CAPÍTULO 4

Kombucha Fermentation and Its Antimicrobial Compounds: A Promising Tool for Food Biopreservation

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Jessyca Caroline Rocha Ribas
Universidade Estadual de Maringá, Maringá, PR, Brasil

ABSTRACT: Kombucha is a fermented tea-based beverage produced by a symbiotic culture of bacteria and yeast (SCOBY), and has gained prominence for its therapeutic properties associated with benefits for human health. Kombucha has recently emerged as a promising natural alternative for food biopreservation. This review examines the current literature concerning the antimicrobial and antifungal activity of kombucha, emphasizing the influence of fermentation conditions, microbial diversity, substrate composition, and metabolite profiles on its inhibitory efficacy against pathogenic and spoilage microorganisms. The antimicrobial activity of kombucha is attributed to the synergistic action of organic acids, polyphenolic compounds, and other metabolites derived from fermentation. In vitro studies demonstrate strong antibacterial activity, particularly against Gram-positive bacteria, while antifungal effects are more species-dependent. Despite promising results, significant challenges persist, including a lack of standardized fermentation protocols, limited validation in real-world food matrices, and an incomplete understanding of the mechanisms underlying antimicrobial activity. This review highlights critical research gaps and future perspectives, including the evaluation of kombucha in complex food systems, the development of kombucha-based active packaging, and the assessment of safety and regulatory aspects. Overall, kombucha represents a multifunctional and sustainable biopreservative with substantial potential to contribute to innovative clean-label food preservation strategies.

KEYWORDS: Antibacterial activity, Antifungal activity; Biopreservation; Fermented beverages; Food safety;

INTRODUCTION

Kombucha tea is a sweetened fermented beverage produced from black, green, or oolong tea by a symbiotic culture of bacteria and yeasts (SCOBY) (Vohra et al. 2019)jaggery (J. It is widely consumed worldwide due to its reported prophylactic and therapeutic properties, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticarcinogenic effects (Silva et al., 2021). However, the use of new raw materials for its fermentation, such as other types of teas, fruits, herbs, milk, spices, milk and food industry by-products has attracted more attention (Leonarski et al. 2022; Wu et al. 2023).

The antimicrobial activity of kombucha against human pathogens was first reported in 1996 by Steinkraus et al. (1996), who attributed this effect mainly to its acetic acid content and the presence of bioactive compounds formed during fermentation (Steinkraus et al., 1996). Since then, this antimicrobial potential has been increasingly explored not only for human health applications, but also for food preservation purposes (Prestinaci et al., 2015).

The antimicrobial properties of kombucha are associated with a wide range of chemical compounds produced during fermentation, including alkaloids, esters, fatty acids, aromatic aldehydes, lactones, alcohols, organic acids, condensed heterocyclic compounds, and antibiotic-like substances produced during fermentation (Al-Mohammadi et al., 2021). These compounds exhibit strong antibacterial and antifungal activity, effectively suppressing mycotoxin production both *in vitro* (Taheur et al., 2019) and *in vivo* (Kilmanoglu et al., 2024).

Food losses caused by microbial spoilage remain a major global challenge, particularly those associated with fungal contamination. Several fungal species belonging to the *Aspergillus*, *Fusarium*, and *Penicillium* genera can produce mycotoxins as secondary metabolites, which can contaminate a wide range of foods and animal feeds (Hernández et al., 2022). Human exposure to mycotoxins may occur either through the direct consumption of contaminated cereals or indirectly through animal-derived products obtained from animals fed with contaminated feed. The contamination of animal feed with mycotoxins such as aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FUMs), and T-2/HT-2 toxins poses a dual threat by compromising both animal and human health. These toxic compounds can be transferred from contaminated feed into animal-derived products, including milk, meat, and eggs, thereby entering the human food chain and increasing the risk of exposure (Muñoz-Solano & González-Peñas, 2023). Mycotoxins can cause serious health complications due to their carcinogenic, mutagenic, teratogenic, and immunosuppressive properties (Kebede et al. 2020)

In addition to food safety concerns, food losses generate important economic, environmental, and social impacts. Losses along the food supply chain result in financial damage, inefficient use of natural resources such as water, energy, and land, and increased greenhouse gas emissions. Moreover, food losses reduce the availability of food and contribute to food insecurity (Sarangi et al. 2024).

Preservatives play a crucial role in extending the shelf life of foods. The use of GRAS (Generally Recognized As Safe) microorganisms for food preservation has been widely investigated, particularly lactic acid bacteria (LAB) (Izzo et al. 2020, Dopazo et al. 2023, Cheong et al. 2014)Penicillium, and Fusarium genera represents a problem in food preservation and consequently, its spoilage. During the fermentation process with lactic acid bacteria, a range of secondary metabolites associated with beneficial health effects were released. In the present study, goat whey fermented by Lactobacillus plantarum (CECT 220, 221, 223, and 748. Through biopreservation—defined as the use of beneficial microorganisms or their metabolites to inhibit spoilage and pathogenic microorganisms—fermentation has emerged as a promising strategy. This approach enables the production of natural antimicrobial compounds capable of replacing or reducing the use of synthetic preservatives (Siedler et al., 2019; Martí-Quijal et al., 2021).

In recent years, interest in the application of kombucha against pathogenic bacteria and fungi responsible for food spoilage and contamination has increased significantly (Zhou et al. 2022, Matei et al. 2017)which can be attributed to its abundant bioactive compounds, especially polyphenols. Kombucha is conventionally prepared by fermentation of a sugared black tea infusion without tea residue. In this study, the effects of black tea residue and green tea residue on kombucha were studied, and its antioxidant activities, total phenolic contents, as well as concentrations of polyphenols at different fermentation stages were evaluated using ferric-reducing antioxidant power, Trolox equivalent antioxidant capacity, Folin-Ciocalteu method and high-performance liquid chromatography with a photodiode array detector. The results showed that fermentation with tea residue could markedly increase antioxidant activities (maximum 3.25 times. In this context, the present review aims to analyze the potential use of kombucha as a natural food additive for preservation purposes, focusing on evidence obtained from both *in vitro* and *in vivo* studies.

METHODOLOGY

For the elaboration of this review, a descriptive review approach was adopted, structured in sequential steps of searching, selecting, and analyzing scientific studies. This approach aimed to gather and organize information available in literature on the use of kombucha as an antimicrobial and antifungal agent and its application in food.

Initially, a bibliographic search was conducted in recognized academic databases, including Scopus, Web of Science, and Google Scholar, using combinations of keywords in English, such as: "kombucha antifungal", "kombucha antimicrobial", "kombucha food preservation". The research encompassed studies published between 2016 and 2026, prioritizing recent works that addressed the topic in question. Peer-reviewed scientific articles, book chapters, and literature reviews relevant to the topic were considered. Duplicate works, those outside the scope of the review, or those that did not present a direct relationship with the use of agro-industrial waste were excluded.

After the initial search, the studies were subjected to a screening process based on reading the titles and abstracts. Subsequently, the selected articles were analyzed in full. The data obtained were organized descriptively, allowing for the synthesis and discussion of the main findings reported in the literature.

RESULTS

Fermentation process

Traditional kombucha fermentation relies on the symbiotic interaction between yeasts and bacteria – in particular, acetic acid bacteria (AAB) although small populations of lactic acid bacteria (LAB) have also been reported (Wang et al. 2022)a sparkling sugared tea beverage fermented by a symbiotic culture of acetic acid bacteria (AAB. The yeast community in kombucha is typically more diverse than the bacterial fraction. Species frequently identified belong to the genera *Zygosaccharomyces*, *Candida*, *Kloeckera/Hanseniaspora*, *Torulaspora*, *Pichia*, *Brettanomyces/Dekkera*, *Saccharomyces*, and *Saccharomycoides* (Marsh et al., 2014).

Among the bacterial community, AAB are consistently present at high cell densities and are considered key functional members of the consortium. The most frequently reported species include *Gluconacetobacter xylinus*, *Acetobacter aceti*, *Acetobacter pasteurianus* and *Gluconobacter oxydans*. Although LAB have been detected in kombucha, they generally occur in lower concentrations (Coton et al. 2017; (Laureys et al. 2020).

The fermentation starts when sucrose is hydrolyzed into glucose and fructose by an invertase enzyme in the periplasm of yeast cells. Yeast fermented glucose and fructose into ethanol, carbon dioxide, and glycerol. The ethanol produced by yeasts is oxidized by Komagataeibacter and Acetobacter into acetaldehyde, which can be further oxidized into acetic acid (Nyhan et al. 2022)slightly acidic beverage traditionally produced by the fermentation of sweetened tea by a symbiotic culture of bacteria and yeast (SCOBY. AAB (in particular Gluconobacter species) can oxidized glucose producing gluconic acid, glucuronic acid, 2-ketogluconic acid, 5-ketogluconic acid, 2,5-diketogluconic acid, and glucuronic acid (Laureys et al. 2020, Bishop et al. 2022). LAB can utilize glucose and fructose molecules to produce lactic acid. Furthermore, many AAB (Gluconacetobacter and Komagataeibacter species) produce bacterial cellulose pellicle from glucose, fructose, sucrose, and other substrates such as ethanol and glycerol. The resulting fermented beverage is low in pH and contains multiple organic acids (mainly acetic and lactic, and to a lesser extent gluconic acid), trace amounts of ethanol and glycerol as well as phenolic compounds (Coton et al. 2017).

The dynamics of kombucha fermentation and the composition of its final products are governed by complex interactions among microbial communities, involving both cooperative and competitive relationships. These interactions are strongly influenced by several factors, including the type of tea or alternative substrate used, the initial sugar concentration, and fermentation conditions such as time and temperature (Villarreal-Soto et al. 2018, Landis et al. 2022)l. Under varying fermentation conditions, microorganisms may activate different metabolic pathways, resulting in significant differences in the production and accumulation of metabolites—particularly organic acids—throughout the process. Consequently, the chemical profile of the final kombucha product can vary substantially between fermentations.

These variations are especially relevant in the context of antimicrobial activity. Changes in substrate composition, SCOBY microbial diversity, and fermentation parameters directly affect both the type and concentration of bioactive compounds, thereby modulating the inhibitory potential of kombucha against pathogenic and spoilage microorganisms (Onsun et al. 2025).

Antimicrobial compounds from kombucha

Most authors consider acetic acid to be the biggest contributor to kombucha's antimicrobial potential. The concentration of 5 g/L of acetic acid is sufficient to affect cell growth and productivity of both Gram-positive and Gram-negative bacteria (Trček et al. 2015).

Acetic acid, as well as other organic acids, can influence antimicrobial activity by two primary mechanisms: cytoplasmic acidification and accumulation of the dissociated acid anion to toxic levels (Ayed et al. 2016). Acetic acid is weakly ionized in aqueous solution, and it suppresses the growth of pathogenic microbes by deactivating the work system of the cell (Yuniarto et al. 2016).

However, the effectiveness of organic acids as antimicrobial agents in food matrices depends on several intrinsic and extrinsic factors, including acid molarity, pH, concentration of the undissociated form, and hydrophobicity (Nieto-Peñalver et al. 2014). Additional variables such as chemical structure, physical state, pKa value, molecular weight, minimum inhibitory concentration, microbial susceptibility, buffering capacity of the food, and exposure time further modulate antimicrobial efficacy (Coban 2020). These factors collectively explain the variability observed in the antimicrobial performance of kombucha under different fermentation and formulation conditions.

In addition to organic acids, catechins and other polyphenolic compounds derived from tea play a significant role in the antimicrobial properties of kombucha (Bhattacharya et al. 2016)"plainCitation":"(Bhattacharya et al. 2016, Fermentation has been shown to enhance the antimicrobial activity of kombucha when compared to unfermented tea, likely due to biotransformation and increased bioavailability of phenolic compounds (Cetojevic-Simin et al. 2008). Catechin derivatives, which represent approximately 30–40% of the dry weight of tea leaves, are among the key functional compounds contributing to these effects (Adriani et al., 2012). Their antimicrobial action occurs through multiple mechanisms, including disruption of bacterial membranes, damage to the cytoplasmic membrane, and the generation of hydrogen peroxide mediated by catechin fractions (Barbosa et al. 2022).

Beyond organic acids and phenolic compounds, other metabolites with antimicrobial potential can be produced during kombucha fermentation. Al-Mohammadi et al found nine chemical groups in kombucha responsible for inhibition of bacterial pathogens such as alkaloids, esters, fatty acids, aromatic aldehydes, lactone, alcohols, acids, condensed heterocyclic compounds and antibiotics. Alkaloids promote membrane disruption, rapid protein denaturation, nutrient leakage, metabolic dysfunction, and ultimately cell lysis. Esters and fatty acid esters reduce cell viability by causing extensive cellular disorganization and act as antibacterial food additives by inhibiting bacterial growth and biofilm formation. Aromatic aldehydes interact with the outer layers of bacterial cells, particularly unprotonated amines on the cell surface, thereby affecting ion transport and enzymatic systems. Lactones can penetrate microbial cells and inactivate sulfhydryl-containing enzymes essential for cellular replication. Additionally, heterocyclic compounds can interact with electrophilic or nucleophilic cellular components, leading to the inhibition of cell wall synthesis, protein synthesis, DNA replication, metabolic pathways, and compromising membrane integrity (Al-Mohammadi et al. 2021).

In synthesis, the antimicrobial activity of kombucha is the result of a synergistic interaction between organic acids, polyphenols, and diverse fermentation-derived metabolites. This chemical and microbial complexity underlies the broad-spectrum inhibitory effects of kombucha against pathogenic and spoilage microorganisms, highlighting its potential as a functional beverage and natural antimicrobial agent.

Antimicrobial Activity of Kombucha: Evidence from In Vitro Studies

Table 1 shows a synthesis of several studies published between 2016 and 2024 that explore the antimicrobial activity of kombucha against strains of gram-positive and gram-negative bacteria and fungi that are important in the agri-food sector. Several studies have demonstrated that kombucha exhibits stronger antibacterial activity against Gram-positive bacteria, particularly *Staphylococcus aureus* and *Bacillus cereus*, than against Gram-negative species. This trend was consistently reported across different substrates and fermentation conditions, including black tea, green tea, fruit-based kombuchas, and herbal formulations (Ayed et al., 2016; Ansari et al., 2017; Cardoso et al., 2020; Al-Mohammadi et al., 2021). Regarding Gram-negative bacteria, *Escherichia coli* appears to be the most susceptible species, whereas *Salmonella* spp. and *Shigella* spp. often show moderate to high resistance, depending on fermentation time and kombucha composition.

Substrate / Fermentation Conditions	Target Microorganisms	Main Findings	Reference
Grape juice kombucha (12 d, 30 °C); neutralized sample tested	Gram+ and Gram– bacteria	Strong inhibition; neutralization reduced activity (only *S. aureus* bacteriostatic)	Ayed et al., 2016
Black tea (21 d, 28 °C)	Enteric bacteria	Maximum antibacterial activity at 14–21 d	Bhattacharya et al., 2016
Banana peel, nettle, black tea (21 d)	Gram+ and Gram– bacteria	No antibacterial activity detected	Pure & Pure, 2016a
Garlic fermented in kombucha (1 month)	Gram+ and Gram– bacteria	Kombucha-fermented garlic more effective than vinegar; fresh garlic strongest	Pure & Pure, 2016b
Black tea (18 d)	Fungi (*A. flavus*, *C. albicans*, *M. gypseum*)	Inhibition observed; highest at early fermentation (6 d)	Yuniarto et al., 2016

Green tea (20–30 °C, 21 d)	*S. aureus*, *S. typhimurium*	Activity increased with fermentation time; no effect on *L. rhamnosus*	Ansari et al., 2017
Green/oolong tea (18 d, 28 °C)	Spoilage fungi	Moderate inhibition of *B. cinerea*; low or no effect on *Aspergillus* spp.	Matei et al., 2017
Black tea kombucha in chitosan film	*E. coli*, *S. aureus*	Enhanced antimicrobial activity in films; *E. coli* most sensitive	Ashrafi et al., 2018
Spiced kombucha (cinnamon, thyme, cardamom) (16 d, 28 °C)	Gram+ and Gram– bacteria	Cinnamon showed strongest inhibition; *E. coli* and *B. cereus* most susceptible	Shahbazi et al., 2018
Yarrow kombucha (7 d, 25 °C)	Bacteria and fungi	Strong antimicrobial activity (low MIC values)	Vitas et al., 2018
Snake fruit kombucha (14 d)	*S. aureus*, *E. coli*	Effective against both; stronger effect on *S. aureus*	Zubaidah et al., 2018
Green/black/oolong tea (15 d); neutralized & heated tested	Enteric bacteria	Fermented kombucha active; neutralized inactive	Kaewkod et al., 2019
Black tea (14 d)	*A. fumigatus* strains	Dose-dependent antifungal activity	Nazemi et al., 2019
Black tea (7 d); neutralized CFS tested	*A. flavus*, *A. carbonarius*	Significant inhibition; neutralized samples inactive	Taheur et al., 2019
Black or green tea (60 d)	Gram+ and Gram– bacteria	Activity increased with fermentation time; no effect on *B. subtilis*	Vohra et al., 2019
Green vs. black tea (10 d)	Foodborne pathogens	Green tea kombucha showed broader activity	Cardoso et al., 2020
Herbal kombucha (7–21 d, 25 °C)	Gram+ and Gram– bacteria	Activity dependent on tea type and sugar concentration	Valiyan et al., 2021
Fermented vs. neutralized vs. heat-treated	Bacteria and fungi	Only fermented kombucha showed strong activity	Al-Mohammadi et al., 2021

Black/green tea (15 d, 28 °C); compared with acetic acid	Enteric bacteria	*E. coli* most susceptible; neutralized inactive	Barbosa et al., 2021
Green tea (7 d); hibiscus second fermentation	*B. cinerea*	Strong inhibition (up to 88.9%)	Souza et al., 2023
Kombucha cellulose film (7 d, 25 °C)	*S. aureus*, *E. coli*	Clear inhibition zones (5–12 mm)	Shiri et al., 2024
Commercial teas (14–21 d, 25 °C)	*E. coli*, *S. aureus*, *S. enteritidis*	Gardenia-based kombucha most effective	Thenuwara et al., 2024

Table 1: Antibacterial and antifungal activity of kombucha produced under different fermentation conditions

The antimicrobial effectiveness of kombucha varies among bacterial species and is strongly influenced by differences in microbial susceptibility to its bioactive compounds. The variation in inhibitory effects observed against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enteritidis* reflects intrinsic differences in cell structure, metabolic activity, and resistance mechanisms, which determine the extent to which these microorganisms are affected by the antimicrobial agents present in tea-based kombucha.

Fermentation time is a key determinant of kombucha's antimicrobial potency. Several studies report a progressive increase in inhibitory activity between 7 and 21 days of fermentation, which coincides with the accumulation of organic acids and other secondary metabolites. In contrast, unfermented kombucha, as well as neutralized or heat-treated preparations, consistently exhibit little or no antimicrobial activity. These findings emphasize the importance of active fermentation and acidic conditions for the development and maintenance of antimicrobial efficacy (Kaewkod et al. 2019, Ben Taheur et al. 2020, Al-Mohammadi et al. 2021).

The loss of antimicrobial activity following neutralization to pH 7.0 further confirms the central role of acidity and pH-dependent mechanisms in kombucha-mediated inhibition. Similarly, thermal treatments reduce antimicrobial effectiveness, suggesting that heat-sensitive metabolites—alongside organic acids—are essential contributors to the overall antimicrobial profile of the beverage (Ayed et al. 2016, Kaewkod et al. 2019, Barbosa et al. 2022).

Although alternative substrates such as fruits, herbs, and spices have been investigated as strategies to enhance kombucha functionality, their antimicrobial performance is highly variable. While kombuchas prepared with substrates such as garlic, cinnamon, or yarrow demonstrated enhanced inhibitory effects, others—including banana peel- and nettle-based kombuchas—showed little to no antibacterial activity. These contrasting outcomes highlight the critical influence of substrate bioactive composition and its interaction with the fermentative microbiota on the antimicrobial potential of kombucha.

Differences in bacterial sensitivity may also be attributed to inherent resistance mechanisms and variations in cell wall composition, which can affect the penetration and action of antimicrobial compounds produced during fermentation (Thenuwara et al. 2024). Overall, the compiled evidence supports the potential of tea-based kombucha as a natural antimicrobial agent against a broad spectrum of bacterial pathogens. Importantly, its antimicrobial activity can be strategically integrated into food preservation systems as part of a multi-hurdle approach—combining pH control, refrigeration, and modified atmosphere packaging—to reduce microbial growth while minimizing reliance on intensive thermal processing.

Beyond antibacterial activity, kombucha has also demonstrated promising antifungal effects against food spoilage and mycotoxigenic fungi, including *Aspergillus flavus*, *A. carbonarius*, *Penicillium expansum*, and *Botrytis cinerea*. Nevertheless, antifungal efficacy appears to be more species-dependent and generally less pronounced than antibacterial activity, indicating distinct sensitivity patterns and potentially different mechanisms of inhibition.

FUTURE PERSPECTIVE

Despite kombucha's recognized antimicrobial power, its application as a biopreservative in food still faces several challenges. The main aspect relates to the marked lack of standardization in fermentation conditions, SCOBY microbial composition, substrate type, and analytical methodologies, which hinders reproducibility and makes direct comparison between studies difficult. A promising strategy would be the establishment of standardized fermentation protocols and antimicrobial evaluation methods, supported by advanced metagenomic and metabolomic approaches, to better correlate microbial dynamics with the production of bioactive compounds (Landis et al. 2022, Villarreal-Soto et al. 2018).

In addition, different substrates can be used for fermentation with SCOBY, which provides different types and concentrations of metabolites formed. Thus, the antimicrobial potential of kombucha may stem from a wide range of compounds, not only organic acids and phenolic compounds, but also from the synergy of these compounds with others developed during fermentation, such as alcohols, alkaloids, fatty acids, bacteriocins, among others (Bhattacharya et al., 2016; Barbosa et al., 2022). However, the relative contribution and mechanisms of action of each compound are not yet fully understood. Future studies should focus on compound fractionation, interaction assays, and molecular-level investigations to elucidate antimicrobial mechanisms and allow for targeted optimization of fermentation processes (Al-Mohammadi et al., 2021).

Another critical gap lies in the limited number of studies evaluating kombucha under real-world food system conditions. Most available data derive from in vitro assays, especially using agar diffusion and minimum inhibitory concentration techniques. These methods do not fully consider the complexity of food matrices, including buffering capacity, fat content, water activity, and interactions with native microbiota. Subsequently, future research should emphasize validation studies in various food products, such as meat, dairy products, bakery products, and fresh products, also evaluating sensory attributes and shelf-life extension under industrial storage conditions (Kaewkod et al., 2019; Martí-Quijal et al., 2021).

The antifungal and antimycotoxigenic potential of kombucha is still poorly explored. Although in vivo studies have shown that this research avenue is particularly promising, it remains largely unexplored (Taheur et al., 2019; Kilmanoglu et al., 2024). Future investigations should therefore evaluate the ability of kombucha-derived metabolites to reduce major mycotoxins, including aflatoxins, ochratoxin A, and fumonisins, in both in vitro and in vivo models, with careful assessment of the safety and stability of the resulting degradation products.

In addition, the use of kombucha in the composition of food films is recent and the results are promising; the results show that edible films, coatings, and active packaging materials based on kombucha can increase antimicrobial efficacy and allow the controlled release of bioactive compounds into the medium, increasing the shelf life of the product (Ashrafi et al., 2018; Shiri et al., 2024). Optimization and scaling up of these technologies could position kombucha as a key component of sustainable and biodegradable food preservation systems.

Finally, safety and regulatory considerations remain essential for industrial implementation. Future efforts should focus on defining safety limits, aligning production practices with regulatory frameworks, and developing kombucha-derived antimicrobial preparations that meet GRAS requirements (Siedler et al., 2019).

FINAL CONSIDERATIONS

Overall, kombucha holds significant potential as a multifunctional natural antimicrobial agent for food biopreservation. However, its successful integration into industrial food systems will depend on overcoming challenges related to standardization, mechanistic understanding, real-matrix validation, and regulatory compliance. By integrating advanced omics technologies, precision fermentation strategies, and multi-hurdle preservation approaches, kombucha may evolve from a traditional fermented beverage into a scientifically validated and sustainable solution for modern food preservation.

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