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Alternative plant infusions as substrates for kombucha production: fermentation performance and functional properties

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ABSTRACT

Kombucha is traditionally produced by fermenting sweetened *Camellia sinensis* tea with a symbiotic culture of bacteria and yeasts. Growing interest in alternative plant-based substrates has created new opportunities for the development of kombucha-type beverages with distinct physicochemical and *in vitro* bioactive characteristics. This study investigated kombucha-type beverages produced from organic sobacha (buckwheat seed infusion), raspberry leaves, dandelion leaves, and nettle leaves, and compared them with a conventional black/green tea kombucha.

Fermentation performance was evaluated through the monitoring of pH evolution, SCOBY biomass production, and metabolite profiling, including carbohydrates, ethanol, and organic acids. In addition, total vitamin C, phenolic and flavonoid contents, antioxidant capacity (DPPH and FRAP assays), and cyclooxygenase-2 (COX-2) inhibitory activity were assessed throughout fermentation.

Apart from nettle leaf infusion, all substrates successfully supported kombucha fermentation, although significant differences in fermentation performance, physicochemical characteristics, and functional properties were observed. Black/green tea kombucha exhibited the highest antioxidant capacity and *in vitro* COX-2 inhibitory activity. Raspberry and dandelion leaf kombuchas showed intermediate antioxidant and COX-2 inhibitory responses while supporting efficient fermentation, whereas sobacha showed comparatively low results for these assays. Nettle-based kombucha developed atypical physicochemical characteristics that limited its suitability under the tested conditions.

Overall, the results demonstrate that botanical substrate selection is a key determinant of kombucha fermentation behaviour, physicochemical characteristics, and *in vitro* bioactive properties. Raspberry and dandelion leaf infusions emerged as promising alternatives to traditional tea, highlighting the potential of locally sourced plant materials for the development of innovative fermented beverages.

ABBREVIATIONS

AA	Ascorbic acid
AAB	Acetic acid bacteria
COX-2	Cyclooxygenase-2
DHAA	Dehydroascorbic acid
DPPH	2,2-diphenyl 1-picrylhydrazyl
DTT	Dithiothreitol
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
LAB	Lactic acid bacteria
MPA	Metaphosphoric acid
QE	Quercetin equivalents
SCOBY	Symbiotic culture of bacteria and yeast
TFC	Total flavonoid content
TPC	Total phenolic content
TPTZ	2,4,6-tripyridyl-S-triazine
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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1. Introduction

Fermentation is a widely used bioprocess that has historically contributed to food preservation and safety, while also improving the nutritional, sensory and physicochemical properties. Among fermented beverages, kombucha – originating from East Asia – has experienced a marked increase in global consumption in recent years. Traditionally, kombucha is obtained by fermenting sweetened black tea with a symbiotic consortium of bacteria and yeasts (SCOBY), which drives a series of biochemical transformations. During fermentation, microbial metabolism converts sugars and tea constituents into a complex mixture of organic acids, polyphenols, flavonoids, vitamins and other compounds that may contribute to the *in vitro* bioactive properties often reported for kombucha compared with non-fermented tea (de Noronha et al., 2022; Liang et al., 2024).

Kombucha consumption has been associated with potential health-related effects, although the available evidence is primarily derived from *in vitro* and preclinical studies and has been summarized in several reviews (Esatbeyoglu et al., 2024; Kapp & Sumner, 2019). The reported effects are often related to antioxidant and other bioactive properties of phenolic acids, flavonoids, and organic acids, which may be modified during fermentation (Teixeira Oliveira et al., 2023). However, evidence remains largely limited to experimental models, and further cell-based, *in vivo*, and clinical studies are required to establish physiological relevance and substantiate potential health effects (Kapp & Sumner, 2019; Morales, 2020).

Recent research has explored alternative fermentation substrates, such as plant infusions (e.g., rooibos, chamomile and sobacha), fruit juices, coconut water, vegetables, and medicinal plant extracts, aiming to diversify the nutritional and functional properties of kombucha (Bortolomedi et al., 2022; Liu et al., 2022; Suffys, Goffin, et al., 2023). Substrate choice strongly influences chemical composition, flavour, and bioactive properties. Herbal teas are often caffeine-free and may provide additional phytochemicals, whereas fruit juices offer sugars that enhance fermentation and supply vitamins, minerals, and antioxidants. Nevertheless, plant infusions can have lower or different phenolic profiles than tea, affecting microbial activity, while fruit-based matrices may be more prone to microbial contamination, requiring strict quality control. Traditional black and green teas remain the most used substrates due to their high catechin and theaflavin content, which serve as microbial substrates and are transformed into derivatives shaping antioxidant potential (Liu et al., 2022; Morales et al., 2023; Suffys, Goffin, et al., 2023).

Using local, organic, plant-based infusions offers additional advantages. Organic ingredients reduce synthetic pesticide and fertilizer use, potentially supporting SCOBY growth and meeting consumer expectations (Bortolomedi et al., 2022). Local plants such as raspberry leaves (*Rubus idaeus* L.), dandelion leaves (*Taraxacum officinale* Weber), nettle leaves (*Urtica dioica* L.), and buckwheat seeds (*Fagopyrum esculentum* Moench) provide phytochemicals, including antioxidants and flavonoids. While these compounds have been investigated for potential biological activities, evidence is derived from traditional use or experimental studies rather than clinical research. Locally sourced plant materials may contribute to more sustainable agricultural systems and support product differentiation for consumers seeking environmental or cultural value (Enthoven & Van den Broeck, 2021). For example, sobacha is a caffeine-free beverage traditionally consumed in Japan, containing rutin and minerals such as magnesium and zinc, with suggested antioxidant and anti-inflammatory activity and a slightly sweet, nutty flavour (Guo et al., 2017; Suffys, Goffin, et al., 2023). Raspberry, dandelion, and nettle infusions are traditionally used in Europe and North America for digestive or reproductive support or general well-being and contain tannins, vitamins, flavonoids, and other phytochemicals, though clinical evidence in humans is limited (Bhusal et al., 2022; Bowman et al., 2021; Buricova et al., 2011; Lis & Olas, 2019; Niu et al., 2022).

In summary, using these infusions as fermentation substrates may diversify the chemical composition and *in vitro* bioactive properties of kombucha-type beverages. Their distinct phytochemical profiles and sensory characteristics make them promising candidates for the development of innovative fermented beverages. However, their adoption requires optimization of fermentation parameters to ensure product consistency, microbial safety, and sensory acceptability. While substrate diversity presents opportunities for innovation, it also poses challenges related to standardization, sensory acceptability, and quality assurance (Jayabalan et al., 2014). The diversification of kombucha substrates is not only relevant from a functional perspective but also represents an opportunity to valorise locally available plant resources and develop innovative fermented beverages responding to consumer demand for sustainable and naturally functional foods.

Although alternative kombucha substrates are increasingly investigated, comparative studies integrating fermentation kinetics, major metabolites, global phytochemical markers, and potential bioactive activity remain limited, particularly for locally sourced plant infusions. This raises the question of to what extent the botanical nature of the substrate influences fermentation dynamics and the formation or transformation of compounds associated with *in vitro* antioxidant capacity and COX-2 inhibitory activity of kombucha.

In this context, this study aimed to evaluate the suitability of organic plant-based infusions, namely buckwheat seeds, raspberry leaves, dandelion leaves and nettle leaves, as alternative substrates for kombucha production. These formulations were compared with a conventional kombucha prepared from black and green tea. Fermentation performance was assessed through the characterization of pH evolution, SCOBY biomass production, and the formation of major metabolites, including carbohydrates, ethanol and organic acids. In addition, the *in vitro* bioactive properties of the resulting beverages were evaluated through the determination of total vitamin C, total phenolic and flavonoid contents, antioxidant activity (DPPH and FRAP assays), and cyclooxygenase-2 (COX-2) inhibition. By integrating fermentation behaviour with physicochemical and functional characteristics, this study provides a comparative assessment of how alternative botanical substrates influence kombucha production and highlights their potential for the development of diversified fermented beverages.

2. Materials and methods

2.1. Materials and chemicals

The Chun Mee Moon Palace green tea and Lien Son black tea leaves (*Camellia sinensis* (L.) Kuntze) used in the fermentation starter (obtained from a previous kombucha batch) were sourced from Zhejiang, China and Yen Bai, Vietnam, respectively, and were certified organically cultivated. Kasha seeds for the sobacha infusion were sourced from Shizuoka, Japan. The three herbal materials – raspberry leaves, dandelion leaves, and nettle leaves – were obtained from L'Oasis des Saveurs (Eghezée, Belgium) and certified organic. Sucrose used for fermentation was refined white beet sugar from Tirlemont Sugar Refinery (Tirlemont, Belgium). The inoculum consisted of a SCOBY obtained from Fairment (Berlin, Germany), a cellulose-based biofilm previously maintained in kombucha.

Standards used for external calibration (sucrose, glucose, fructose, ethanol, acetic and lactic acids, $FeSO_4{\cdot}7H_2O$, gallic acid, quercetin, ascorbic acid (AA) and dehydroascorbic acid (DHAA)) were purchased from Merck (Darmstadt, Germany). Sulfuric acid, DPPH, TPTZ, $FeCl_3{\cdot}6H_2O$, HCl, methanol, Folin-Ciocalteu reagent, sodium carbonate, aluminium chloride, potassium acetate, metaphosphoric acid (MPA), and dithiothreitol (DTT) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). A fluorometric COX-2 inhibitor screening kit (ab283401, Abcam, Cambridge, UK), containing recombinant human COX-2, arachidonic acid, OxiRed probe and celecoxib as positive control, was used for the evaluation of *in vitro* COX-2 inhibition assay.

2.2. Preparation and sampling of kombucha-type beverages

Tea infusion was prepared by steeping Chun Mee Moon Palace green tea and Lien Son black tea leaves (1:1) at a final concentration of 8 g/L (w/v) in mineral water preheated to 75 °C. Sobacha solution was prepared by infusing kasha (10 g/L) in mineral water preheated to 92 °C. Raspberry, dandelion, and nettle leaf infusions were prepared at 10 g/L in mineral water preheated to 75 °C. After 10 min of infusion (except 20 min for sobacha), solutions (1 L each, in triplicate) were filtered and sweetened with white beet sugar to 40 g/L. Once cooled to ~35 °C, all infusions were inoculated under identical starter conditions with a fresh SCOBY pellicle (0.5% w/v) and liquid kombucha starter (10% v/v), both obtained from the same maintained culture. The inoculation conditions were kept constant across all experimental treatments. Glass jars (1.5 L total volume) were covered with gauze secured by an elastic band to maintain aerobic conditions and incubated for 14 days at 25 °C in an oven (Memmert UF55, Schwabach, Germany) (Suffys, Goffin, et al., 2023; Suffys, Richard, et al., 2023). Sampling was performed on days 0, 2, 4, 7, 9, 11, and 14 (D0 – D14). Aliquots (10 mL) were collected and stored at −80 °C for subsequent analyses. Prior to all analytical determinations, samples were thawed, centrifuged (1 mL, 14,000 × g, 10 min, 25 °C; Eppendorf Mini Spin, Eppendorf, Hamburg, Germany), and filtered through 0.22 μm nylon syringe filters.

The reference formulation and fermentation conditions were based on our previously established protocol (Suffys, Richard, et al., 2023).

Kombucha samples prepared from nettle leaf infusion were excluded from *in vitro* functional potential analyses due to the development of atypical physicochemical properties during fermentation (e.g., excessive turbidity and unstable texture), which compromised sample consistency and analytical reliability.

2.3. Dynamical analysis of the physico-chemical parameters of fermented beverages

pH and concentrations of the main fermentation metabolites (sucrose, glucose, fructose, ethanol, acetic acid and lactic acid) were monitored throughout fermentation. The pH was measured directly in the fermentation medium using a pH meter (SensION + pH1, Hach Company, Loveland, CO, USA). At the end of fermentation, SCOBY biofilms were collected and their fresh and dry weights were determined following the method proposed by (Suffys, Goffin, et al., 2023). Briefly, pellicles were separated from the inoculum, gently blotted to remove surface moisture, weighed, and then dried at 65 °C for 4 h (Memmert IPP55plus, Memmert GmbH + Co.KG, Schwabach, Germany) before determination of dry mass using an analytical balance (Sartorius BC-EB, Sartorius Lab Instruments, Göttingen, Germany).

Carbohydrates (sucrose, glucose and fructose), ethanol and organic acids (acetic and lactic acids) were quantified by HPLC using an Agilent 1200 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump (G1311A), an autosampler (G1329A), a thermostatic column compartment (G1316B) and a multiple wavelength detector (G1365D) coupled to a refractive index detector (G1362A). Chromatographic separations were performed in isocratic mode using Aminex HPX-87H and HPX-87C columns (300 × 7.8 mm; Bio-Rad Laboratories, Hercules, CA, USA) equipped with Micro-Guard Carbo-C cartridges (30 × 4.6 mm; Bio-Rad Laboratories, Hercules, CA, USA). Ultrapure water was used as the mobile phase for carbohydrate analysis, while 5 mM H_2SO_4 was used for ethanol and organic acid determination. The flow rate was set at 0.6 mL/min, the injection volume was 10 μL, and column temperatures were maintained at 50 °C and 85 °C for the HPX-87H and HPX-87C columns, respectively. Detection was performed at 210 nm using the multiple wavelength detector, while the refractive index detector was maintained at 30 °C. Compounds were quantified by external calibration using appropriate standards (Suffys, Goffin, et al., 2023).

2.4. Assessment of in vitro functional potential of the fermented beverages

2.4.1. Total vitamin C content

Total vitamin C content was determined at days 0, 7, and 14 of fermentation by quantifying ascorbic acid (AA) and dehydroascorbic acid (DHAA) using HPLC.

For AA determination, 400 μL of sample were mixed with 200 μL of metaphosphoric acid (MPA) solution to stabilise ascorbic acid and precipitate interfering compounds. The mixture was sonicated for 10 min in an ultrasonic bath (MT5800, Branson Ultrasonics, Brookfield, CT, USA) while maintaining the bath temperature with ice to prevent overheating, diluted with ultrapure water to a final volume of 800 μL, and centrifuged for 5 min at 10,000 × g at 4 °C (5424R, Eppendorf, Hamburg, Germany) prior to analysis.

Total vitamin C was determined after reduction of DHAA to AA using dithiothreitol (DTT). Briefly, 400 μL of sample were mixed with 200 μL of DTT solution (40 mg w/v in 10 mL Tris buffer). The reaction was stopped by adding 200 μL of MPA, followed by sonication and centrifugation under the same conditions as described above.

Chromatographic analyses were performed using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump (G1311A), an autosampler (G1329A), and a diode-array detector (G1315D). Separation was achieved on a reversed-phase C18 column (Merck Lichrocart RP-18, 250 × 4.6 mm, 5 μm; Merck, Darmstadt, Germany). A 20 μL aliquot of extract was injected and eluted isocratically with ortho-phosphoric acid (0.022% w/v, pH 2) at a flow rate of 0.7 mL/min, with detection at 254 and 225 nm.

Total vitamin C corresponded to the amount of AA measured after reduction of DHAA. DHAA content was calculated by difference between total vitamin C and native AA concentrations. This approach is widely used for the determination of vitamin C in food matrices due to its reliability and ability to account for both reduced and oxidised forms. Quantification was performed using external calibration with ascorbic acid standard (Nováková et al., 2008).

2.4.2. Total phenolic and flavonoid contents

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, as previously described, with minor modifications (Mfopa et al., 2024). Briefly, 20 μL of each sample or standard were added to 100 μL of diluted Folin-Ciocalteu reagent (1:10, v/v). Then, 80 μL of aqueous sodium carbonate solution (75 g/L) were added to each well. After incubation at room temperature, absorbance was measured at 700 nm, in end-point mode (SpectraMax ABS Plus, Molecular Devices, San José, CA, USA). External calibration was performed using gallic acid. TPC values were expressed as mg of gallic acid equivalent per 100 mL of kombucha (mg GAE/100 mL).

Total flavonoid content (TFC) were determined using the aluminium chloride colorimetric method (Mfopa et al., 2024). Briefly, 30 μL of sample or standard were introduced into a 96-well microplate, followed by 168 μL of distilled water. Then, 90 μL of MeOH, 6 μL of aluminium chloride solution (10%, w/v) and 6 μL of potassium acetate solution (1 M) were added to each well. The mixture was incubated for 30 min at room temperature, under constant shaking, in the dark. Absorbance was measured at 420 nm (SpectraMax ABS Plus, Molecular Devices, San José, CA, USA). Quercetin was used as a calibration standard. TFC values were expressed as mg quercetin equivalents per 100 mL of kombucha (mg QE/100 mL).

2.4.3. Antioxidant capacity

2.4.3.1. DPPH inhibition assay. Antioxidant capacity was determined using the inhibition of DPPH radical scavenging assay, following previously described methods with minor modifications (Su & Silva, 2006). First, a DPPH methanolic solution (2.5 mg/L) was prepared by ultrasonication (Sonorex, Bandelin, Berlin, Germany) for 15 min at room

temperature. In a 96-well microplate, 20 μL of sample or standard were added. Then, 200 μL of MeOH and 200 μL of DPPH methanolic solution were added. Absorbance of all wells was read at 517 nm using a microplate reader (SpectraMax ABS Plus, Molecular Devices, San José, CA, USA). Trolox was used as the reference antioxidant standard. The blank contained MeOH instead of DPPH-methanol solution, while the control contained DPPH-methanol solution without the sample. The scavenging activity was calculated by using the following equation:

$$Scavenging\ activity\ (\%) = 1 - \left(\frac{Abs_{Sample} - Abs_{Blank}}{Abs_{Control}}\right) \times 100$$

Results were expressed as the scavenging activity (%) which reflects the ability of the samples to neutralize free radicals by donating electrons or hydrogen atoms.

2.4.3.2. FRAP assay. The FRAP assay was performed according to previously described methods, with minor modifications (Amjadi et al., 2023). Briefly, the FRAP working solution (10:1:1, v/v) was prepared by mixing 10 mL of acetate buffer (300 mM, pH 3.6), 1 ml of TPTZ solution (10 mM in 40 mM HCl), and 1 mL of ferric chloride 20 mM. The mixture was warmed to 37 °C before use. In a 96-well microplate, 10 μL of sample or standard were added to each well, followed by 220 μL of FRAP working solution. After incubation for 4 min at room temperature under constant shaking, absorbance was read at 593 nm (SpectraMax ABS Plus, Molecular Devices, San José, CA, USA). A ferrous sulphate solution (1 mM) was used to generate the calibration curve. Results were expressed as FRAP values (μM Fe^{2+}/mL of kombucha).

2.4.4. COX-2 inhibition assay

The assay was performed at days 0, 7, and 14 of fermentation. The COX-2 inhibitor screening assay was carried out in accordance with the manufacturers' instructions. Samples were first diluted to the appropriate concentration in COX assay buffer to obtain a final working concentration corresponding to 1× in the reaction mixture. Enzyme control (EC, without inhibitor), sample wells (S), and inhibitor control (IC, celecoxib) were prepared in parallel. Briefly, 10 μL of diluted samples or assay buffer (EC) were added to each well, while 2 μL of celecoxib were used as a positive control.

The reaction was initiated by adding a reaction master mix containing COX assay buffer, OxiRed probe, cofactor and recombinant human COX-2 enzyme, followed by arachidonic acid substrate. Fluorescence was measured using a microplate reader (Tecan Spark, Tecan Group, Männedorf, Switzerland) at excitation/emission wavelengths of 535/587 nm in kinetic mode every 30 s at 25 °C for 30 min. Background fluorescence was corrected by subtracting initial signal values, and reaction rates were calculated from the slope of fluorescence increase within the linear range.

Relative inhibition was calculated as:

$$Relative\ inhibition\ (\%) = \frac{Slope\ of\ EC - Slope\ of\ S}{Slope\ of\ EC} \times 100$$

2.5. Statistical analysis

All fermentations were performed in biological triplicate ($n = 3$). For the health-related assays, analytical determinations were carried out in technical duplicate ($n = 2$), and the mean of the two technical replicates was used as the representative value. Data were expressed as mean ± standard deviation of three independent biological replicates ($n = 3$).

Basic data processing was performed using GraphPad Prism (8.0.1 software, GraphPad software Corporation, San Diego, USA) and Microsoft office Excel (Excel 2021 software, Microsoft Corporation, Redmond, USA).

The effects of fermentation time and infusion type on physico-chemical parameters and *in vitro* bioactive properties were analysed using a two-way repeated-measures analysis of variance (ANOVA), with time and infusion type as fixed factors and biological replicate as a random factor ($n = 3$; $\alpha = 0.05$). When only one factor was considered (e.g., comparison between infusion types at a given time point), a one-way ANOVA was performed ($n = 3$; $\alpha = 0.05$). When significant main effects or interactions were detected, Tukey's multiple comparisons test was applied for post hoc pairwise comparisons. Statistical significance was set at $p < 0.05$.

For the *in vitro* bioactive parameters, fermentation-associated changes were calculated for each biological replicate relative to its corresponding day-0 value (Δ = value at time t − value at day 0). Baseline-subtracted values were used for statistical analyses and graphical representation. The complete dataset, including raw values for all biological replicates and detailed statistical analyses, is provided in the Supplementary Material (Table S1–S3).

3. Results and discussion

3.1. Dynamical analysis of the physico-chemical parameters of fermented beverages

The decrease in pH (Fig. 1A) and the SCOBY biomass production (Fig. 1B) was both influenced by the type of plant infusion used. Raw data and full statistical outputs are provided in the Supplementary Material (Table S1–S3).

In all formulations, pH decreased rapidly during the first four days of fermentation and then stabilized around 3.0 – 3.5 by day 14, which is consistent with typical kombucha fermentation patterns (Suffys, Goffin, et al., 2023; Suffys, Richard, et al., 2023). A two-way repeated-measures ANOVA revealed significant effects of fermentation time ($p < 0.05$) and infusion ($p < 0.05$), as well as a significant interaction between both factors ($p < 0.05$). These findings indicate that both fermentation duration and plant matrix affect kombucha acidity. Post hoc Tukey's tests showed that pH values differed significantly between several infusion types, particularly at the early stages: raspberry and dandelion leaves supported faster acidification, sobacha exhibited a slower pH decline, whereas black/green tea and nettle displayed intermediate profiles. Multiple reviews and experimental studies reported a fast drop in pH during the first days of kombucha fermentation followed by stabilization at low pH values, a pattern driven by organic-acid production and commonly observed across tea and plant substrates (Bishop et al., 2022; Villarreal-Soto et al., 2018).

SCOBY biomass production was significantly affected by infusion type ($p < 0.05$). Tukey's post hoc tests indicated that dandelion leaf infusion yielded significantly higher SCOBY biomass than black/green tea for both fresh weight ($p < 0.05$) and dry weight ($p < 0.05$). Nettle infusion also showed higher dry biomass than black/green tea ($p < 0.05$), although this result should be interpreted with caution due to its atypical physicochemical behaviour. In contrast, raspberry leaf and sobacha infusions generated lower biomass values compared with tea-based kombucha. These results indicate that rapid acidification is not directly associated with increased cellulose biosynthesis. Instead, culture conditions and infusion composition, particularly phenolic content and nutrient availability, may influence bacterial cellulose production, as previously reported for kombucha-associated acetic acid bacteria, including strains belonging to the genus *Komagataeibacter*. (Chen et al., 2025). However, the presence and relative abundance of this genus were not determined in the present study. Although traditional black/green tea media are often reported to support efficient cellulose production, the present results highlight that certain plant substrates, such as dandelion leaves, may also promote substantial biomass formation depending on their composition (Ramírez Tapias et al., 2022).

Fresh and dry biomass of the SCOBY pellicle produced during fermentation provided complementary information on cellulose production during kombucha fermentation. Fresh weight reflected the overall pellicle mass, including variable amounts of water retained

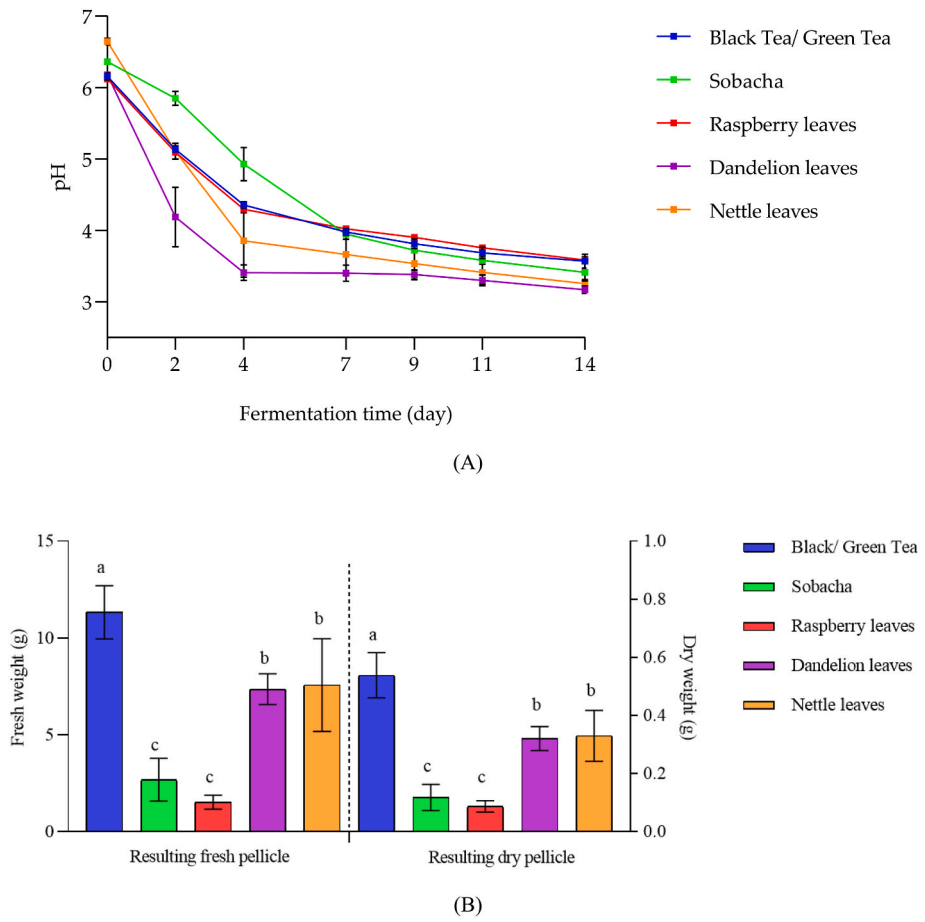


Fig. 1. Characterization of the fermentation process of kombucha based on alternative infusions (A) pH evolution of the formulated beverages as a function of fermentation time ($n = 3$, error bar = SD) and (B) resulting SCOBY production (fresh weight and dry weight (g)) of the newly formed SCOBY pellicle collected at day 14 ($n = 3$, error bar = SD). Bars with different letters are significantly different ($p < 0.05$) according to one-way ANOVA followed by Tukey's multiple comparison test.

within the biofilm, whereas dry weight offered a more accurate estimate of actual cellulose synthesis. This indicates that although hydration affects the apparent pellicle mass, the trends in cellulose accumulation remain robust when corrected for water content (Tapias et al., 2023).

Taken together, these results indicate that, although all infusions reached a similar final acidity, their ability to support SCOBY development varied markedly. Thus, the choice of substrate shapes both fermentation kinetics and the SCOBY development, which is likely to influence the sensory profile and potential functional properties of the final beverage.

The metabolic profiles of kombucha fermentation are presented in Fig. 2.

Sucrose, the initial carbon source, decreased progressively throughout fermentation, accompanied by a concomitant increase in glucose and fructose, confirming the activity of yeast-derived invertase (May et al., 2019; Suffys, Richard, et al., 2023). Glucose and fructose profiles showed substrate-dependent changes over fermentation time. Nettle infusion exhibited a pronounced transient increase in glucose during the early stages of fermentation, followed by a marked decrease. This pattern may reflect an initial imbalance between sucrose hydrolysis and monosaccharide utilization, whereby glucose was released faster than it was consumed during the first days of fermentation, before subsequent utilization became predominant. Fructose displayed similar substrate-dependent fluctuations, although its accumulation and consumption patterns differed from those of glucose, suggesting that the relative rates of sucrose hydrolysis and monosaccharide utilization varied according to the botanical matrix. The relatively large standard deviations observed for some metabolites at specific sampling points reflect variability among the three independent biological fermentations and warrant cautious interpretation of these transient changes.

Indeed, two-way repeated-measures ANOVA revealed significant effects of fermentation time, infusion type, and their interaction on carbohydrate levels ($p < 0.05$). Fermentation time strongly influenced sugar depletion, while infusion type modulated both the initial sugar composition and its utilization rate. Based on the temporal profiles of sucrose, glucose, and fructose, kombucha-type beverages prepared with raspberry and dandelion leaf infusions showed faster sucrose depletion and monosaccharide utilization, whereas the sobacha formulation retained higher residual sugar concentrations and showed a slower decrease in pH.

Ethanol concentrations increased transiently during fermentation, reaching a maximum between days 7 and 9 before decreasing due to oxidation by acetic acid bacteria. Ethanol production was significantly affected by fermentation time, infusion type, and their interaction ($p < 0.05$), with fermentation time accounting for most of the variance. Black/green tea and raspberry leaf kombuchas showed the highest ethanol accumulation, whereas sobacha and nettle produced lower amounts. Final ethanol concentrations remained below 1.2% (v/v), the European Union legislation level (1169/2011) compatible with the classification of non-alcoholic beverages.

Contrasting patterns were observed for organic acids. Acetic acid production was significantly influenced by fermentation time, infusion type, and their interaction, with concentrations increasing steadily and reaching the highest levels in dandelion and raspberry leaf beverages,

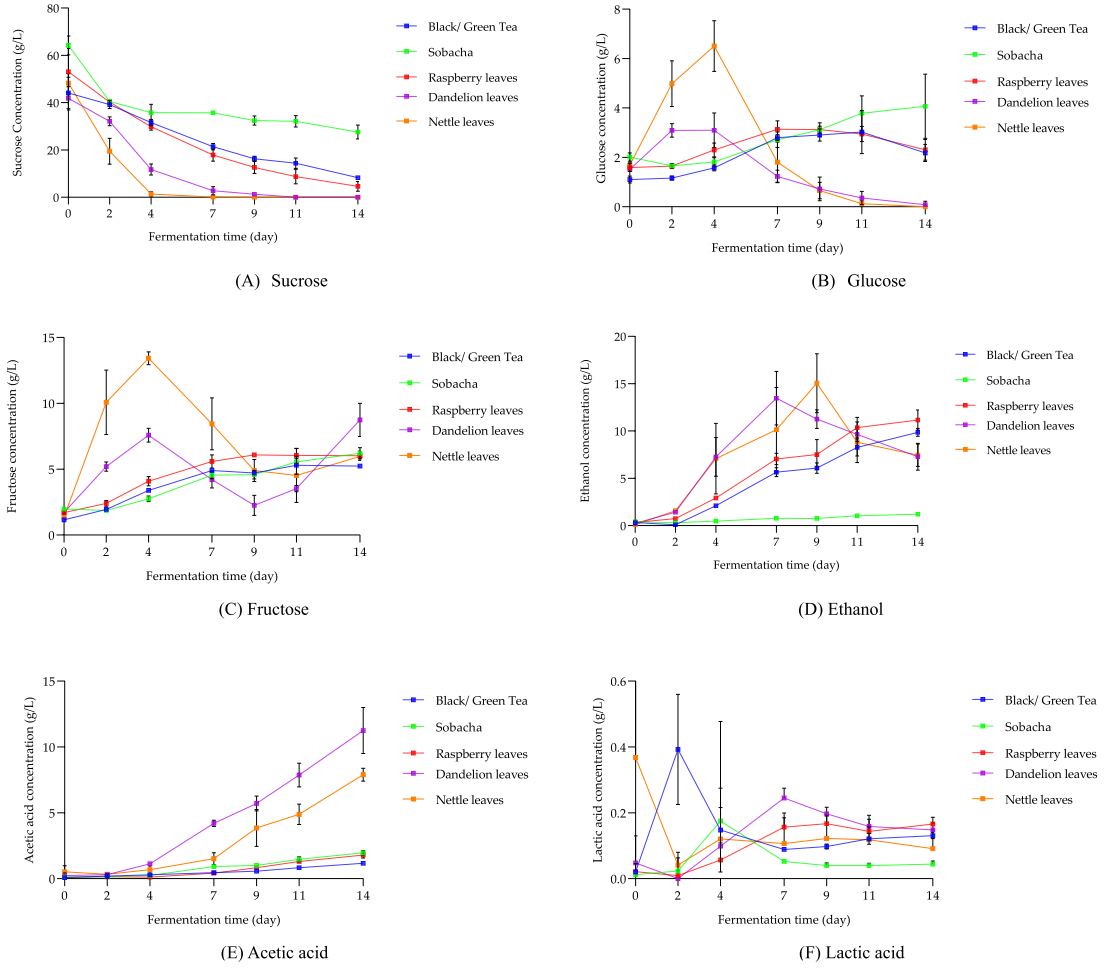


Fig. 2. Changes in the kinetics of major components namely (A) sucrose, (B) Glucose, (C) Fructose, (D) Ethanol, (E) Acetic acid, and (F) Lactic acid synthesized during fermentation (g/L) in function of kombucha beverages based on alternative infusions ($n = 3$, error bar = SD).

while sobacha remained lower.

Lactic acid concentrations remained relatively low throughout fermentation and showed greater variability than acetic acid. Although some herbal infusions tended to exhibit higher lactic acid concentrations than the traditional tea formulation at specific sampling points, neither fermentation time nor infusion type alone had a significant effect. The significant time × infusion interaction nevertheless indicated that lactic acid evolution differed among substrates over the course of fermentation. As microbial populations were not quantified, these differences cannot be directly attributed to changes in LAB abundance or activity and may also reflect substrate-dependent metabolic dynamics within the microbial consortium. Overall, these metabolic profiles are consistent with the complementary activities of yeasts and bacteria in kombucha fermentation: yeasts hydrolyse sucrose and produce ethanol, while acetic acid bacteria oxidize ethanol into acetic acid, driving acidification. The infusion substrate modulates the balance between these processes, thereby influencing carbohydrate consumption dynamics and organic acid production. As microbial populations and cell viability were not quantified in the present study, changes in metabolite production cannot be directly attributed to variations in the abundance or activity of specific microbial groups. Future studies should therefore include enumeration (e.g., CFU determination) and/or molecular characterization of AAB, yeasts, and LAB throughout fermentation.

In contrast to the other substrates, nettle-based kombucha exhibited atypical physicochemical properties, including a rapid and pronounced acidification, marked turbidity, and the formation of an unusual flocculent or gelatinous texture during fermentation, which negatively affected both visual quality and handling. These observations were accompanied by a sharp decrease in clarity and the presence of visible precipitates, suggesting the aggregation of macromolecular components. Such behaviour may be related to the relatively high protein content of nettle leaves, including soluble proteins that can undergo denaturation and precipitation under acidic conditions typically reached during kombucha fermentation. In addition, the specific phenolic composition of nettle and potential interactions between proteins may further promote complex formation and phase separation, thereby altering matrix structure (Kilic & Sengun, 2023; Ozdal et al., 2013; Sanwal et al., 2023).

Although the underlying mechanisms were not directly investigated in this study, these physicochemical alterations may reflect differences in nutrient availability, matrix composition, and microbial accessibility compared with other substrates. Importantly, the combination of excessive turbidity and unstable texture indicated that the resulting product did not meet the expected physical characteristics of kombucha beverages. For these reasons, nettle-based kombucha was considered unsuitable under the tested conditions and was therefore excluded from subsequent analyses.

3.2. *Assessment of in vitro functional potential of the fermented beverages*

3.2.1. *Total vitamin C content*

Total vitamin C content differed markedly among the initial

botanical infusions, reflecting the intrinsic composition. Absolute concentrations and full statistical outputs are provided in the Supplementary Material (Table S2–S3), whereas Fig. 3 presents change relative to the corresponding day-0 values.

Black/green tea kombucha showed the highest vitamin C concentrations (19.8–23.4 mg/L) throughout fermentation, which is consistent with the naturally occurring vitamin C content and antioxidant precursors present in tea leaves. Raspberry leaf kombucha showed intermediate initial values (11.6–12.6 mg/L), suggesting that the fermentation process may facilitate the release or stabilization of ascorbic compounds from the plant matrix. In contrast, sobacha showed initial vitamin C concentrations of 2–3 mg/L, consistent with the relatively low ascorbic acid contribution of buckwheat infusions. Dandelion leaf kombucha showed moderate concentrations of approximately 4–5 mg/L.

When expressed as changes from baseline, the response differed according to the botanical substrate (Fig. 3). Two-way repeated-measures ANOVA revealed a significant effect of infusion type ($p < 0.05$), whereas fermentation time and the time × infusion interaction were not significant ($p > 0.05$). Black/green tea showed a modest increase in total vitamin C relative to baseline, reaching approximately +1.4 mg/L at day 14. Raspberry leaf kombucha exhibited the largest mean increase, from approximately +2.1 mg/L at day 7 to +3.3 mg/L at day 14, although substantial inter-replicate variability was observed. In contrast, sobacha showed a slight decrease relative to baseline at both day 7 and day 14 (approximately −0.8 mg/L), while dandelion leaf kombucha displayed only small positive changes. Tukey's multiple-comparison test showed that the change observed for raspberry leaves was significantly greater than that of sobacha at both day 7 and day 14 ($p < 0.05$), whereas no other pairwise differences were significant.

Overall, total vitamin C content showed only limited variation over the 14-day fermentation across the tested formulations. The acidic environment generated during kombucha fermentation may contribute to limiting oxidative degradation of ascorbic acid, while the increases observed in some substrates may reflect improved extraction, stabilization effects, or limited microbial transformations. However, these mechanisms were not specifically investigated in this study. Previous studies have suggested that microbial activity in fermented systems may contribute to the maintenance of vitamin C rather than significant de novo synthesis (Júnior et al., 2022; Phung et al., 2023).

3.2.2. Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of formulated beverages during fermentation were presented in Fig. 4(A) and (B), respectively, as changes relative to their corresponding day-0 values. Absolute concentrations and full statistical outputs are provided in the Supplementary Material (Table S2–S3).

Total phenolic content (TPC) differed markedly among the initial botanical matrices. Black/green tea kombucha consistently exhibited the highest TPC levels (>2000 mg GAE/100 mL), followed by raspberry and dandelion leaf beverages, which showed intermediate values (1500 – 1800 mg GAE/100 mL). Sobacha displayed substantially lower TPC values throughout fermentation, remaining below 500 mg GAE/100 mL. When expressed as changes from baseline (Fig. 4A), TPC generally increased during fermentation, although the magnitude and temporal pattern of these changes depended on the botanical substrate. Black/green tea showed the largest positive changes, particularly during the early stages of fermentation, while raspberry leaf kombucha also exhibited sustained positive changes. Sobacha showed smaller increases, whereas dandelion leaf kombucha displayed a more variable pattern, with moderate increases during the intermediate stages followed by a decline toward the end of fermentation. These changes may partly reflect modifications in phenolic extractability or the release of bound phenolic compounds during fermentation, although these mechanisms were not directly investigated in the present study.

Two-way repeated-measures ANOVA of baseline-subtracted TPC values revealed a significant interaction between fermentation time and infusion type ($p < 0.05$), as well as a significant effect of infusion type ($p < 0.05$), whereas fermentation time alone was not significant ($p > 0.05$). This interaction indicates that the evolution of TPC during fermentation depended on the botanical substrate. Tukey's multiple-comparison test showed that the increase in TPC was significantly greater in black/green tea kombucha than in dandelion leaf kombucha at day 9 ($p < 0.05$), and significantly greater than in sobacha at days 11 and 14 ($p < 0.05$). No other pairwise differences were significant.

Total flavonoid content (TFC, Fig. 4B) showed a different distribution in terms of absolute concentrations. Raspberry leaf kombucha exhibited the highest TFC values (approximately 200 – 250 mg QE/100 mL), while black/green tea and dandelion leaf beverages generally showed values closer to 100–150 mg QE/100 mL. Sobacha again displayed the lowest flavonoid concentrations, remaining below 50 mg QE/100 mL. However, when expressed as changes relative to baseline, TFC showed only limited fluctuations throughout fermentation, with substantial variability among biological replicates. Raspberry leaf kombucha fluctuated around its initial value, whereas black/green tea and dandelion leaf beverages tended to show modest decreases and sobacha remained comparatively close to baseline. Two-way repeated-measures ANOVA detected no significant effect of fermentation time, infusion type, or their interaction on baseline-subtracted TFC values ($p > 0.05$). Consistently, Tukey's multiple-comparison test revealed no significant pairwise differences between infusions at any fermentation time. Thus, although the botanical matrices differed considerably in their flavonoid

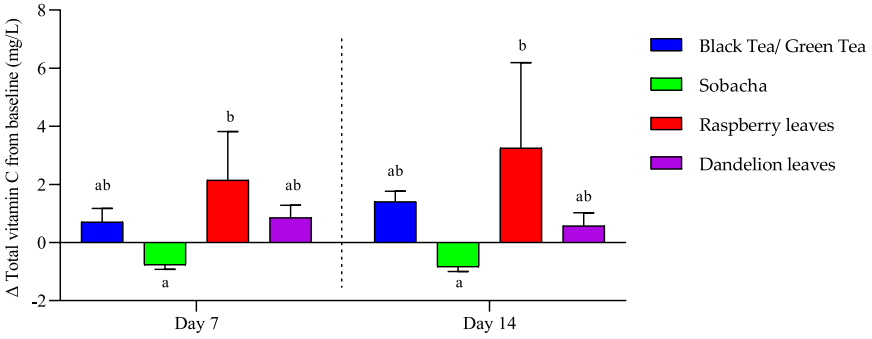
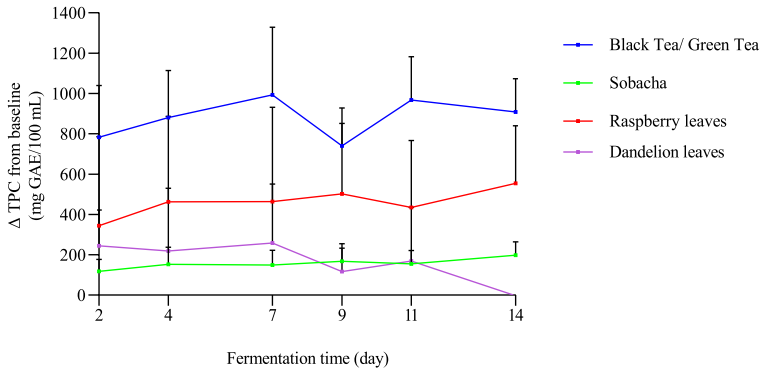
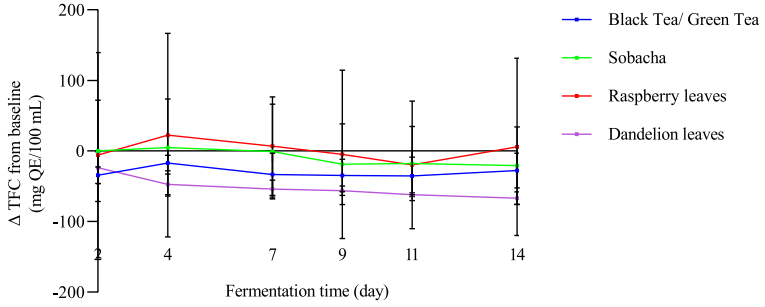


Fig. 3. Changes in total vitamin C content relative to baseline (day 0) in kombucha-type beverages prepared with different plant infusions during fermentation. For each biological replicate, baseline-subtracted values were calculated as Δ = value at each fermentation time point − corresponding value at day 0. Data are expressed as mean ± SD of three independent biological replicates (n = 3). Data were analysed using a two-way repeated-measures ANOVA followed by Tukey's multiple-comparison test. Bars with different letters within the same fermentation time are significantly different ($p < 0.05$).



(A) Total phenolic content



(B) Total flavonoid Content

Fig. 4. Changes in total phenolic and flavonoid contents relative to baseline (day 0) in kombucha-type beverages prepared with different plant infusions over fermentation time: (A) total phenolic content (TPC, expressed as mg gallic acid equivalents (GAE)/100 mL) and (B) total flavonoid content (TFC, expressed as mg quercetin equivalents (QE)/100 mL). For each biological replicate, baseline-subtracted values were calculated as Δ = value at each fermentation time point − corresponding value at day 0. Data are expressed as mean ± SD of three independent biological replicates (n = 3). Data were analysed using a two-way repeated-measures ANOVA followed by Tukey's multiple-comparison test.

contents, fermentation did not induce statistically significant changes in TFC under the tested conditions.

Overall, these results indicate that the botanical substrate strongly influence the initial phenolic and flavonoid composition of the beverages, while the fermentation-associated changes differed between TPC and TFC. TPC evolution was substrate-dependent, whereas TFC remained comparatively stable after accounting for differences in initial composition. This is consistent with previous studies showing that the substrate used for kombucha fermentation strongly influences phenolic and flavonoid profiles (Bortolomedi et al., 2022).

3.2.3. *Antioxidant capacity*

The antioxidant capacity of the kombucha-type beverages assessed using DPPH and FRAP assays, showed distinct fermentation-associated changes depending on the botanical substrate. Absolute antioxidant values and full statistical outputs are provided in the Supplementary Material (Table S2–S3), whereas Fig. 5 presents change relative to the corresponding day-0 values.

In terms of DPPH radical-scavenging activity, all formulations exhibited high activity (>80%) during the early stages of fermentation. Black/green tea and raspberry leaf kombuchas maintained consistently high values (>90%), indicating that fermentation preserved or enhanced their antioxidant potential, in line with previous observations (Morales et al., 2023). Dandelion leaf kombucha showed slightly lower and more variable activity, whereas sobacha displayed a marked decline in DPPH activity after day 2, reaching approximately 50 – 60% by day 14.

When expressed as changes relative to baseline (Fig. 5A), however, distinct substrate-dependent trajectories became apparent. Sobacha showed the largest initial positive change in DPPH radical-scavenging activity at day 2, followed by a progressive decrease toward its initial value by day 14. Black/green tea and raspberry leaf kombuchas exhibited smaller but generally positive changes throughout fermentation, whereas dandelion leaf kombucha showed predominantly negative changes relative to baseline, which became more pronounced toward the end of fermentation. Two-way repeated-measures ANOVA revealed significant effects of fermentation time and infusion type, together with a significant time × infusion interaction ($p < 0.005$). The significant interaction indicates that changes in DPPH radical-scavenging capacity over fermentation depended on the botanical substrate. Tukey's multiple-comparison test revealed several time-specific differences among infusions, particularly involving sobacha during the early stages of fermentation and dandelion leaves at later sampling points. Specifically, at day 2 sobacha differed significantly from black/green tea, raspberry leaves, and dandelion leaves ($p < 0.05$); at day 4, sobacha differed from dandelion leaves ($p < 0.05$); at day 7, dandelion leaves differed from both black/green tea and raspberry leaves ($p < 0.05$); and additional differences involving black/green tea, raspberry leaves, and dandelion leaves were observed at days 9, 11, and 14 ($p < 0.05$). These results indicate that fermentation-associated changes in DPPH radical-scavenging capacity were strongly dependent on both substrate and fermentation stage.

The FRAP assay showed a different pattern (Fig. 5B). In terms of reducing power, black/green tea kombucha exhibited the highest values, reaching more than 5000 μM Fe^{2+}/mL during fermentation, consistent with its comparatively high phenolic content. Raspberry and

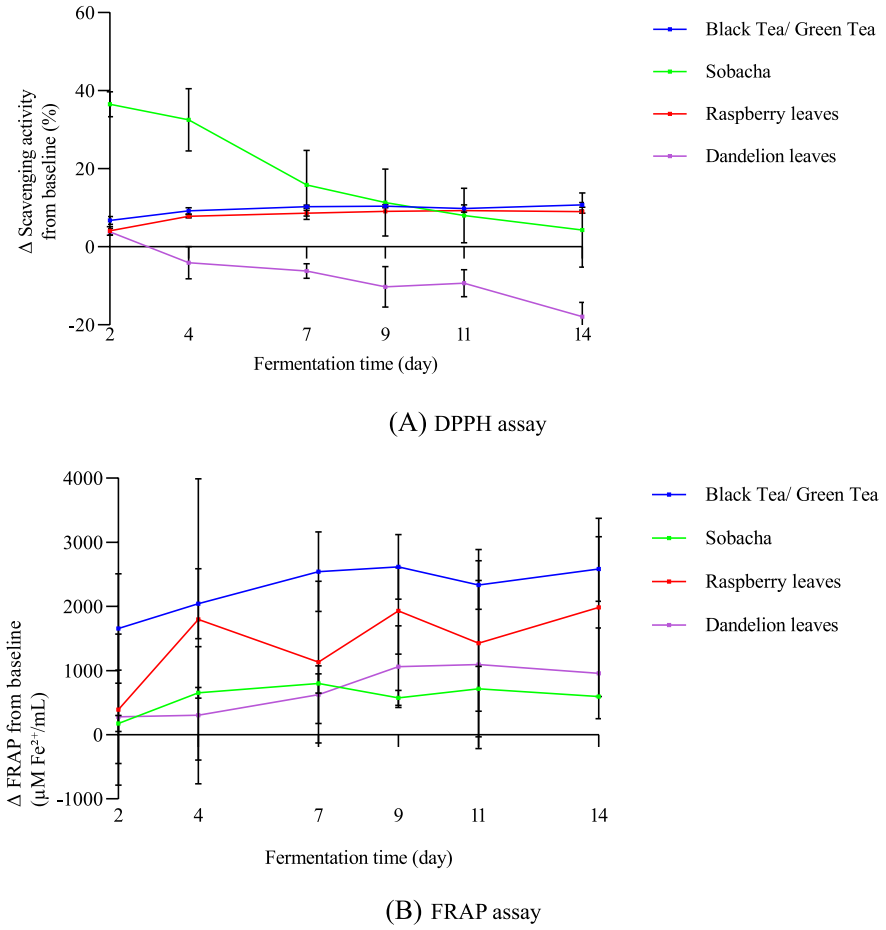


Fig. 5. Changes in antioxidant capacity relative to baseline (day 0) in kombucha-type beverages prepared with different plant infusions over fermentation time: (A) DPPH radical scavenging activity (expressed as % inhibition) and (B) ferric reducing antioxidant power (FRAP, expressed as μM Fe^{2+}/mL). For each biological replicate, baseline-subtracted values were calculated as Δ = value at each fermentation time point − corresponding value at day 0. Data are expressed as mean ± SD of three independent biological replicates (n = 3). Data were analysed using a two-way repeated-measures ANOVA followed by Tukey's multiple-comparison test.

dandelion leaf kombuchas showed intermediate FRAP values, whereas sobacha displayed the lowest reducing power, remaining below 2000 μM Fe^{2+}/mL.

When changes relative to baseline were considered, black/green tea showed the largest overall positive increases in FRAP, while raspberry leaf kombucha also displayed substantial but more variable increases. Dandelion leaves showed moderate positive changes, whereas sobacha generally exhibited smaller increases relative to its initial value. Two-way repeated-measures ANOVA showed a significant effect of fermentation time on baseline-subtracted FRAP values ($p < 0.05$), whereas neither infusion type nor the time × infusion interaction was significant ($p > 0.05$). Overall, FRAP changes were therefore primarily associated with fermentation time rather than with a statistically significant effect of infusion type.

These differences observed between DPPH and FRAP responses may partly reflect the chemical nature and relative proportions of antioxidant compounds present in the beverages. The DPPH assay evaluates radical scavenging through hydrogen atom or electron donation, a mechanism to which flavonoids containing multiple hydroxyl groups can contribute substantially. In contrast, the FRAP assay reflects the ability of sample constituents to reduce Fe^{3+} to Fe^{2+} through electron transfer and can be influenced by the broader pool of phenolic compounds, including catechins, tannins, and phenolic acids. Consequently, measured *in vitro* antioxidant capacity depends not only on the total phenolics or flavonoid content, but also on compound structure, relative abundance, and interaction within the beverage matrix.

The qualitative composition of the different botanical substrates may therefore contribute to the distinct antioxidant profiles observed. Black and green teas contain catechins, including epigallocatechin gallate and epicatechin, as well as theaflavins, and tannins, compounds with strong electron-donating properties that may contribute to the high FRAP values. Raspberry leaves contain flavonoids such as quercetin and kaempferol glycosides and ellagic acid derivatives, which may contribute to their high DPPH radical-scavenging activity despite a lower total phenolic content than tea. Dandelion leaves contain hydroxycinnamic acids, including chicoric and caffeic acids, as well as luteolin and apigenin glycosides, which may contribute to their intermediate antioxidant capacity. Sobacha is notably characterized by rutin and displayed a comparatively low total phenolic content in the present study, which may partly account for its lower DPPH and FRAP responses (Cardoso et al., 2020). These compositional explanations should nevertheless be interpreted cautiously, as individual antioxidant compounds were not quantified in the present study.

Antioxidant capacity cannot, however, be attributed solely to polyphenols and flavonoids. Other constituents, including vitamin C, carotenoids, reducing peptides, and organic acids may also contribute to radical-scavenging and reducing capacities. This may help explain why matrices with relatively low phenolic content, such as sobacha, still exhibited measurable antioxidant capacity. Interactions among compounds may also influence the measured response: synergistic interactions between polyphenols and other antioxidants may increase apparent antioxidant capacity, whereas antagonistic interactions, such

as complexation with proteins or metal ions, may reduce measurable *in vitro* activity. Such effects could contribute to discrepancies between total phenolic content and antioxidant capacity among infusions (Liang et al., 2024; Rudrapal et al., 2022).

Overall, the combined use of DPPH and FRAP assays provided complementary information on the *in vitro* radical-scavenging and reducing properties of the beverages. The baseline-subtracted analysis further showed that fermentation-associated changes differed according to the analytical endpoint: DPPH exhibited pronounced substrate-dependent kinetics, whereas FRAP was influenced primarily by fermentation time. These findings reinforce the importance of considering both the initial composition of the botanical substrate and its evolution during fermentation when interpreting *in vitro* antioxidant properties (Prior et al., 2005).

3.2.4. COX-2 inhibition assay

The *in vitro* COX-2 inhibitory activity of kombucha-type beverages was presented in Fig. 6 as changes relative to the corresponding day-0 values. Absolute inhibition values and full statistical outputs are provided in the Supplementary Material (Table S2–S3).

COX-2 is an inducible enzyme involved in the biosynthesis of prostaglandins associated with inflammatory responses. Unlike the constitutively expressed COX-1 isoform, COX-2 is selectively upregulated during inflammatory responses. The COX-2 inhibition assay is particularly relevant for assessing anti-inflammatory potential, as it specifically targets the inflammation-associated isoform and discriminates between COX-2 and COX-1 activities (González et al., 2011). Therefore, inhibition of COX-2 activity can be used as an initial *in vitro* screening approach for compounds capable of interacting with this enzyme. However, this acellular assay does not reproduce cellular uptake, metabolism, bioavailability, or the complexity of inflammatory pathways *in vivo* and should therefore not be interpreted as direct evidence of anti-inflammatory effects.

When expressed as changes from baseline, all formulations showed increased *in vitro* COX-2 inhibition at days 7 and 14, although the magnitude of the increase differed among substrates (Fig. 6). Two-way repeated-measures ANOVA revealed significant effects of fermentation time and infusion type ($p < 0.05$), whereas the time × infusion interaction was not significant ($p > 0.05$). This indicates that COX-2 inhibition increased over time and differed among botanical substrates, but that the overall temporal pattern was not significantly different between infusions.

Tukey's multiple-comparison test showed that sobacha exhibited significantly lower increases in COX-2 inhibition than black/green tea, raspberry leaves, and dandelion leaves at both day 7 and day 14 ($p < 0.05$), whereas no significant differences were detected among black/green tea, raspberry leaf, and dandelion leaf kombuchas. In absolute terms, black/green tea kombucha showed high inhibitory values, exceeding 80% by day 14, while raspberry and dandelion leaf beverages also displayed substantial inhibition (approximately 60–70%). Sobacha consistently showed lower inhibition (<30%). These results suggest that fermentation-related changes in matrix composition may influence the ability of beverage constituents to inhibit COX-2 under the assay conditions.

The observed differences may be partly related to the distinct phenolic and flavonoid profiles of the respective substrates. Compounds such as catechins, quercetin derivatives, and phenolic acids have previously been reported to interact with COX-2 or other inflammation-related pathways. (González et al., 2011). Raspberry leaf kombucha may derive its inhibitory capacity from flavonoids such as quercetin and kaempferol and from ellagic acid derivatives. Dandelion leaf kombucha showed a similar but slightly lower effect, likely associated with hydroxycinnamic acids (e.g., chicoric and caffeic acids) and flavones such as luteolin and apigenin, which could modulate inflammatory pathways. In contrast, sobacha kombucha exhibited weak inhibition, consistent with its lower phenolic density and the predominance of rutin, which shows comparatively limited COX-2 inhibitory potency (García-Lafuente et al., 2009). However, these mechanistic interpretations remain hypothetical because individual compounds and their transformation products were not directly quantified in the present study.

Fermentation may modify the availability or chemical form of phenolic compounds, including conversion between glycosylated and aglycone forms, although such transformations were not directly investigated in this study. The temporal evolution of COX-2 inhibition showed some similarities with the antioxidant responses measured by DPPH and FRAP, which may indicate overlapping contributions from redox-active compounds. Nevertheless, the present data do not establish a causal relationship between antioxidant capacity and COX-2 inhibition, nor do they demonstrate anti-inflammatory effects *in vivo* (Liang et al., 2024).

4. Conclusion

The present study demonstrates that the botanical nature of the substrate plays a major role in shaping kombucha fermentation behaviour and the associated physicochemical and *in vitro* bioactive properties. Black/green tea showed consistent fermentation behaviour under the tested conditions, with progressive acidification and metabolite production, and exhibited the highest antioxidant capacity and *in vitro* COX-2 inhibitory activity. Among alternative substrates, raspberry and dandelion leaves emerged as the most promising, exhibiting

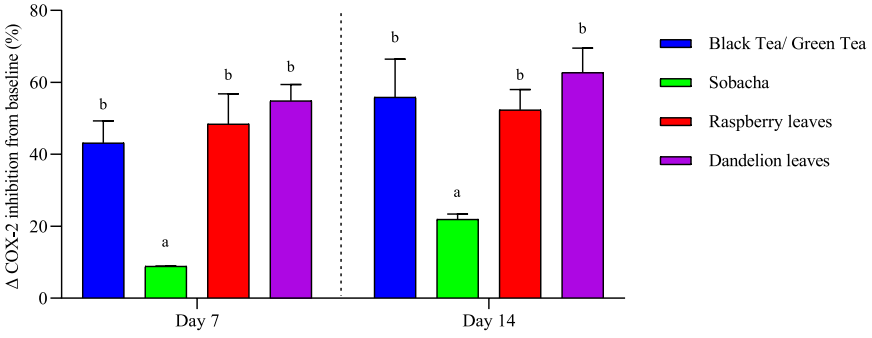


Fig. 6. Changes in *in vitro* COX-2 inhibition relative to baseline (day 0) in kombucha-type beverages prepared with different plant infusions during fermentation. For each biological replicate, baseline-subtracted values were calculated as Δ = value at each fermentation time point − corresponding value at day 0. Data are expressed as mean ± SD of three independent biological replicates (n = 3). Data were analysed using a two-way repeated-measures ANOVA followed by Tukey's multiple-comparison test. Bars with different letters within the same fermentation time are significantly different ($p < 0.05$).

intermediate to high responses in the measured *in vitro* assays, whereas sobacha, although fermentable, displayed comparatively lower antioxidant and COX-2 inhibitory values. In contrast, nettle-based kombucha showed instability of the matrix and atypical physicochemical characteristics, leading to its exclusion from further analyses.

This study provides an integrated comparison of fermentation dynamics, metabolite production, and several *in vitro* bioactivity markers across plant-based kombucha formulations. Variations in phytochemical composition, particularly phenolic compounds, may contribute to the differences observed in antioxidant capacity and *in vitro* COX-2 inhibitory activity, although no direct mechanistic relationship was established. In addition, the relatively limited changes in vitamin C content observed during fermentation indicate that kombucha processing conditions may help preserve this compound across different substrates.

The antioxidant and COX-2 inhibition assays used in this study represent *in vitro* screening approaches and do not account for gastrointestinal stability, bioavailability, cellular uptake, metabolism, or systemic physiological responses. Therefore, the results should not be interpreted as evidence of health-promoting or anti-inflammatory effects *in vivo*. Further studies using cell-based models, digestion and bioavailability approaches, and ultimately *in vivo* investigations are required to substantiate any potential functional claim. In addition, a sensory evaluation to ensure product acceptability, and a characterization of the microbial consortium and its role in compound transformation could provide valuable insight into the mechanisms underlying fermentation-dependent changes in bioactivity. Such approaches would support a more comprehensive understanding of kombucha as a complex fermented system and guide the development of optimized functional beverages.

From an industrial perspective, raspberry and dandelion leaf infusions appear particularly promising as alternative kombucha substrates because they supported efficient fermentation while maintaining comparatively high antioxidant capacity and *in vitro* COX-2 inhibitory activity. These findings support the use of alternative plant infusions as viable substrates for kombucha production and provide useful knowledge for the development of diversified fermented beverages with functional potential. More broadly, the results highlight opportunities for the valorisation of locally sourced botanical resources and contribute to ongoing efforts toward sustainable and innovative food systems.

CRediT authorship contribution statement

Sarah Suffys: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dorothée Goffin:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Olivia Denies:** Validation, Software, Resources, Investigation, Formal analysis. **Marie-Laure Fauconnier:** Writing – review & editing, Supervision. **Éric Haubruge:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used ChatGPT for language editing and improvement of clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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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.fbio.2026.110151.

Data availability

Data will be made available on request.

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