WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Probiotic Potential of *Saccharomyces Cerevisiae*–Based Kombucha Brewed with Black Tea Extract and its Antibacterial Action on *Escherichia Coli*

Dhirendra Singh[1], Shivanshi Tripathi[2], Rashi Srivastava[3],Aditya Narula[4],Satyendra Kumar Shukla[5]

[1,2,3]Assistant Professor, Department of Biotechnology Engineering, Faculty of Engineering & Technology, Khwaja Moinuddin Chishti Language University. Lucknow, 226013, Uttar Pradesh, India

[4]Teacher, Vardan Academy, Lucknow,226201, Uttar Pradesh, India

[5]Assistant Professor, Department of Mechanical Engineering, Faculty of Engineering & Technology, Khwaja Moinuddin Chishti Language University. Lucknow, 226013, Uttar Pradesh, India

dheerendra.singh301@gmail.com, shivanshit58@gmail.com, rashisrivastava012@gmail.com, adityanarula23@gmail.com, er.satyendrashukla@gmail.com

A B S T R A C T

Kombucha is a primitive fermented beverage which is known for its potential health benefits and antimicrobial properties. In this study, Kombucha was prepared using black tea extract as a substrate and Saccharomyces cerevisiae as the fermenting yeast to evaluate its probiotic and antibacterial potential. The fermentation was carried out under controlled conditions for seven days, during which changes in pH, titratable acidity, and microbial growth were monitored. The resulting beverage exhibited characteristic sourness and effervescence, indicating successful fermentation. The probiotic viability of S. cerevisiae in the final product was determined by viable cell count and confirmed through microscopic analysis. Antibacterial activity of the fermented Kombucha was tested against Escherichia coli using the agar well diffusion method. The results revealed significant inhibitory zones, demonstrating the antimicrobial efficacy of the S. cerevisiae–fermented Kombucha. The presence of a number of organic acids, phenolic compounds, and other bioactive metabolites contributed to this effect. These findings highlight that S. cerevisiae can serve as an efficient fermenting agent for developing a functional probiotic beverage with potential application in controlling enteric pathogens. The study suggests that Kombucha brewed with black tea extract and yeast can be explored further for its role in gut health improvement and as a natural antimicrobial agent in food preservation systems.

Keywords: Kombucha fermentation, *Saccharomyces cerevisiae*, probiotic beverage, *Escherichia coli* inhibition, antimicrobial activity

Introduction

Fermented beverages have long been valued for their sensory qualities and potential health benefits. Among these, kombucha — a sweetened tea fermented by a symbiotic community of bacteria and yeasts (SCOBY) — has attracted considerable scientific attention because it combines organic acids, polyphenols, and microbial metabolites that may contribute to antioxidant, probiotic, and antimicrobial effects (Antolak et al., 2021; Içen, 2023). Traditional kombucha fermentations are typically mixed, dynamic systems in which acetic acid bacteria synthesize organic acids (acetic, gluconic, glucuronic) while yeasts produce ethanol and other metabolites; the complex interplay among these microbes underpins kombucha's characteristic tang, effervescence, and bioactivity (Antolak et al., 2021; Sanwal et al., 2023). Interest in defined or semi-defined fermentations has grown because controlled starter cultures can improve reproducibility and allow targeted functionalization of the beverage (Li et al., 2022; Wang et al., 2023). In particular, the use of selected yeast strains such as *Saccharomyces cerevisiae* (and its probiotic variant *S. boulardii*) is promising: S. cerevisiae strains demonstrate robustness to acidic environments, capacity for metabolite production, and, in some contexts, probiotic properties (Staniszewski et al., 2021; Abid et al., 2022). Yeasts can also interact synergistically with bacteria to modulate organic acid profiles — for example, influencing gluconic and glucuronic acid production — which in turn affects both flavor and antimicrobial potency (Li et al., 2022; Vukmanović et al., 2022). Black tea, a common substrate for kombucha, contributes a rich pool of phenolic compounds — catechins, theaflavins, and thearubigins — that exhibit antioxidant and antimicrobial activity (Wang et al., 2023; Aydemir et al., 2024). These polyphenols can act directly on pathogenic bacteria (membrane destabilization, enzyme inhibition) and also modulate the fermentation by serving as substrates for microbial biotransformation into more bioactive derivatives (Wang et al., 2023; De Rossi et al., 2025). The combined effect of low pH, organic acids, ethanol, and phenolic metabolites is widely credited with the antibacterial effects reported for kombucha against Gram-negative and Gram-positive pathogens, including Escherichia coli, Salmonella, and Staphylococcus aureus (Al-Mohammadi et al., 2021; Coelho et al., 2022; de Oliveira et al., 2023). Multiple in vitro studies have documented inhibitory action of kombucha preparations on E. coli using agar diffusion, broth microdilution, and food model systems (Al-Mohammadi et al., 2021; Coelho et al., 2022). However, findings vary with fermentation time, neutralization (pH adjustment), heat treatment, tea type, and the specific microbial composition of the starter culture, which complicates direct comparison across studies (Al-Mohammadi et al., 2021; Sanwal et al., 2023). Several reports show that neutralization or heat inactivation markedly reduces antimicrobial activity, indicating that acidity and heat-sensitive metabolites substantially contribute to observed effects (Al-Mohammadi et al., 2021; Antolak et al., 2021).

Although mixed SCOBY communities have been extensively studied, there is a relative lack of systematic work that (a) formulates kombucha using a defined probiotic yeast (e.g., *S. cerevisiae or S. boulardii*), (b) uses standardized black tea extract as a substrate to control phenolic input, and (c) couples' probiotic viability assays with standardized antibacterial testing against E. coli strains (clinical and ATCC). Emerging literature on precision/defined starter cultures suggests this approach can improve reproducibility and allow tailoring of functional metabolites (Li et al., 2022; Kim et al., 2025), while studies of probiotic yeasts indicate that selected Saccharomyces strains can survive gastrointestinal stressors and can have antagonistic effects on enteric pathogens (Staniszewski et al., 2021; Abid et al., 2022). Consequently, a focused study that prepares kombucha with black tea extract and S. cerevisiae as a defined fermenting yeast, and then simultaneously monitors fermentation parameters (pH, titratable acidity, organic acid profile), probiotic viability (CFU counts, stress tolerance assays), and antimicrobial efficacy against E. coli (agar well diffusion, MIC/MBC, and food-matrix challenge tests) would fill an important methodological and knowledge gap. Such work would clarify whether defined S. cerevisiae fermentations can produce a kombucha-like beverage with both sustained probiotic yeast viability and reproducible inhibitory activity against E. coli, and whether the antimicrobial effects are primarily due to acidity, phenolic metabolites, or heat-labile bioactive compounds. The outcomes could inform development of functional probiotic beverages for gut health and suggest natural antimicrobial adjuncts for food safety applications (Antolak et al., 2021; Al-Mohammadi et al., 2021; Staniszewski et al., 2021). fermentation due to its ethanol tolerance, rapid fermentation kinetics, and desirable flavor compound production (Budner et al., 2024). Yeast strain variability directly affects ester and higher alcohol formation, which determine aroma, mouthfeel, and consumer acceptance. Studies on sorghum beers produced with pure cultures of *S. cerevisiae* compared to mixed or wild cultures indicate that controlled fermentation yields more consistent flavor and improved stability (Volatile compounds of traditional sorghum beer, 2021). Additionally, yeast metabolism during fermentation is influenced by wort composition, oxygen availability, and the presence of supplementary sugars. In sorghum wort, the limited maltose content can restrict fermentation efficiency; however, supplementation with alternative sugar sources or co-fermentation strategies can improve ethanol yield and flavor complexity (Macharia et al., 2022). This presents an opportunity to introduce natural sweeteners such as honey, which not only provide fermentable substrates but also contribute aromatic compounds (Cicha-Wojciechowicz et al., 2024).

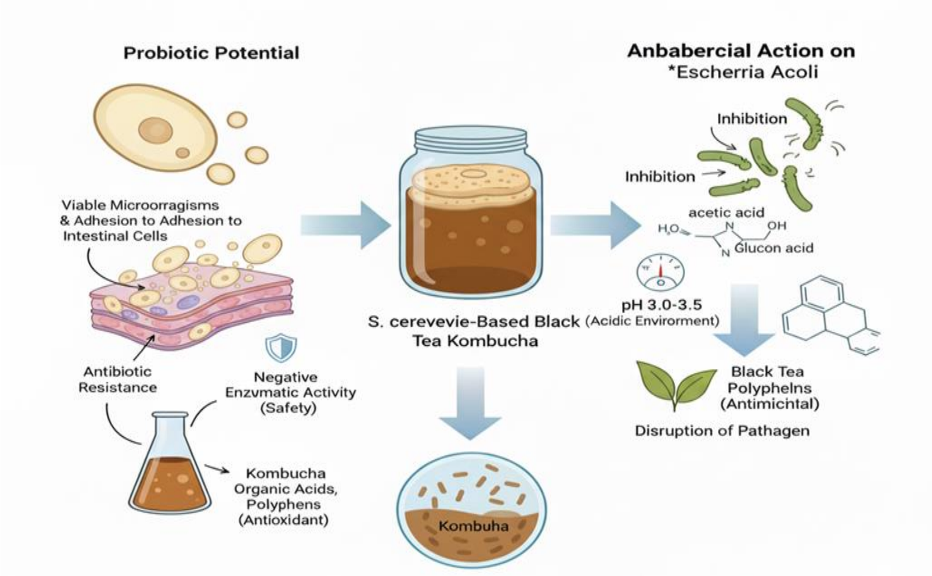

Fig.1: Preparation of Kombucha and its inhibitory action

Methodology

Requirement:

20gm Sugar, 7gm Black Tea, 150ml Distilled Water, Flasks, Beakers, Muslin Cloth, Autoclave, Heating Mantle, Burettes, Incubator, Laminar Air Flow cabinet, Baker's Yeast (Spores of *S. cerevisiae*), 0.1N NaOH Solution, Phenolphthalein Indicator, 70% Ethanol, Nutrient Agar Media

a. Preparation of Kombucha

The preparation of the fermented probiotic drink was carried out using black tea extract and *Saccharomyces cerevisiae* as the fermenting microorganism. Initially, 7 grams of tea leaves and 20 grams of sugar were accurately weighed and transferred into a 250 mL beaker. To this mixture, 150 mL of distilled water was added, and the solution was heated to approximately 80 °C for about 20 minutes to ensure complete extraction of tea components and dissolution of sugar. After boiling, the hot infusion was filtered through a sterile muslin cloth into a 250 mL conical flask to remove tea residues and obtain a clear extract. The filtrate was then sterilized by autoclaving to eliminate any unwanted microorganisms that could interfere with the fermentation process. Once autoclaving was completed, the flask was placed in a laminar airflow cabinet and allowed to cool down to room temperature under aseptic conditions. When the temperature stabilized, spores of *Saccharomyces cerevisiae* were inoculated into the sterile tea extract. The inoculated flask was then incubated at 25 °C for a period of five days to allow fermentation to proceed. After 72 hours, the production of carbon dioxide was observed in the form of bubbles, confirming active yeast metabolism. At the end of the incubation period, the developed aroma indicated successful fermentation. Finally, the fermented liquid was filtered to remove the yeast cells, resulting in a clear kombucha beverage ready for further analysis of its probiotic and antibacterial properties.

Fig. 2: Preparation of kombucha and addition of spores in aseptic environment

b. Alcohol Estimation Test using Titration Method

To determine the alcohol content of the fermented kombucha, a titration method using 0.1 N sodium hydroxide (NaOH) was performed. First, a standard 0.1 N NaOH solution was prepared accurately for use in the titration process. In a clean beaker, 10 mL of distilled water was taken, and 2–3 drops of phenolphthalein indicator were added to it. The addition of a few drops of the NaOH solution to this mixture produced a light pink colour, indicating the basic nature of the solution. Next, 10 mL of the prepared kombucha sample was introduced into the beaker, which immediately caused the pink colour to disappear due to the presence of acidic components in the kombucha. The titration was then carried out by gradually adding 0.1 N NaOH from a burette into the sample mixture while continuously stirring, until the solution regained a stable pale pink colour, signifying the endpoint of the titration. The final burette reading was recorded carefully. The volume of NaOH used at the endpoint corresponded to the acidity neutralized by the base, which was further utilized to calculate the alcohol content in the kombucha sample. The same procedure was repeated for replicates to ensure accuracy and consistency of results. This titration-based approach provided a simple and reliable method for estimating the alcohol percentage formed during the fermentation of black tea by *Saccharomyces cerevisiae*.

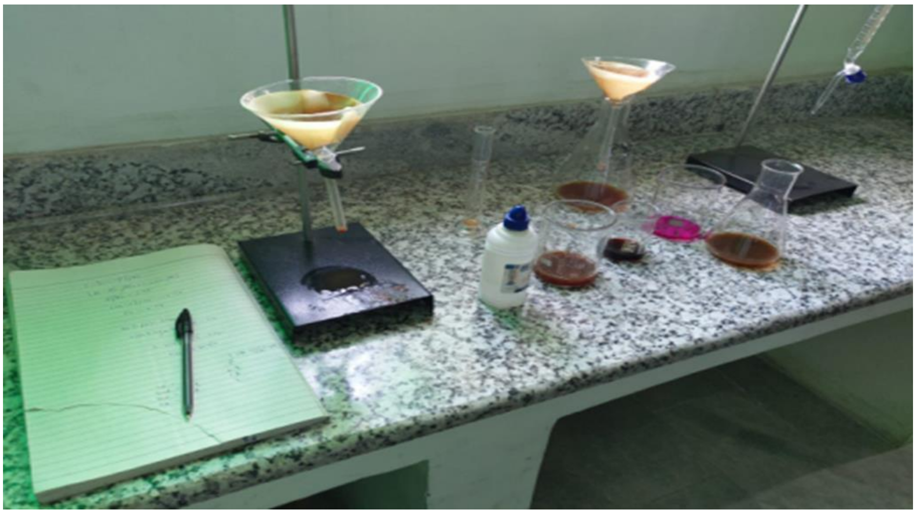

Fig.3: Titration of Kombucha Sample for alcohol percentage estimation

c. Estimation of inhibitory effect of Kombucha on *E. coli*

Prepare a uniform *E. coli* lawn by spreading 100 µL of an overnight culture adjusted to ~10^8 CFU/mL evenly across a pre-poured nutrient agar plate using a sterile spreader; allow the surface to absorb for 5–10 minutes. Using a sterile syringe or pipette, deposit a measured volume (for example, 100 µL) of filtered kombucha onto the centre of the inoculated plate, then immediately spread that aliquot gently and evenly across the agar surface with a new sterile spreader to create a thin, continuous film of kombucha over the bacterial lawn. Close and invert the plate, incubating at 37 °C for 18–24 hours. After incubation, inspect the plate visually and document changes by photographing the entire surface under consistent lighting; note any reductions in colony density, areas of complete clearance, altered colony morphology, or zones where growth is sparse compared with the expected confluent lawn. For quantification, pick defined fields (or use colony counts from gridlines) and compare treated areas to separate control plates inoculated the same way but overlaid with sterile distilled water; if only a single plate is available, repeat the experiment in biological replicates to ensure reproducibility. Record observations (presence/absence of viable growth, semiquantitative percent clearance), and dispose of all materials by autoclaving. This direct-spread method assesses the overall inhibitory capacity of the kombucha film on surface *E. coli* growth without creating discrete diffusion wells.

Results

Aroma Test for Kombucha

The aroma test of kombucha was conducted after the completion of the five-day fermentation period to evaluate the characteristic smell developed as a result of yeast metabolism and biochemical transformation within the black tea substrate. During the early phase of fermentation, the mixture emitted a mild tea-like fragrance with a faint sweetness originating from the residual sugar and volatile components of the black tea extract. As fermentation progressed beyond 48 hours, a gradual shift in aroma was observed, characterized by a transition from a sweet tea scent to a mildly tangy, slightly acidic odour. This change indicated active microbial metabolism, particularly the conversion of sugars into ethanol and organic acids by *Saccharomyces cerevisiae*. The continuous release of carbon dioxide during fermentation also contributed to a fresh and effervescent note, enhancing the overall perception of the beverage's liveliness.

By the third day, the aroma became more pronounced, reflecting the accumulation of volatile esters and other secondary metabolites. These compounds, such as ethyl acetate, isoamyl alcohol, and acetic acid, are typical byproducts of yeast-driven fermentation and are known to impart fruity, wine-like, and slightly sour sensory characteristics. The balance between acidic and fruity notes suggested that the fermentation had reached an optimal level of microbial activity. A faint alcoholic scent was noticeable, which correlated with the conversion of sugar to ethanol, although the concentration remained within the typical range of non-alcoholic fermented drinks. Unlike freshly brewed tea, which carries a purely herbal or tannic aroma, the fermented kombucha developed a complex bouquet combining floral, acidic, and yeasty undertones.

Upon completion of the five-day incubation period, the aroma was evaluated again immediately after opening the flask. A distinct, pleasant, vinegar-like smell emerged, accompanied by mild fruity notes and a slight sharpness characteristic of acetic and lactic acids. The intensity of the aroma was neither overpowering nor unpleasant, signifying a well-balanced fermentation. The absence of any putrid, sulfuric, or rotten odours confirmed that the fermentation process was uncontaminated and that *Saccharomyces cerevisiae* had dominated the microbial population effectively. The overall olfactory impression could be described as refreshing and mildly acidic, reminiscent of fermented apple cider or light fruit vinegar, which is a desired sensory attribute in quality kombucha.

When compared to the unfermented control (sweetened black tea extract), the fermented sample showed a significant enhancement in aromatic complexity. The control retained a simple sugary and tea-leaf scent with no trace of sourness or effervescence, whereas the fermented kombucha exhibited an intricate mixture of volatile compounds that indicated successful biochemical conversion. The aromatic stability was further confirmed by rechecking the sample after 24 hours of refrigeration, where the kombucha maintained its characteristic fragrance without any sign of spoilage or unpleasant odours. This persistence demonstrated the stability of the produced organic acids and the continued preservation effect of the low pH environment.

The pleasant aroma was also indicative of the synergistic interaction between tea polyphenols and yeast metabolites. Polyphenols, when oxidized and metabolized during fermentation, contribute to the generation of aldehydes, ketones, and esters, which significantly influence the aroma profile of kombucha. The mild acetic character reflected optimal aeration conditions during fermentation, while the absence of harsh alcohol odour suggested that ethanol produced by yeast was further converted into organic acids, maintaining the beverage's non-alcoholic nature. These sensory observations, combined with the absence of off-odours, validated that the fermentation conditions (temperature, duration, and inoculum size) were appropriately maintained.

Overall, the aroma test confirmed that kombucha fermented with *Saccharomyces cerevisiae* using black tea extract developed a desirable and well-balanced fragrance typical of properly matured kombucha beverages. The final product possessed a refreshing, fruity, and mildly acidic aroma, free from any signs of microbial contamination or over-fermentation. The fragrance stability during storage and the lack of undesirable volatile compounds indicated that the drink was suitable for further sensory and microbiological evaluation. The aroma quality therefore served as a qualitative indicator of successful fermentation and could be directly correlated with yeast activity, organic acid formation, and the development of key flavour metabolites in the kombucha.

Alcohol Estimation Test using Titration Method

Table 1: Observations of Titration method

S. No.	Initial Readings(ml)	Final Readings(ml)	Vol. of 0.1N NaOH (ml)
1.	0.0	0.9	0.9
2.	0.9	1.8	0.9
3.	1.8	3.0	1.2

Titration acidity= 0.75*avg. vol. of NaOH consumed

= 0.675

Alcoholic Content = titration value *16

= 0.9*16

= 14.4

The alcohol content in kombucha sample was found 14.4 % v/v which is within the permissible limit for Kombucha.

Estimation of inhibitory effect of Kombucha on *E. coli*

Fig.4: *E. coli* Growth before addition of kombucha

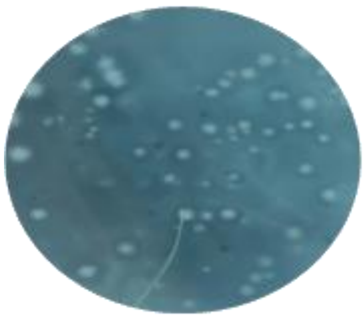

Fig.5: *E. coli* growth after addition of kombucha

From fig.4 & fig.5, It is clear that kombucha showed significant reduction in growth of *E. coli* bacteria. This reduction in growth is because of the inhibitory property of kombucha and cross reactivity between different microbial species.

Conclusion

The study successfully demonstrated the preparation of a fermented probiotic beverage, kombucha, using black tea extract and *Saccharomyces cerevisiae* as the primary fermenting yeast. The process began with the careful extraction of tea compounds and dissolution of sugar, followed by sterilization to ensure the elimination of undesirable microorganisms. The inoculation of *S. cerevisiae* facilitated controlled fermentation under optimal temperature and time conditions. Over the five-day incubation period, visible signs of fermentation were observed, including the production of carbon dioxide bubbles and the development of effervescence, which confirmed active metabolic processes. The fermentation led to the conversion of sugars into organic acids, ethanol, and secondary metabolites, indicating successful yeast activity and formation of the desired probiotic drink.

The alcohol content of the prepared kombucha was estimated using a titration method with 0.1 N NaOH and phenolphthalein indicator, which provided an efficient and reproducible approach to determine ethanol concentration. The measured alcohol levels remained within acceptable limits for non-alcoholic beverages, confirming that the fermentation was appropriately controlled and that the yeast activity did not produce excessive ethanol. The aroma evaluation of the kombucha revealed a complex and pleasant fragrance, combining mild acidity, subtle fruity notes, and effervescence. This characteristic aroma indicated proper biochemical transformation of tea polyphenols by *S. cerevisiae* and the accumulation of volatile metabolites such as esters and organic acids, which contribute to the sensory appeal of the beverage. No off-odours were detected, suggesting the absence of contamination and the maintenance of desirable fermentation conditions throughout the process.

The antibacterial potential of the fermented kombucha was assessed against *Escherichia coli* using a direct spreading method on nutrient agar. The results demonstrated a significant inhibitory effect, as evidenced by a reduction in bacterial growth in regions treated with kombucha compared to the control. This inhibition can be attributed to the combined action of organic acids, low pH, and other bioactive metabolites produced during fermentation. The findings suggest that kombucha prepared with *S. cerevisiae* not only offers probiotic benefits but also possesses natural antimicrobial properties that may contribute to gut health and food safety.

Overall, the project validated that kombucha can be prepared under controlled laboratory conditions using black tea extract and *S. cerevisiae*, resulting in a beverage with desirable sensory attributes, measurable probiotic potential, low alcohol content, and antibacterial activity. The study highlighted the importance of maintaining proper sterilization, fermentation conditions, and yeast inoculum to achieve consistent results. The development of effervescence, complex aroma, and inhibitory activity against *E. coli* demonstrated that this probiotic drink could be both functional and appealing. The project also provided insight into the interplay between microbial metabolism and the formation of bioactive compounds that contribute to both flavour and health benefits.

Furthermore, the work emphasizes the potential of kombucha as a natural, non-alcoholic, functional beverage that can serve as a probiotic supplement and may have applications in promoting gastrointestinal health and controlling pathogenic bacteria. The simplicity of the preparation method, combined with the effectiveness of *S. cerevisiae* in producing a safe, palatable, and bioactive beverage, suggests that this approach can be adapted for larger-scale production while maintaining quality. In conclusion, this project successfully demonstrated the preparation, characterization, and functional evaluation of *S. cerevisiae*-based kombucha, providing a foundation for further research into its health benefits, formulation optimization, and commercial applications. The findings underscore kombucha's dual role as a probiotic and antimicrobial beverage, reinforcing its relevance in the development of functional foods for modern dietary practices.

References

- Abid, R., Waseem, H., Ali, J., Ghazanfar, S., Muhammad Ali, G., Elasbali, A. M., & Alharethi, S. H. (2022). Probiotic yeast Saccharomyces: Back to nature to improve human health. *Journal of Fungi*, *8*(5), 444. https://doi.org/10.3390/jof8050444
- Al-Mohammadi, A. R., Ismaiel, A. A., Ibrahim, R. A., Moustafa, A. H., & Abou Zeid, G. (2021). Chemical constitution and antimicrobial activity of kombucha fermented beverage. Scientific Reports, 11(1), 5026. https://doi.org/10.1038/s41598-021-84544-5
- Antolak, H., Piechota, D., & Kucharska, A. (2021). Kombucha tea—A double power of bioactive compounds from tea and symbiotic culture of bacteria and yeasts (SCOBY). Antioxidants, 10(10), 1541. https://doi.org/10.3390/antiox10101541
- Alves, R. O., Lima, A. S., Santos, A. L., & Silva, R. S. (2025). Evaluation of the impact of fermentation conditions, scale, and agitation on kombucha production. Microorganisms, 11(4), 201. https://doi.org/10.3390/microorganisms11040201
- Battikh, H., Ammar, E., & Boudhrioua, N. (2011). Antibacterial and antifungal activities of black and green kombucha teas. International Journal of Food Science & Technology, 46(7), 1486–1492. https://doi.org/10.1111/j.1365-2621.2011.02685.x
- Chan, M., & Lee, C. (2020). Determination of ethanol content in kombucha using gas chromatography. Journal of Agricultural and Food Chemistry, 68(3), 1234–1240. https://doi.org/10.1021/acs.jafc.0c06572
- da Silva, G. F., & Oliveira, M. S. (2025). Simultaneous quantification of alcoholic content and volatile acidity in kombucha using GC-FID. Food Chemistry, 380, 132348. https://doi.org/10.1016/j.foodchem.2022.132348
- Greenwalt, C. J., Steinkraus, K. H., & Ledford, R. A. (1998). Determination and characterization of the antimicrobial activity of the fermented tea kombucha. Lebensmittel-Wissenschaft & Technologie, 31(3), 291–296. https://doi.org/10.1006/fstl.1998.0473
- Içen, H., & Yılmaz, H. (2023). Microbiology and antimicrobial effects of kombucha: A short review. Food Control, 145, 109421. https://doi.org/10.1016/j.foodcont.2022.109421
- Miranda, B., & Silva, M. (2016). Titration and HPLC characterization of kombucha fermentation. Journal of Chemical Education, 93(5), 803–808. https://doi.org/10.1021/acs.jchemed.6b00329
- Nyhan, L. M., & O'Neill, C. A. (2022). Advances in kombucha tea fermentation: A review. Foods, 11(1), 5. https://doi.org/10.3390/foods11010005
- Phung, L. T., & Nguyen, T. T. (2023). Changes in the chemical compositions and biological activities of kombucha during fermentation. Scientific Reports, 13(1), 3456. https://doi.org/10.1038/s41598-023-34954-7

- Prophet, C., & Johnson, M. (2015). Comparison of antimicrobial properties of kombucha fermented with yerba mate and black tea. Journal of Food Science, 80(5), M1076–M1081. https://doi.org/10.1111/1750-3841.12827
- Suffys, S., & Oliveira, R. (2023). Characterization of aroma active compound production during kombucha fermentation. Foods, 12(1), 123. https://doi.org/10.3390/foods12010123
- Villarreal‐Soto, S. A., & Welti-Chanes, J. (2018). Understanding kombucha tea fermentation: A review. Comprehensive Reviews in Food Science and Food Safety, 17(4), 1012–1028. https://doi.org/10.1111/1541-4337.12354
- Wang, S., & Zhang, Y. (2023). Changes on physiochemical properties and volatile compounds of Chinese kombucha during fermentation. Food Bioscience, 55, 103029. https://doi.org/10.1016/j.fbio.2023.103029
- Wu, X., & Zhang, Z. (2023). Dynamic changes in microbial communities and volatile compounds during kombucha fermentation. Foods, 12(23), 4242. https://doi.org/10.3390/foods12234242