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Artichoke as a novel substrate for kombucha fermentation: Fermentation characteristics, functional properties, and sensory evaluation

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Abstract: This study examined the feasibility of using artichoke (*Cynara scolymus* L.) as a non-traditional substrate for kombucha fermentation and evaluated its effects on fermentation performance, functional properties, and sensory quality. Fermentation with artichoke supported pronounced SCOBY development by day 21, alongside progressive utilisation of soluble solids, increasing total acidity, and a concomitant decrease in pH. Total phenolic and flavonoid contents increased significantly during fermentation, reaching peak values at day 14 [342.47 ± 16.89 mg gallic acid equivalents (GAE)·L^{-1} and 44.54 ± 3.35 mg quercetin equivalents (QE)·L^{-1}, respectively], which coincided with enhanced antioxidant activity as assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The fermented beverage also demonstrated antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, and *Staphylococcus aureus*, together with moderate α-amylase inhibitory activity. Sensory evaluation showed higher overall acceptability for artichoke-based kombucha compared with the conventional tea-based formulation. Collectively, these findings indicate that artichoke can be effectively utilised as a substrate for kombucha fermentation, resulting in a beverage with promising *in vitro* functional properties and favourable sensory quality.

Keywords: *Cynara scolymus*; alternative substrate; SCOBY fermentation; phenolic biotransformation; functional beverage; antioxidant activity; antibacterial activity; α-amylase inhibition

Plant-derived medicinal products have long contributed to human health and remain of considerable interest. *Cynara scolymus* L. (artichoke), native to the Mediterranean, is widely consumed as food or herbal tea and valued for both sensory and health-promoting properties (Negro et al. 2012). It is rich in polyphenols with demonstrated antioxidant and antibacterial activities (Salekzamani et al. 2019), no-

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tably caffeoylquinic acid derivatives (e.g. chlorogenic acid and cynarin) and flavonoids such as luteolin glycosides (Negro et al. 2012). Consequently, artichoke represents a natural source of bioactive compounds associated with a range of health-promoting effects, including anticancer activity (Shallan et al. 2020), lipid-lowering properties (Sahebkar et al. 2018), and hepatoprotective effects (Gebhardt 1997). Accordingly, artichoke is increasingly incorporated into functional foods and beverages to enhance nutritional and functional value.

Kombucha is a fermented beverage of Asian origin that has gained global attention for its potential health benefits. It is produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeasts (SCOBY), a cellulose-based biofilm. During fermentation, yeasts convert sugars into ethanol, which is subsequently oxidised by acetic acid bacteria to acetic acid (Leal et al. 2018). Concurrently, a range of organic acids, such as gluconic, lactic, malic, citric, and tartaric acids, are generated through the biotransformation of tea constituents. These metabolites contribute to kombucha's characteristic aroma and exhibit antibacterial activity, potentially inhibiting pathogenic microorganisms in the gastrointestinal tract (Leal et al. 2018). Furthermore, beneficial microbes within the SCOBY, including *Lactobacillus* spp. and *Lactococcus* spp., might support gut microbial balance (Vargas et al. 2021). Moreover, kombucha-associated microbiota also produce minerals, antimicrobial compounds, and bioactive metabolites, particularly phenolic acids and flavonoids, which exhibit antioxidant and anti-inflammatory properties (Leal et al. 2018). Collectively, these properties have been associated with disease-preventive effects, including the protection of organs such as the pancreas, liver, kidneys, and heart against oxidative stress in diabetic models, the modulation of immune responses, and the potential inhibition of cancer development (Leal et al. 2018).

Traditionally, teas derived from *Camellia sinensis* have served as the primary substrates for kombucha fermentation. However, increasing attention has been directed towards alternative substrates, including fruits, herbs, vegetables, spices, and flowers, to replace *C. sinensis* and diversify kombucha products (Emiljanowicz and Malinowska-Pańczyk 2020). Artichoke, with its favourable flavour profile and phytochemical composition exhibiting established pharmacological activities, represents a promising alternative substrate for the development of novel fermented beverages. Accordingly, this study aims to develop a kombucha beverage based on artichoke tea and to evaluate its sensory attributes and biological activities. This approach contributes to the expansion of the functional food and beverage sector by introducing a novel product that is both nutritionally valuable and potentially beneficial to consumer health.

MATERIAL AND METHODS

Preparation of tea infusion. Black tea (*C. sinensis*), commercially available under the Cozy® brand (Eco-Product Joint Stock Company, Vietnam), was used as the conventional substrate. Dried artichoke (*C. scolymus* L.) was sourced from Lam Dong Province, Vietnam. Flower buds were harvested 90–100 days after planting and dried at 55–60 °C to a final moisture content below 10% prior to use.

Starter cultures (SCOBY) were obtained from a commercial supplier (Foodplus Ltd., Vietnam) and comprised a fermented broth and a cellulosic pellicle, stored at 4 °C until use. According to the supplier, the SCOBY contained yeast species, including *Brettanomyces bruxellensis* and *Saccharomyces cerevisiae*, and acetic acid bacteria (AAB), including *Komagataeibacter pomaceri* and *K. rhaeticus*. The microbial load was approximately 5×10^6 CFU·g^{-1}, as determined by the Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi (VNU), Vietnam. Prior to fermentation, colony-forming unit (CFU) enumeration was performed to verify microbial consistency between batches.

Fermentation of kombucha. The substrate concentration (10 g·L^{-1}) and fermentation conditions (30 °C, 3% SCOBY, 10% sour broth) were selected based on established kombucha protocols (Cardoso et al. 2020) to ensure comparability with conventional black tea kombucha and consistent SCOBY development. Briefly, black tea and artichoke infusions were prepared by steeping 10 g of each substrate in 1 000 mL of hot water for 20 min, followed by filtration through a sterile sieve. Sucrose was added to a final concentration of 100 g·L^{-1} and dissolved completely. After cooling to room temperature, the sweetened infusions were dispensed into sterile bottles (90 mL per bottle). SCOBY was inoculated at 3 g per bottle (3%, *w/v*), together with 10 mL of sour broth (10%, *v/v*). Fermentation was conducted at 30 °C, with samples collected at 7, 14, and 21 days. Three independent batches were prepared and analysed for each substrate.

Determination of SCOBY growth, total soluble solids, pH, and total acidity. During fermentation

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(days 0, 7, 14, and 21), the cellulosic pellicle was harvested from each batch to determine SCOBY biomass ($g \cdot L^{-1}$). Total soluble solids were measured as °Brix using an RA500 KEM refractometer (Kyoto Electronics Manufacturing Co., Ltd., Japan) at 20 °C. Sample pH was determined using a Mettler Toledo J12683 pH meter (Mettler-Toledo, USA). Total titratable acidity was measured by potentiometric titration and expressed as $g \cdot L^{-1}$ acetic acid equivalents following titration with 0.1 N sodium hydroxide (NaOH) to pH 8.1. All measurements were performed in triplicate.

Quantification of total phenolic content and total flavonoid content. Total phenolic content (TPC) of artichoke and black tea kombucha was determined using the Folin-Ciocalteu method (Singleton and Rossi 1965). Briefly, 100 µL of sample was mixed with 900 µL of distilled water and 500 µL of 10% Folin–Ciocalteu reagent, followed by the addition of 500 µL of 7.5% sodium carbonate (Na_2CO_3). The mixture was incubated at 40 °C for 30 min, and absorbance was measured at 765 nm. TPC was quantified using a gallic acid standard curve and expressed as mg gallic acid equivalents per litre (mg $GAE \cdot L^{-1}$).

Total flavonoid content (TFC) was determined using a colourimetric method (Amjadi et al. 2023). Briefly, 0.5 mL of sample was mixed with 1.5 mL of 99% ethanol and incubated for 5 min. Subsequently, 0.1 mL of 10% aluminium chloride ($AlCl_3$) was added and incubated for a further 5 min at room temperature. Then, 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of distilled water were added, followed by incubation at room temperature for 45 min. Absorbance was measured at 415 nm. TFC was calculated using a quercetin standard curve and expressed as mg quercetin equivalents per litre (mg $QE \cdot L^{-1}$).

Evaluation of antioxidant activity. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays (Le et al. 2024). For the DPPH assay, 50 µL of sample was mixed with 1 mL of DPPH solution (40 $mg \cdot L^{-1}$; CAS 1898-66-4; TCI Chemicals, China) and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. The ABTS assay was conducted according to (Xia et al. 2019). ABTS solution (7 mM) was mixed with 2.45 mM potassium persulfate ($K_2S_2O_8$) and incubated in the dark for 12–16 h prior to use. Then, 1 mL of diluted ABTS solution was combined with 50 µL of sample and incubated in the dark at room temperature for 6 min, followed by absorbance measurement at 734 nm. Radical scavenging activity (%) for both assays was calculated using the following equation:

$$Inhibition\ (\%) = \left[\left(Abs_{control} - Abs_{sample}\right) / Abs_{control}\right] \times 100 \quad (1)$$

Where: $Abs_{control}$ – the absorbance of the control reaction (without sample); Abs_{sample} – the absorbance of the test sample (Jakubczyk et al. 2020).

Evaluation of antibacterial activity. Prior to antibacterial assays, kombucha samples were neutralised to pH 7.0 using sterile 0.1 M NaOH and sterilised by membrane filtration (0.22 µm). Antibacterial activity was evaluated using the agar diffusion method against *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, and *Staphylococcus aureus* (Balouiri et al. 2016). Bacterial strains were cultured overnight in Mueller–Hinton Broth (HiMedia, India) and adjusted in phosphate-buffered saline (PBS) to 1×10^7 $CFU \cdot mL^{-1}$. Aliquots (100 µL) of each suspension were spread onto Mueller–Hinton Agar (MHA) plates. Three wells (5 mm diameter) were created in each plate using a sterile metallic borer, and 100 µL of each kombucha sample was added. Sterile distilled water served as the negative control. Plates were incubated at 37 °C for 24 h, after which inhibition zones were measured.

Evaluation of the α-amylase inhibitory activity. Alpha-amylase inhibitory activity was evaluated using the starch–iodine method as described by (Ononamadu et al. 2020) with minor modifications. Briefly, 400 µL of sample was mixed with 400 µL of α-amylase solution (Sigma-Aldrich, USA) in 0.04 M phosphate buffer (pH 6.9) and incubated at 36 °C for 10 min. Subsequently, 400 µL of 1% starch solution was added and incubated for a further 10 min at 36 °C. The reaction was terminated by adding 100 µL of 1 M hydrochloric acid. Thereafter, 100 µL of 5 mM iodine solution was mixed with 200 µL of reaction mixture and diluted with 1 mL of distilled water. Absorbance was measured at 570 nm. PBS served as negative control, while acarbose was used as the positive control.

The percentage inhibition of α-amylase activity was calculated as:

$$Inhibition\ (\%) = (C - S) / C \times 100 \quad (2)$$

Where: C – the absorbance of the control reaction (starch + α-amylase without inhibitor); S – the absorbance of the sample reaction (starch + α-amylase in the presence of the kombucha sample or acarbose).

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Sensory evaluation. Sensory evaluation was conducted with 30 untrained consumers (approximately equal numbers of males and females, aged 22–35 years), all regular consumers of fermented beverages and representative of the target population. Prior to evaluation, panellists attended a 30 min orientation session to familiarise them with the sensory attributes, evaluation criteria, and the nine-point hedonic scale (1 = dislike extremely; 9 = like extremely) (Everitt 2009). To minimise bias, samples were coded with random three-digit numbers and presented in a fully randomised order using a balanced Latin square design. Evaluations were conducted in individual sensory booths under controlled white lighting (6500 K) at 22 ± 1 °C to limit environmental variation and interaction among panellists (Carpenter et al. 2000). Panellists assessed appearance, colour, odour, sweetness, sourness, astringency, and overall acceptability. Sensory evaluation of artichoke and black tea kombucha was performed after 14 days of fermentation.

Statistical analysis. The experiment followed a completely randomised factorial design with two substrates and four fermentation times, with three independent biological replicates per treatment. Data were analysed using SAS 9.4 (SAS Institute Inc., USA) and are presented as mean ± standard deviation (SD). Differences among groups were assessed by two-way analysis of variance, followed by Tukey's post hoc test, with statistical significance set at $P \leq 0.05$. For antibacterial activity, one-way ANOVA was performed separately for each substrate and bacterial strain across fermentation times. Lowercase letters indicate significant differences among fermentation times, whereas uppercase letters denote differences between substrates. Graphs were generated using GraphPad Prism 10 (GraphPad Software, USA).

RESULTS AND DISCUSSION

The effect of artichoke substrate on the growth of SCOBY layer at different time points. The formation and development of the SCOBY are key indicators of kombucha fermentation performance, reflecting the metabolic activity and functional interactions between yeasts and acetic acid bacteria (Tran et al. 2020). In the present study, SCOBY biomass increased progressively during fermentation in both substrates, with consistently greater accumulation in artichoke-based kombucha (ATK) than in black tea kombucha (BTK) at all time points (Figure 1A). A distinct SCOBY layer was evident from day 7 and exhibited substantial growth by day 21. At day 7, SCOBY biomass in ATK reached 51.17 ± 2.91 $g{\cdot}L^{-1}$, significantly higher than in BTK (27.53 ± 1.76 $g{\cdot}L^{-1}$). This trend persisted at days 14 and 21, with ATK reaching 59.53 ± 2.37 $g{\cdot}L^{-1}$ and 78.00 ± 1.20 $g{\cdot}L^{-1}$, respectively, compared with 45.57 ± 2.06 $g{\cdot}L^{-1}$ and 72.97 ± 1.66 $g{\cdot}L^{-1}$ in BTK. These findings indicate substantial microbial biomass accumulation during fermentation, with the artichoke substrate supporting enhanced SCOBY formation (Figure 1A).

Sucrose in the fermentation medium serves as the primary energy source for SCOBY-associated microorganisms. During fermentation, sugars and substrate-derived components are metabolised, leading to changes in soluble solids, pH, and organic acid composition (Leal et al. 2018). Prior to fermentation, soluble solids (°Brix) were comparable between ATK (9.33 ± 0.06 °Bx) and BTK (9.40 ± 0.10 °Bx). As fermentation progressed, °Brix decreased significantly in both systems, reaching 8.30 ± 0.10 °Bx for ATK and 8.07 ± 0.15 °Bx for BTK by day 21 (Figure 1B), indicating active carbohydrate utilisation.

Concomitantly, pH in ATK declined from 5.13 ± 0.01 to 2.87 ± 0.08 at day 14 and further to 2.71 ± 0.09 at day 21, with a comparable trend observed in BTK (Figure 1C). pH is a critical parameter for assessing the microbiological safety of fermented beverages. During kombucha fermentation, SCOBY microorganisms produce organic acids, including acetic, gluconic, lactic, malic, citric, and tartaric acids, resulting in progressive acidification (Leal et al. 2018). Kombucha beverages with pH values within the safe range of 2.5–4.2 are considered favourable for preserving the biological activity of phenolic compounds while inhibiting the growth of pathogenic microorganisms (Coban 2020). In addition, SCOBY cultivated in the ATK medium exhibited total acidity expressed as acetic acid equivalents of 9.16 ± 0.18 $g{\cdot}L^{-1}$ and 9.84 ± 0.12 $g{\cdot}L^{-1}$ after 14 and 21 days of fermentation, respectively, with similar levels recorded in BTK. These data indicate that decreasing pH was accompanied by increasing total acidity during fermentation (Figure 1D).

Collectively, these findings are consistent with established kombucha fermentation models and demonstrate the feasibility of producing artichoke-based kombucha. Accordingly, artichoke appears to be a suitable substrate for SCOBY development. Although pre-fermentation CFU enumeration confirmed comparable microbial loads ($\approx 5 \times 10^6$ $CFU{\cdot}g^{-1}$) across batches, the taxonomic stability of the SCOBY community was not characterised using molecular approach-

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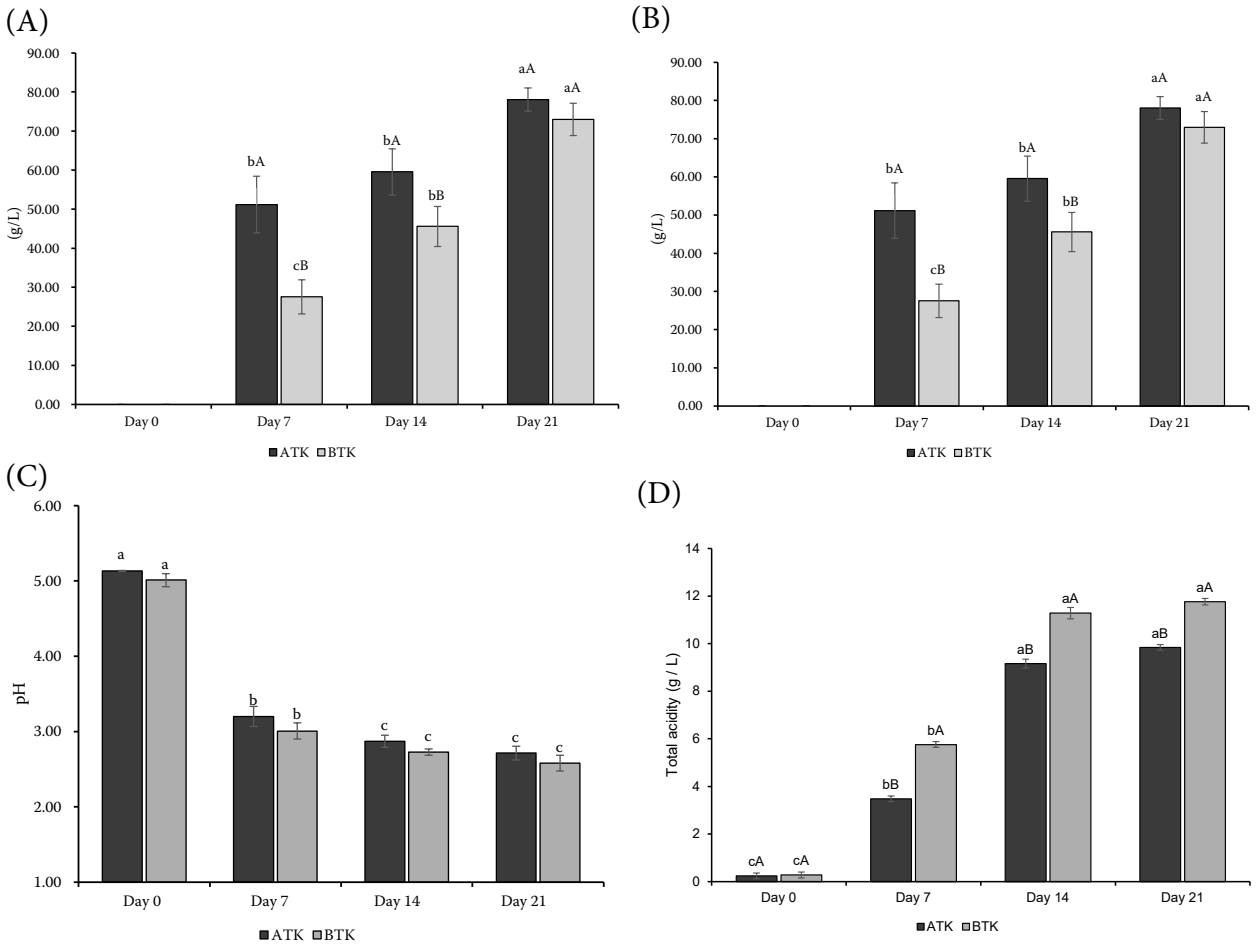

Figure 1. (A) Effects of substrate type on the growth of nascent SCOBY pellicles in artichoke kombucha (ATK) and black tea kombucha (BTK) over fermentation time. Changes in total soluble solids (°Brix) (B), pH (C), and total acidity (D) during fermentation in ATK and BTK

$^{a–c}$different letters indicate significant differences across fermentation time points ($P < 0.05$), $^{A–B}$indicate significant differences between substrates at the same time point ($P < 0.05$); data are presented as mean ± standard deviation of triplicate analyses

es (e.g. 16S rRNA or ITS sequencing). Consequently, minor variations in microbial composition between batches cannot be excluded. Nevertheless, the consistent trends observed across replicates in biomass accumulation, soluble solids, pH reduction, and total acidity indicate stable functional fermentation under the applied conditions. Future studies should prioritise the isolation and selection of defined yeast–acetic acid bacterial consortia and employ controlled starter inoculation strategies to standardise microbial inputs. This would enhance batch-to-batch reproducibility, enable mechanistic investigation of metabolite production, and support scalable industrial applications.

Evaluation of total phenolic and flavonoid contents of artichoke kombucha. Phenolic and flavonoid compounds are key contributors to the biological functionality of kombucha, owing to their strong association with antioxidant capacity and other health-related effects. During fermentation, microbial activity within the SCOBY can modify plant-derived polyphenols through enzymatic hydrolysis, oxidation, and biotransformation (Leal et al. 2018). Accordingly, changes in TPC and TFC were monitored in ATK throughout fermentation (Figure 2).

Prior to fermentation, ATK exhibited a TPC of 223.67 ± 3.45 mg·L^{-1} and a TFC of 23.61 ± 1.27 mg·L^{-1} (Figure 2). Both parameters increased significantly during fermentation, reaching maxima at day 14 (342.47 ± 16.89 mg·L^{-1} for TPC and 44.54 ± 3.35 mg·L^{-1} for TFC). Comparable trends were observed in BTK. The increase in TPC and TFC during the early and mid-fermentation stages is likely attributable to enzymatic activities of yeasts and acetic acid bacteria, which hydrolyse complex polyphenol–protein and poly-

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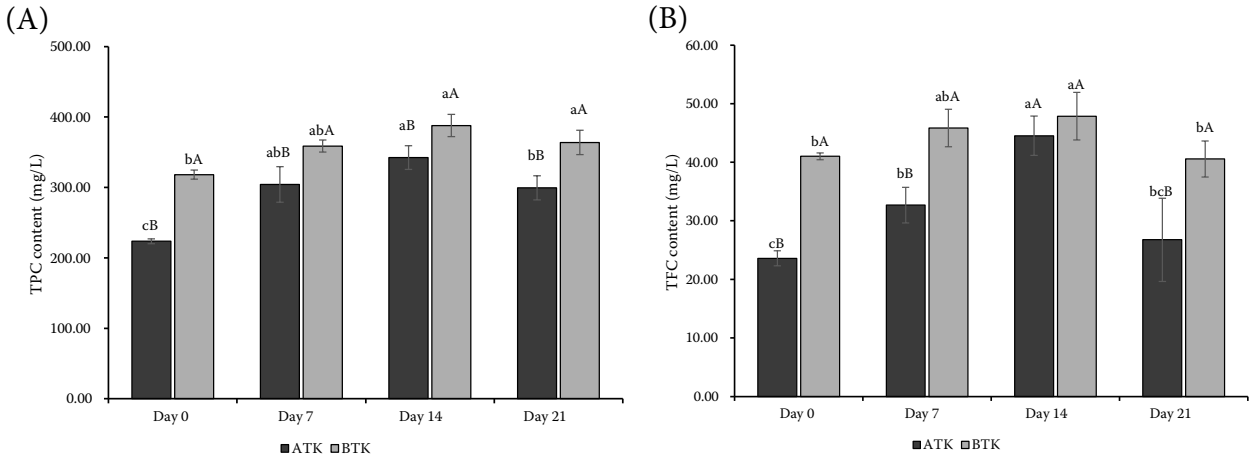

Figure 2. Total phenolic content (TPC) (A) and total flavonoid content (TFC) (B) of artichoke kombucha (ATK) and black tea kombucha (BTK) over fermentation time

[a–c]different letters indicate significant differences across fermentation time points ($P < 0.05$), [A–B]indicate significant differences between substrates at the same time point ($P < 0.05$); data are presented as mean ± standard deviation of triplicate analyses

phenol-polysaccharide conjugates into more readily detectable low-molecular-weight forms. Similar increases in phenolic and flavonoid contents have been reported in kombucha derived from both conventional and alternative substrates, supporting microbial biotransformation rather than de novo synthesis as the primary mechanism (Amjadi et al. 2023).

In contrast, the decline observed at day 21 (Figure 2) may reflect secondary degradation, polymerisation, or oxidative transformation of phenolic compounds under prolonged acidic conditions. Similar patterns have been reported in black tea, green tea, and cascara kombucha, indicating that fermentation-driven modulation of phenolics and flavonoids follows comparable kinetics across substrates (Jakubczyk et al. 2020; Le et al. 2024).

From a functional perspective, the enrichment of phenolic and flavonoid contents during fermentation is particularly relevant, given their well-documented antioxidant, antibacterial, anti-inflammatory, and immunomodulatory activities (Leal et al. 2018). The peak observed at day 14 therefore suggests an optimal fermentation duration for maximising bioactive compound availability in artichoke kombucha.

Analysis of the antioxidant capacity of artichoke kombucha. Antioxidant capacity is a key quality attribute of kombucha and is closely linked to fermentation-driven modifications of phenolic and flavonoid compounds (Amjadi et al. 2023). To characterise these changes, the antioxidant activity of ATK was evaluated using DPPH and ABTS radical scavenging assays, representing hydrogen atom transfer and electron transfer mechanisms, respectively (Figure 3).

Both DPPH and ABTS scavenging activities increased progressively during fermentation, reaching maxima at day 14 (Figure 3). This trend parallels the temporal changes in total phenolic and flavonoid contents, indicating a strong association between polyphenol enrichment and antioxidant capacity during mid-fermentation. Such behaviour is consistent with microbial biotransformation during kombucha fermentation, whereby enzymatic hydrolysis and metabolic conversion enhance the availability of redox-active phenolic compounds (Kumar et al. 2025). Although ATK exhibited lower absolute antioxidant activity than BTK, both beverages displayed similar kinetic profiles, characterised by an increase during early fermentation, a peak at mid-fermentation, and a subsequent decline (Figure 3). This pattern suggests that fermentation duration is the primary determinant of antioxidant dynamics, whereas substrate composition mainly influences the magnitude of the response. The higher activity observed in BTK is likely attributable to the intrinsic abundance of catechins and theaflavins in black tea, which are well-established contributors to antioxidant capacity (Leung et al. 2001).

Overall, the results demonstrate that fermentation effectively enhances the antioxidant properties of artichoke kombucha, with maximal activity observed at day 14. Beyond this point, the decline in scavenging capacity indicates that prolonged fermentation does not confer additional antioxidant benefits, consistent with the concurrent reduction in phenolic and flavonoid contents.

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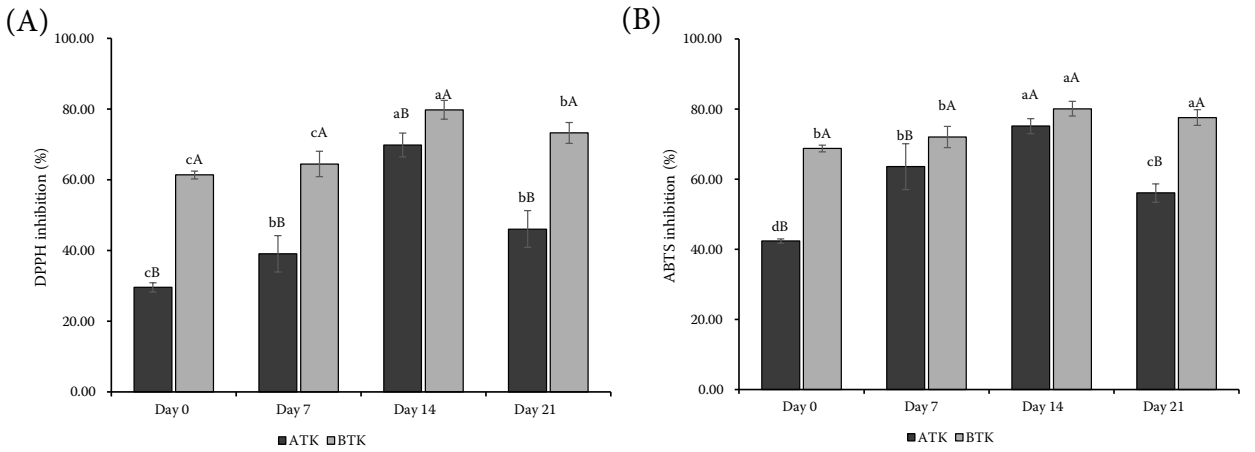

Figure 3. Antioxidant activity of kombucha assessed by DPPH radical scavenging (A) and ABTS radical scavenging (B) assays over fermentation time

[a–d]different letters indicate significant differences across fermentation time points ($P < 0.05$), [A–B]indicate significant differences between artichoke kombucha (ATK) and black tea kombucha (BTK) at the same time point ($P < 0.05$); data are presented as mean ± standard deviation of triplicate analyses

Analysis of antibacterial activity of artichoke kombucha. Antibacterial activity is a key functional attribute of kombucha, with relevance to food safety and potential health benefits (Bhattacharya et al. 2016). The antibacterial activity of ATK was evaluated using an agar diffusion assay against common human pathogens, including *E. coli*, *S. typhi*, *V. cholerae*, and *S. aureus*. The unfermented solution exhibited no inhibitory effect against any tested pathogen (Table 1). After 7 days of fermentation, the reference kombucha showed stronger inhibition of Gram-negative bacteria, particularly *E. coli* and *S. typhi*. In contrast, ATK reached maximal antibacterial activity at day 14, exceeding the inhibitory effects observed at earlier and later stages (Table 1). This temporal pattern mirrors fermentation-dependent changes in total phenolic and flavonoid contents (Figure 2).

Antibacterial efficacy is more plausibly attributed to fermentation-induced accumulation or transformation of bioactive compounds, particularly phenolics and flavonoids. This interpretation is supported by studies on herbal kombucha derived from medicinal plants such as cinnamon, cardamom, and Shirazi thyme, in which enhanced antibacterial activity against *E. coli* and *Salmonella* spp. was positively associated with increased phenolic and flavonoid contents (Shahbazi et al. 2018). Similarly, the antibacterial activity of traditional tea-based kombucha against *E. coli*

Table 1. Antibacterial activity of artichoke (ATK) and black tea (BTK) kombucha against selected bacterial pathogens during fermentation determined by agar well diffusion assay

Bacterial strains		Inhibition halo diameter (mm)			
		Day 0	Day 7	Day 14	Day 21
ATK	*Escherichia coli*	–	–	14.17 ± 1.17^{a}	9.5 ± 1.64^{b}
	Salmonella typhi	–	–	16.83 ± 1.47^{a}	13.17 ± 1.17^{b}
	Vibrio cholerae	–	13.83 ± 1.47^{a}	12.50 ± 1.05^{ab}	10.17 ± 1.33^{b}
	Staphylococcus aureus	–	15.5 ± 3.08^{a}	19.00 ± 2.19^{a}	19.17 ± 2.23^{a}
BTK	*E. coli*	–	17.17 ± 1.83^{a}	15.00 ± 1.41^{b}	15.17 ± 1.60^{b}
	S. typhi	–	17.67 ± 1.21^{a}	11.50 ± 2.07^{c}	14.50 ± 1.87^{b}
	V. cholerae	–	15.83 ± 1.94^{ns}	16.50 ± 2.17^{ns}	15.67 ± 1.51^{ns}
	S. aureus	–	17.50 ± 1.52^{b}	21.17 ± 2.93^{a}	20.00 ± 3.29^{ab}

[a–c]different lowercase letters within the same row indicate significant differences across fermentation time points for each kombucha type and bacterial strain ($P < 0.05$); data are presented as mean ± standard deviation of triplicate analyses

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and *S. aureus* has been attributed to specific phenolic constituents, including catechins and verbascoside, rather than to acidity alone (Cardoso et al. 2020).

Accordingly, the pronounced antibacterial activity of ATK on day 14 is most likely attributable to the accumulation and biotransformation of phenolic and flavonoid compounds during fermentation. The subsequent decline on day 21 may reflect degradation, polymerisation, or reduced bioavailability of these compounds under prolonged acidic conditions, consistent with the concurrent decreases in total phenolic and flavonoid contents. Comparable over-fermentation effects have been reported in other non-traditional kombucha systems, underscoring the importance of optimising fermentation duration to maximise antibacterial activity (Le et al. 2024).

It should also be acknowledged that kombucha contains organic acids, including acetic, gluconic, and glucuronic acids, produced during fermentation and potentially contributing to antibacterial activity. Although samples were pH-adjusted prior to testing to minimise the direct effect of acidity, the contribution of organic acids cannot be excluded. The absence of acid-matched neutralised controls limits discrimination between organic acid-mediated effects and those attributable to other fermentation-derived metabolites. In particular, undissociated acid fractions or synergistic interactions between organic acids and phenolic compounds may still influence antimicrobial activity (Ormeneanu et al. 2025). Therefore, while phenolic constituents are likely contributors, the antibacterial activity observed here should be considered multifactorial, and the relative roles of organic acids and other fermentation-derived metabolites remain to be further clarified.

Table 2. Alpha-amylase inhibition activity of kombucha beverages from artichoke (ATK) and black tea (BTK) at different fermentation times

	α-amylase inhibition (%)		
	ATK	BTK	Acarbose
Day 0	18.56 ± 3.55^{cA}	22.20 ± 1.78^{dA}	72.46 ± 1.96
Day 7	25.51 ± 2.78^{bB}	34.45 ± 2.84^{cA}	
Day 14	56.22 ± 1.18^{aA}	58.47 ± 2.20^{aA}	
Day 21	30.54 ± 6.66^{bB}	47.09 ± 3.36^{bA}	

$^{a-c}$different letters indicate significant differences in α-amylase inhibition ability for each kind of kombucha over fermentation time ($P < 0.05$), $^{A-B}$indicate significant differences in α-amylase inhibition ability between ATK and BTK ($P < 0.05$); data are presented as the means of triplicate analysis ± standard deviation

Evaluation of the α-amylase inhibitory activity of artichoke kombucha. Alpha-amylase and α-glucosidase are key enzymes involved in starch digestion. In the gastrointestinal tract, these enzymes hydrolyse dietary starch into glucose, which is subsequently absorbed into the bloodstream, increasing postprandial blood glucose levels. Accordingly, inhibition of α-amylase is an established strategy for limiting carbohydrate digestion and moderating glycaemic response (Delorme and Chiasson 2005). On this basis, the present study evaluated the α-amylase inhibitory activity of artichoke kombucha in comparison with a conventional tea-based formulation. As shown in Table 2, the fermented beverage exhibited significant α-amylase inhibitory activity, with maximal inhibition observed at day 14 (56.22 ± 1.18%). This value was slightly, but not significantly, lower than that of the reference kombucha (58.47 ± 2.20%) and markedly lower than that of the positive control, acarbose (Table 2). These results indicate that fermentation enhances α-amylase inhibitory potential, with maximal activity occurring at mid-fermentation.

The observed inhibitory effect is most plausibly attributable to fermentation-induced accumulation or transformation of phenolic and flavonoid compounds, which can interact with digestive enzymes via hydrogen bonding and hydrophobic interactions (Sun et al. 2020). Consistent with this interpretation, polyphenol-rich kombucha and related fermented beverages have been reported to exhibit moderate α-amylase inhibitory activity, supporting their potential as dietary adjuncts for glycaemic management (Phan-Van et al. 2024). However, the present evaluation was conducted at a single concentration and did not include dose-response analysis or IC_{50} determination, limiting quantitative comparison and mechanistic insight. Furthermore, these findings are restricted to in vitro conditions and require validation through appropriately designed *in vivo* studies.

Sensory evaluation of artichoke kombucha. The physicochemical properties of fermented beverages directly influence sensory perception; therefore, sensory evaluation is essential for the development of acceptable and marketable kombucha products (Neffe-Skocinska et al. 2017; Kim and Adhikari 2020). Accordingly, the sensory attributes of artichoke kombucha fermented for 14 days were evaluated and compared with those of a conventional tea-based formulation, focusing on appearance, colour, odour, sweetness, sourness, astringency, and overall acceptability (Figure 4). Sensory analysis showed that the artichoke-based for-

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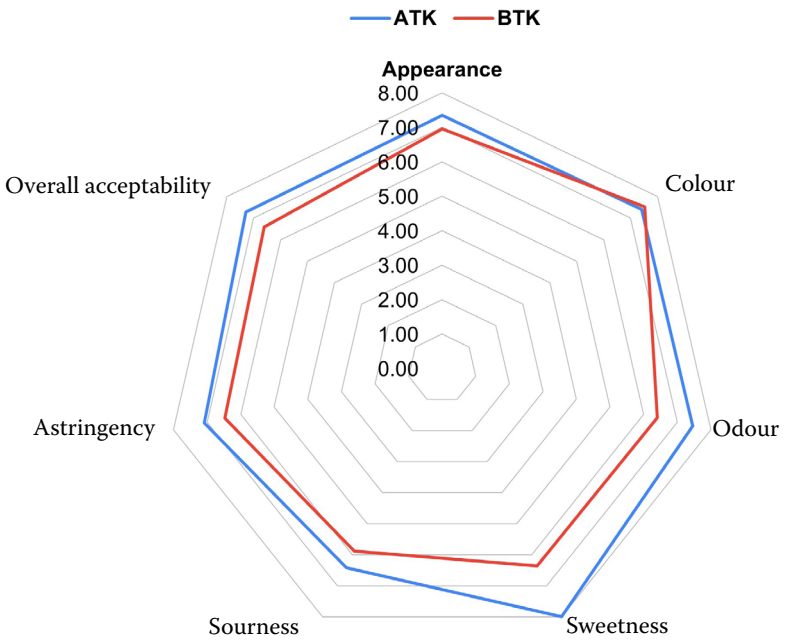

Figure 4. Sensory evaluation of artichoke-based kombucha (ATK) and black tea kombucha (BTK) after 14 days of fermentation

Data represent mean hedonic scores for appearance, colour, odour, sweetness, sourness, astringency, and overall acceptability, evaluated using a nine-point hedonic scale

mulation fermented for 14 days received significantly higher scores for most attributes than the reference sample ($P < 0.05$) (Figure 4). Among the attributes, sweetness received the highest rating, indicating a favourable balance and palatability. The overall score was 7.09 ± 1.07, significantly higher than that of the reference kombucha (6.11 ± 1.63; $P < 0.05$) (Figure 4). Importantly, both samples achieved mean overall scores above 6, indicating that artichoke is a suitable alternative to black tea while maintaining consumer acceptance and improving several sensory attributes.

Overall, the superior sensory performance observed at day 14 aligns with the concurrent optimisation of physicochemical and functional properties at this stage, including phenolic enrichment, antioxidant capacity, antibacterial activity, and α-amylase inhibition. This convergence indicates that mid-fermentation provides a favourable balance between bioactivity and sensory acceptability, rather than a trade-off between functionality and palatability.

Limitations of the study. Despite these promising findings, several limitations should be acknowledged. The use of a 10 g·L^{-1} substrate concentration, although consistent with published protocols, warrants artichoke-specific optimisation in future studies. Fermentation was performed using a conventional mixed SCOBY consortium, which reflects practical production conditions but limits control over fermentation kinetics and metabolite formation. The use of defined starter cultures would enhance reproducibility and enable clearer mechanistic interpretation. Bioactive components were assessed as total phenolic and flavonoid contents, capturing overall trends but not resolving individual compounds; thus, compound-specific analyses are required. Functional properties were evaluated using *in vitro* models, which provide preliminary evidence of bioactivity but do not fully reflect *in vivo* physiological relevance. Accordingly, *in vivo* studies are necessary to substantiate functional claims and support translation into food production applications. Finally, systematic optimisation of fermentation parameters, processing conditions, and storage stability is needed to ensure functional consistency and facilitate industrial scalability.

CONCLUSION

This study demonstrates that artichoke (*Cynara scolymus* L.) is a viable non-traditional substrate for kombucha fermentation, maintaining characteristic fermentation behaviour. Artichoke-based fermentation followed established kinetics while exhibiting measurable physicochemical changes and time-dependent modulation of phenolic and flavonoid contents. The concurrent optimisation of phenolic content, functional bioactivities, and sensory acceptability at day 14 indicates that fermentation duration is a key determinant of product characteristics, rather than intrinsic substrate superiority. The observed *in vitro* antioxidant, antibacterial, and α-amylase inhibitory activities, together with favourable sensory performance, support the potential for product diversification. However, these findings are limited to controlled laborato-

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ry conditions and do not demonstrate *in vivo* health benefits; dedicated human studies are required before functional or therapeutic claims can be substantiated. Overall, the results support the technological feasibility and product relevance of artichoke as an alternative kombucha substrate and provide a foundation for future compound-specific, *in vivo*, and process optimisation studies aimed at scalable production.

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