a]
28	**Terpene**	β-myrcene	7.065	0.40 ± 0.04[a]		1.28 ± 0.11[b]
29		Limonene	7.568	0.96 ± 0.04[a]	0.60 ± 0.06[b]	0.57 ± 0.01[b]
30		Linalool oxide	8.099	0.34 ± 0.01[a]	0.4 ± 0.01[ab]	0.43 ± 0.04[b]

*K-coffee: coffee fermented with black tea kombucha fermented after 18 h.
**CK-coffee: coffee fermented with coffee kombucha fermented after 18 h.

baked goods. The contents of pyrazines, including 2-methyl pyrazine, 2-ethyl-6-methyl pyrazine, 2-ethyl-5-methyl pyrazine and 3-ethyl-2, 5-dimethylparazine were decreased during fermentation. In contrast, the contents of 2,5-dimethylpyrazine, found in fermented food and characterised by a cocoa flavour, were 4.89% in K-coffee and 4.57% in CK-coffee (Table 1) (Ren et al., 2024). *Bacillus subtilis* and *Lactococcus lactis* can produce 2,5-dimethylpyrazine during fermentation (Lin et al., 2019). In addition, β-myrcene with a mildly sweet flavor profile belonging to the terpene group was increased 3.2-fold in CK coffee (Table 1). The results outlined above suggested that the fermentation process in K-coffee and CK-coffee led to notable changes in contents of specific volatile and non-volatile components, highlighting the transformative impact of microbial activity on the chemical profile of coffee. The study demonstrates that the yeast *Dekkera bruxellensis* and bacteria *Komagataeibacter* spp. in kombucha significantly influence the flavour compounds in fermented coffee, enriching its aroma profile with increased aldehydes and distinct sour notes, which showcases the profound impact of microbial interactions on coffee's sensory qualities. Kombucha fermentation introduces a novel method for coffee processing that may attract both home brewers and commercial producers. At home, enthusiasts can use simple equipment and kombucha cultures to create unique, potentially healthier coffee varieties. Commercially, this method offers a way to differentiate products in the market with distinctive flavours and improved nutritional benefits, appealing to health-conscious consumers. However, before commercial adoption, it's crucial to assess the scalability, maintain consistency in flavour and quality, obtain necessary regulatory approvals, and ensure consumer acceptance of the new flavour profiles.

4. Conclusion

Going beyond traditional single-strain fermentation research, this study explored the complex dynamics of coffee fermentation using kombucha containing a symbiotic culture of bacteria and yeasts. Unlike previous studies focusing solely on antioxidant activities and component analysis, this research involved analysis of the multifaceted impacts of kombucha fermentation under controlled conditions, assessing how different substrates influence microbial community dynamics and coffee biochemistry.

The findings confirmed significant biochemical transformations during the fermentation process. There was a marked decrease in pH and an increase in TA, indicating robust acid production by the microbial community. Analysis by HPLC revealed substantial increases in key coffee compounds, such as trigonelline, chlorogenic acid and caffeic acid—products of chlorogenic acid decomposition. Interestingly, CK-coffee also showed an increase in caffeine level, suggesting substrate-specific microbial interactions. Moreover, the results indicated enhanced antioxidant capacity in the fermented coffee, primarily due to increased TPC and TFC. These compounds significantly bolstered the ability of coffee to inhibit α-glucosidase activity, suggesting potential antidiabetic effects. Analysis of post-fermentation flavour profiles revealed considerable changes, identifying specific substances that were produced, increased or reduced during the fermentation process. Comparative analysis between K-coffee and CK-coffee highlighted distinct differences in component profiles, functionality and taste, underscoring the influence of varying microbial communities within black tea kombucha and coffee kombucha on the fermentation outcomes.

The findings illuminate the intricate relationships between microbial actions and coffee biochemistry. This research underscores the potential of using kombucha-based fermentation to tailor the properties of coffee, offering new avenues for enhancing the flavour, health benefits, and overall quality of coffee. Further exploration to optimize fermentation parameters, microbial consortia, *in vivo*, and/or clinical studies is required to refine and expand the applications of fermented coffee products in the beverage industry. Furthermore, the sensory evaluation

of fermented coffee was conducted using an electronic tongue sensor system, which could not capture the full flavour perception like human sensory panel evaluations. Therefore, the sensory evaluation of fermented coffee by sensory panelists needs further study.

CRediT authorship contribution statement

Hayeong Kim: Conceptualization, Writing – original draft, Writing – review & editing, Methodology, Formal analysis, Validation. **Jihyeon Jeon:** Writing – original draft, Methodology, Formal analysis. **Jiyeon Lee:** Methodology, Data curation. **Chaeri Song:** Methodology, Data curation. **Boncheol Gu:** Methodology, Formal analysis. **Nahyun Mariah Kim:** Methodology, Validation. **Tae-hui Yang:** Resources, Investigation, Methodology. **Sejin Oh:** Funding acquisition, Validation. **Soochul Park:** Formal analysis, Validation. **Kunal Pal:** Methodology, Validation. **Ghahyun Jeffrey Kim:** Writing – review & editing, Validation. **Doman Kim:** Project administration, Funding acquisition, Investigation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.100996.

Data availability

Data will be made available on request.

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