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"ANALYTICAL APPROACHES FOR PROFILING BIOACTIVE COMPOUNDS IN KOMBUCHA: CURRENT STATUS AND FUTURE PERSPECTIVES"

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ABSTRACT

The fermented tea beverage known as kombucha has garnered increasing attention due to its abundance of bioactive components and potential health advantages. Many significant substances, including polyphenols, flavonoids, organic acids, vitamins, and microbial metabolites, are produced during fermentation by a symbiotic culture of bacteria and yeast. To comprehend the chemical makeup, quality, and functional usefulness of kombucha, it is crucial to identify and quantify these substances. For this goal, a variety of analytical methods have been employed, such as UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS). This review covers standard sample preparation and extraction methods as well as the main analytical approaches used to investigate bioactive chemicals in kombucha. Additionally, it emphasizes new developments and cutting-edge technology that could enhance further study in this area. To give a detailed picture of the state of kombucha today, essential conclusions from earlier research and significant analytical techniques are also compiled analysis.

KEYWORDS: Kombucha; Bioactive compounds; Analytical techniques; High-performance liquid chromatography (HPLC); Mass spectrometry; Metabolomics; Fermentation; Phenolic compounds; Spectroscopic analysis; Quality control.

INTRODUCTION

A symbiotic culture of bacteria and yeast (SCOBY) in a sugared tea media produces kombucha, a Fermented herbal drink. Due to its designation as a functional beverage with possible health benefits, kombucha has attracted more scientific and commercial attention in In the past few years. Numerous bioactive substances, like as polyphenols, organic acids, vitamins, amino acids, and microbial metabolites created during fermentation, are probiotics in the drink are the main reason for these benefits.[1] Significant biochemical changes occur during the fermentation of sweetened tea, producing substances including acetic acid, gluconic acid, glucuronic acid, catechins, flavonoids, and other antioxidant molecules that support kombucha's Effects on living organisms.

Multiple factors, including as the kind of tea, sugar concentration, length of fermentation, microbial composition, and ambient conditions, may affect how much of these good chemicals are present and what they contain.[2] Reliable analytical methods are necessary for the precise identification and quantification of these chemicals because of the complexity of the kombucha matrix and the variety of metabolites produced during fermentation. The chemical informaton of kombucha has been characterized using a analytical techniques. While high-performance/Pressure liquid chromatography (HPLC) makes it possible to separate and quantify individual phenolic compounds and organic acids and conventional techniques like UV–visible spectrophotometry. Uv Spectroscopy are frequently employed to determine or detect total phenolic content and antioxidant activity. Furthermore, for thorough metabolomic profiling and structural identification of bioactive compounds in fermented beverages, olive analytical methods such as nuclear magnetic resonance (NMR), gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS) have been used and maintain properly.[3]

Kombucha's complex fermentation matrix and dynamic metabolic processes create it difficult to completely characterize its beneficial components, including tremendous advancements in maine analytical techniques. Therefore, to more/ increase the sensitivity, selectivity, and accuracy of chemical detection, ongoing development of sophisticated analytical techniques is need.Due to their potential to improve gut flora and promote human health, fermented beverages have garnered more interest in pastly years. But its distinct fermentation process and the variety of metabolites generated during microbial activity, kombucha has become one of these beverages' most well-liked functional drinks. Acetic acid bacteria further oxidize ethanol into organic acids such as acetic acid and gluconic acid, while yeasts ferment sucrose into ethanol and carbon dioxide. Numerous bioactive substances are formed as a result of these metabolic changes, which give kombucha its distinctive taste, aroma, and possible health benefites.[4]

Kombucha is in number of other physiologically active substances, such as vitamins, amino acids, and microbial enzymes, in added to organic acids and polyphenolic compounds. These substances have been attached to a number of biological functions, including detoxifying, anti-inflammatory, antioxidant, and antibacterial properties. althrough, the microbial consortia in the SCOBY, the length of the fermentation process, the temperature, and the kind of tea employed as the substrate can all have a substantial impact on the concentration and makeup of these metabolites.[5] consequently, a thorough analytical assessment is necessary to gain a deeper understanding of kombucha's chemical makeup and biological potential.

Better instruments for the thorough characterisation of bioactive substances in intricate food matrices have been made possible by few day before developments in analytical chemistry. The sensitivity and selectivity of compound identification have been increased because to contemporary analytical platforms that combine chromatographic divided in with spectroscopic and mass spectrometric detection. The metabolic profile of fermented beverages, in that kombucha, is increasingly being work using methods like NMR spectroscopy, LC–MS/MS, GC–MS, and HPLC combined with diode array detection.[2] These cutting-edge methods permission researchers to assess the nutritional and physiological qualities of kombucha, track fermentation dynamics, and discover less metabolites more precisely.

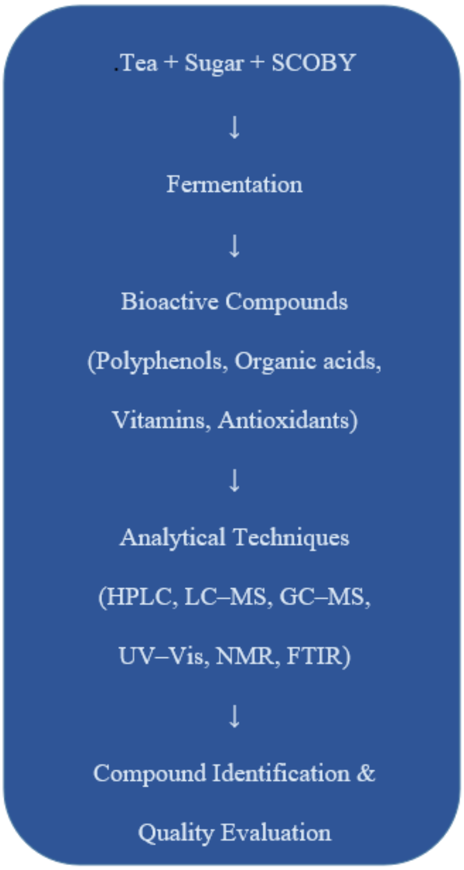

Figure No. 1: Schematic Overview of Kombucha Fermentation, Bioactive Compounds, And Analytical Techniques Used For Their Determination.

BIOACTIVE COMPOUNDS IN KOMBUCHA

Because a symbiotic culture of bacteria and yeast (SCOBY) ferments sweetened tea to produce a variety of bioactive chemicals, kombucha is widely acknowledged as a functional fermented beverage. Both the tea substrate and the microbial metabolic processes that take place during fermentation are the sources of these chemicals. Polyphenols, organic acids, vitamins, amino acids, and microbial metabolites are the main types of bioactive chemicals found in kombucha. These components work together to provide the beverage its antioxidant, antibacterial, and health-promoting qualities.[1]

In kombucha, polyphenolic compounds are among the most significant classes of bioactive chemicals. These substances, which include gallic acid, catechins, epicatechins, and other flavonoids, are mostly obtained from tea leaves.

Enzymatic processes and microbial metabolism can change these substances into new metabolites with increased biological activity during fermentation. According to studies, the fermentation process may boost kombucha's antioxidant activity because it breaks down complex polyphenols into simpler phenolic compounds with greater bioavailability.[2]

Another important class of molecules found in kombucha are organic acids. Acetic acid, gluconic acid, glucuronic acid, lactic acid, and malic acid are the most often reported organic acids. These acids are mostly created by acetic acid bacteria during fermentation. These organic acids are linked to a number of biological activities, such as antibacterial properties and detoxifying processes in the human body, and they also contribute to the distinctively sour flavor of kombucha.[6]

Because of its possible involvement in detoxification processes, glucuronic acid has drawn special interest among these substances.

B-complex vitamins, amino acids, enzymes, and volatile compounds produced during fermentation are among the additional biologically active substances found in kombucha in addition to polyphenols and organic acids. The type of tea, the length of the fermentation process, the microbial makeup of the SCOBY, and environmental elements like temperature and oxygen availability can all affect the concentration of these chemicals.[4]

Table No. 1: Major Bioactive Compounds Identified in Kombucha and Their Analytical Determination Methods.

Bioactive Compound	Chemical Class	Analytical Technique Used	Typical Instrument	Reference
Catechin	Flavonoid	HPLC-UV / LC–MS	HPLC system with UV detector	1
Epicatechin	Flavonoid	HPLC / LC–MS/MS	HPLC–MS	2
Gallic acid	Phenolic acid	HPLC-UV	Reverse-phase HPLC	3
Caffeic acid	Phenolic acid	HPLC / LC–MS	HPLC-MS	4
Glucuronic acid	Organic acid	HPLC / GC-MS	HPLC-RID or GC-MS	5
Acetic acid	Organic acid	HPLC / GC-MS	GC-MS	6
Gluconic acid	Organic acid	HPLC	HPLC-UV or RID	7
Ethanol	Fermentation metabolite	GC-MS / GC-FID	Gas chromatography	8
Vitamin C (Ascorbic acid)	Vitamin	HPLC-UV	RP-HPLC	9
Polyphenols (total)	Antioxidant compounds	UV–Vis spectrophotometry	UV-Vis spectrometer	10

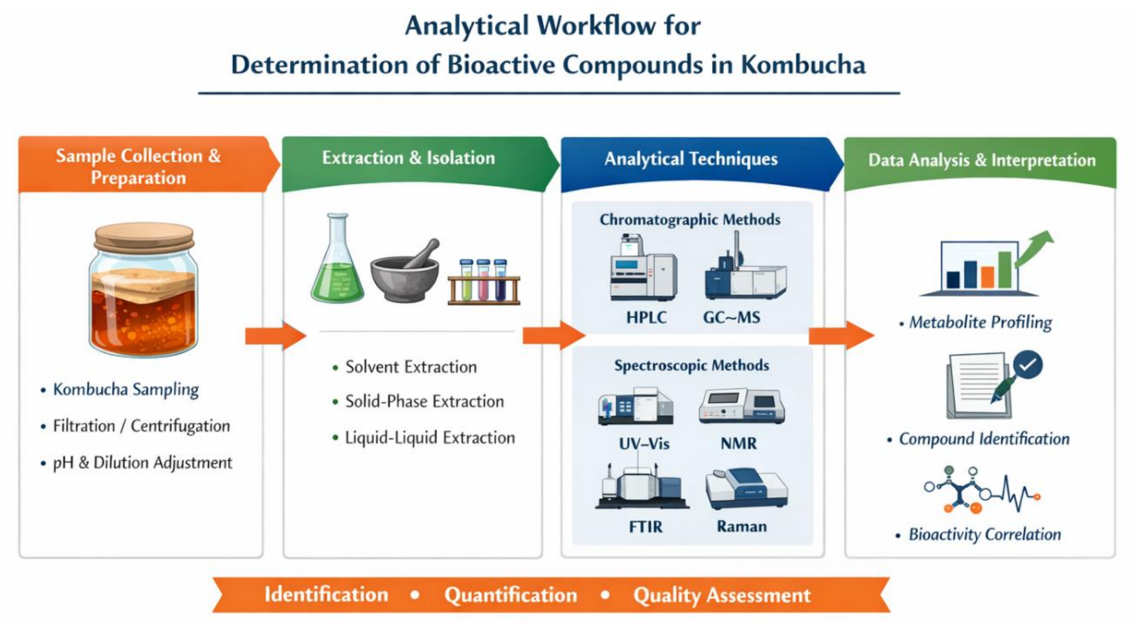

Figure No. 2: Analytical workflow for determination of bioactive compounds in kombucha.

"A number of studies have used various analytical techniques to quantify the bioactive compounds present in kombucha. Table 2 summarizes the reported concentration ranges of key compounds from various studies."

Table No. 2: Reported Concentrations of Bioactive Compounds in Kombucha from Different Studies.

Bioactive Compound	Concentration Range	Analytical Method	Kombucha Substrate	Reference
Gallic acid	5–45 mg/L	HPLC-UV	Black tea kombucha	Jayabalan et al., 2014
Catechin	10–120 mg/L	HPLC	Green tea kombucha	Villarreal-Soto et al., 2018
Epicatechin	8–95 mg/L	LC–MS	Green tea kombucha	Coton et al., 2017
Glucuronic acid	0.5–2.3 g/L	HPLC	Traditional kombucha	Jayabalan et al., 2014
Acetic acid	1–10 g/L	HPLC / GC	Fermented tea	Villarreal-Soto et al., 2018
Gluconic acid	2–15 g/L	HPLC	Black tea kombucha	Chakravorty et al., 2016
Ethanol	0.1–1.5 % (v/v)	GC-MS	Kombucha beverage	Coton et al., 2017
Total phenolic content	200–1200 mg GAE/L	UV–Vis spectrophotometry	Tea kombucha	Jayabalan et al., 2014
Ascorbic acid	20–60 mg/L	HPLC	Fruit-based kombucha	Chakravorty et al., 2016

Current Analytical Techniques for Determination of Bioactive Compounds in Kombucha

Organic acids, polyphenols, flavonoids, vitamins, and microbial metabolites are just a few of the bioactive substances found in kombucha, a fermented beverage. To comprehend the nutritional value, safety, and functional qualities of kombucha, these substances must be accurately determined. These chemicals have been characterized during the last ten years using a variety of analytical techniques, from sophisticated chromatographic and mass spectrometric approaches to traditional spectrophotometric methods.

The most popular methods for determining the bioactive chemicals in kombucha both qualitatively and quantitatively are spectroscopic techniques, chromatographic methods, and hyphenated analytical techniques. These techniques are appropriate for complicated fermentation matrices because they offer great sensitivity, selectivity, and reproducibility.

Table No. 3: Common Analytical Techniques Used for Determination of Bioactive Compounds in Kombucha.

Analytical technique	Target compounds	Advantages	Limitations
UV–Visible spectroscopy	Total phenolics, antioxidants	Simple, low cost	Low specificity
HPLC	Phenolic compounds, organic acids	High accuracy and sensitivity	Requires sample preparation
LC–MS/MS	Metabolites, polyphenols	High selectivity and identification capability	Expensive instrumentation
GC–MS	Volatile compounds, organic acids	High resolution for volatile compounds	Requires derivatization
NMR spectroscopy	Metabolite profiling	Structural identification	Lower sensitivity compared to MS

SAMPLE PREPARATION AND EXTRACTION METHODS FOR BIOACTIVE COMPOUNDS IN KOMBUCHA[7,8]

The analytical identification of bioactive components in kombucha requires careful sample preparation and extraction. Effective extraction techniques are necessary to get accurate analytical data because of the complex content of this fermented beverage, which includes organic acids, polyphenols, flavonoids, sugars, vitamins, and microbial metabolites. The recovery, stability, and detection of target chemicals are greatly impacted by the choice of suitable extraction methods and solvents.

Prior to instrumental analysis, sample preparation for kombucha analysis often entails filtration, centrifugation, dilution, and extraction procedures. The goal of these processes is to eliminate particles, microorganisms, and chemicals that could interfere with spectroscopic or chromatographic measurements.

i. Filtration and Centrifugation

In kombucha, filtration and centrifugation are frequently employed as initial sample preparation methods. analysis. Kombucha that has fermented Samples frequently include insoluble particles, cellulose made by acetic acid bacteria, and suspended microbial biomass. Thus, centrifugation at high speeds or filtration through membrane filters (usually 0.22 µm or 0.45 µm) are required prior to chromatographic analysis.

These procedures enhance analytical accuracy and reproducibility while preventing column clogging in chromatographic systems like HPLC and LC-MS. Centrifugation and membrane filtering are often carried out before organic acids and phenolic compounds are determined.

ii. Solvent Extraction

One of the most popular techniques for separating bioactive substances from kombucha is solvent extraction. Depending on the polarity of the target molecules, organic solvents like methanol, ethanol, acetone, and water mixes are commonly employed.

Aqueous methanol or ethanol solutions are commonly used to extract phenolic compounds and flavonoids because of their high polarity and superior solubility in these solvents. Typically, the extraction procedure include blending the kombucha sample with the solvent, then sonication, shaking, or vortexing to improve component recovery. Following extraction, the samples are centrifuged, and the supernatant is gathered for further chromatographic or spectroscopic analysis.

Solvent extraction techniques are comparatively easy and efficient, but in order to enhance extraction efficiency and reduce compound degradation, extraction conditions and solvent selection must be tuned.

iii. Solid Phase Extraction (SPE)

For the concentration and purification of bioactive components in kombucha, solid phase extraction is a commonly used sample cleanup method. In chromatographic analyses, SPE is very helpful for eliminating interfering substances and increasing detection sensitivity.

This method involves passing the kombucha extract through a cartridge that contains a particular sorbent substance, like polymeric phases or C18. While undesirable components are washed away, target compounds are kept on the sorbent. After that, the analytes are eluted using the appropriate solvents in preparation for HPLC or LC-MS analysis.

Reduced solvent usage, increased analytical sensitivity, and greater selectivity are just a few benefits of SPE. As a result, investigations looking into phenolic compounds and other secondary metabolites in fermented beverages often use it.

iv. Advanced Extraction Techniques

Advanced extraction methods such solid-phase microextraction (SPME), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) have been investigated recently for the extraction of bioactive components from food and beverage matrices, including kombucha.

By using sonic cavitation to facilitate mass transfer, ultrasound-assisted extraction shortens extraction times and increases extraction efficiency. Microwave energy is used in microwave-assisted extraction to speed up solvent penetration and chemical release from the sample matrix.

When compared to traditional solvent extraction techniques, these contemporary extraction techniques offer greater efficiency, reduced solvent use, and quicker processing times, making them appealing substitutes for the examination of bioactive chemicals in fermented drinks.

CHROMATOGRAPHIC AND SPECTROSCOPIC TECHNIQUES FOR BIOACTIVE COMPOUND ANALYSIS[9-13]

The identification, isolation, and measurement of the bioactive chemicals found in kombucha depend heavily on chromatographic and spectroscopic methods. This fermented beverage's complex chemical makeup necessitates the use of sophisticated analytical techniques to precisely describe its metabolites. For the analysis of phenolic compounds, organic acids, vitamins, and other metabolites found in kombucha, methods like high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR), and UV–visible spectroscopy are frequently used.

One of the most popular methods for identifying organic acids and phenolic chemicals in kombucha is high-performance liquid chromatography (HPLC). Compounds like catechins, gallic acid, caffeic acid, and other polyphenols generated from tea substrates can be separated and quantified using HPLC in conjunction with UV or diode array detectors (DAD). The method is very useful for routine kombucha since it has great sensitivity, good repeatability, and the capacity to evaluate several chemicals at once. Analysis.

For the thorough analysis of bioactive chemicals in fermented beverages, liquid chromatography combined with mass spectrometry (LC–MS) has become a potent method. Unknown metabolites produced during fermentation can be identified thanks to this method's high sensitivity and structural information. For profiling complex phenolic chemicals, flavonoids, and microbial metabolites that could be difficult to identify using traditional chromatographic techniques, LC–MS and LC–MS/MS procedures are particularly helpful.

For the measurement of volatile chemicals and certain organic acids in kombucha, gas chromatography (GC) is frequently employed in conjunction with mass spectrometry (GC–MS). Kombucha's distinctive flavor and scent are influenced by volatile substances such ethanol, acetic acid derivatives, and aromatic chemicals. GC-MS is a crucial method for flavor and metabolite profiling because it offers high resolution and accurate detection of volatile metabolites.

Spectroscopic methods are also crucial for kombucha analysis. bioactive substances. Using colorimetric tests like the Folin-Ciocalteu method, UV-visible spectroscopy is frequently used to quickly determine total phenolic content and antioxidant activity. This method offers a rapid and economical alternative for preliminary investigation of phenolic chemicals, although being less specific than chromatographic approaches.

Another potent analytical method for the structural clarification of metabolites in intricate matrices like kombucha is nuclear magnetic resonance (NMR) spectroscopy. Without requiring a lot of sample preparation, NMR offers comprehensive information about molecular composition and structure. Additionally, the use of NMR-based metabolomics techniques to examine metabolic alterations during kombucha has grown. fermentation and to find new bioactive substance.

a. High-Performance Liquid Chromatography (HPLC)

One of the most popular analytical methods for identifying the bioactive components in kombucha is high-performance liquid chromatography (HPLC). This method makes it possible to effectively separate, identify, and measure organic acids, phenolic compounds, and other fermentation-related metabolites. C18 columns are frequently used in reverse-phase HPLC (RP-HPLC), which effectively separates flavonoids and phenolic acids generated from tea leaves.

Compounds including gallic acid, catechins, caffeic acid, chlorogenic acid, and other polyphenols found in kombucha are often quantified using HPLC in conjunction with ultraviolet (UV) or diode array detection (DAD). By enabling simultaneous monitoring at several wavelengths, DAD detectors enhance compound identification and detection sensitivity.

Additionally, HPLC provides high reproducibility and accuracy, making it suitable for routine quality control and compositional analysis of fermented beverages.

b. Liquid Chromatography–Mass Spectrometry (LC–MS)

For the thorough evaluation of bioactive chemicals in complex matrices like kombucha, liquid chromatography combined with mass spectrometry (LC–MS) has emerged as a crucial analytical method. This method combines the high sensitivity and structural identification offered by mass spectrometry with the separation power of liquid chromatography. For the purpose of identifying unknown metabolites produced during the fermentation process, LC–MS and LC–MS/MS technologies are especially helpful. These techniques make it possible to find low-concentration substances such microbial metabolites, phenolic derivatives, and flavonoids. Advanced mass spectrometers' high resolution and precise mass measurements greatly enhance the discovery of new bioactive compounds and offer a greater understanding of the metabolic changes taking place during kombucha fermentation.

c. Gas Chromatography–Mass Spectrometry (GC–MS)

Kombucha's volatile components and aroma-related metabolites are frequently analyzed using gas chromatography coupled with mass spectrometry (GC–MS). The distinctive flavor and scent of the beverage are mostly attributed to volatile organic molecules produced during fermentation.

Compounds like ethanol, acetic acid, esters, aldehydes, and other volatile metabolites can be identified and quantified using GC-MS. Sample preparation methods like solid-phase microextraction (SPME) or headspace analysis are frequently used to separate volatile chemicals before GC analysis. GC-MS is a useful instrument for analyzing flavor profiles in kombucha products and researching metabolic changes associated with fermentation due to its excellent sensitivity and resolution.

d. UV–Visible Spectroscopy

A quick and easy method for the initial examination of bioactive substances in kombucha is UV-visible spectroscopy. It is frequently used to measure antioxidant activity, total phenolic content, and other colorimetric tests.

Assays like DPPH or ABTS are used to assess antioxidant capability, whereas techniques like the Folin-Ciocalteu assay are commonly employed to measure total phenolic content. UV-visible spectroscopy has advantages such as low cost, minimum sample preparation, and quick analysis, but it lacks the specificity of chromatographic procedures. As a result, it is frequently employed in conjunction with chromatographic techniques as a supplementary procedure.

e. Nuclear Magnetic Resonance (NMR) Spectroscopy

A potent analytical method for metabolomic investigation and structural clarification of intricate biological materials is nuclear magnetic resonance (NMR) spectroscopy. NMR has been used in kombucha research to detect and measure a variety of metabolites, including as sugars, amino acids, organic acids, and other fermentation products.

The fact that NMR allows for non-destructive sample analysis and requires little sample preparation is one of its main benefits. Additionally, thorough analysis of metabolic changes during fermentation is made possible by NMR-based metabolomics techniques, which offer important insights into the biochemical pathways involved in kombucha production.

The bioactive chemicals in kombucha have been analyzed using a variety of spectroscopic and chromatographic methods. Table No. 4 provides a comparative summary of different analytical techniques, including their benefits and drawbacks.

Table No. 4: Comparison of Analytical Techniques Used for Determination of Bioactive Compounds in Kombucha.

Analytical Technique	Target Compounds	Advantages	Limitations	Typical Applications
UV–Visible Spectroscopy	Phenolics, organic acids, antioxidant compounds	Simple, rapid, low cost	Low selectivity and sensitivity	Total phenolic content, antioxidant activity assays
HPLC-UV	Organic acids, polyphenols, catechins	High resolution, good reproducibility	Requires standards and longer analysis time	Quantification of phenolic compounds and organic acids
LC–MS/MS	Polyphenols, flavonoids, metabolites	High sensitivity and selectivity; structural identification	Expensive instrumentation and complex data analysis	Comprehensive metabolite profiling
GC–MS	Volatile compounds, organic acids	High sensitivity for volatile metabolites	Requires derivatization for non-volatile compounds	Aroma compound analysis
NMR Spectroscopy	Sugars, organic acids, metabolites	Non-destructive and highly reproducible	Lower sensitivity compared with MS-based methods	Metabolomics and structural elucidation
FTIR Spectroscopy	Functional groups in biomolecules	Rapid and minimal sample preparation	Limited quantitative capability	Fingerprint analysis and quality control

ADVANCED ANALYTICAL APPROACHES AND FUTURE RESEARCH DIRECTIONS[14-18]

Analytical technologies for the study of bioactive chemicals in fermented drinks like kombucha have advanced significantly in recent years. Even though conventional methods like UV-visible spectroscopy, gas chromatography–mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC) are still frequently used, contemporary analytical techniques are being used more frequently to gather more precise information about the intricate chemical makeup of kombucha.

The use of analytical systems based on metabolomics is one of the most promising advancements in this field. The thorough examination of small molecule metabolites in biological systems is known as metabolomics, and it offers important information about the metabolic changes that take place during fermentation. The detection of many metabolites, including as polyphenols, organic acids, amino acids, and microbial metabolites, has been made possible by the combination of liquid chromatography with high-resolution mass spectrometry (HRMS).

These methods enable the identification of hitherto unknown bioactive chemicals and aid researchers in comprehending the metabolic pathways involved in kombucha fermentation.

The advancement of biosensor technology and quick detection systems is another new trend. The detection of phenolic compounds and antioxidant molecules in fermented beverages has shown significant promise using electrochemical and optical biosensors. High sensitivity, quick analysis times, and less sample preparation are some benefits of these analytical tools. Additionally, prospects for on-site beverage quality and safety monitoring are provided by the integration of biosensors with portable devices and smartphone-based systems.

Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy are two vibrational spectroscopic techniques that have drawn interest as quick screening methods for food and beverage analysis. These techniques produce molecular fingerprints that can be used to assess kombucha's fermentation stages and compositional variations. samples. These spectroscopic methods can reliably classify and quantify chemical constituents without requiring a lot of sample preparation when used in conjunction with chemometric analysis.

Additionally, the interpretation of complicated analytical datasets has been substantially improved by the incorporation of data science techniques like chemometrics and machine learning. Large datasets from spectroscopic and chromatographic methods can be analyzed using machine learning algorithms, allowing for more precise metabolite identification, prediction, and classification.

In food chemistry research, these computational tools are being employed more and more to increase analytical dependability and efficiency.

Even with the availability of sophisticated analytical tools, there are still a number of obstacles to overcome in order to fully characterize kombucha. Significant variations in chemical composition can result from variations in raw materials, fermentation conditions, microbial communities, and processing parameters. Therefore, in order to gain a more thorough understanding of kombucha, future research should concentrate on developing standardized analytical approaches and integrating various analytical techniques.

Bioactive chemicals in kombucha have been identified and quantified using a variety of analytical techniques. Table 5 summarizes a comparison of the popular methods together with their benefits and drawbacks.

Table No 5: Advantages and Limitations of Analytical Techniques Used for Kombucha Analysis.

Analytical Technique	Target Compounds	Advantages	Limitations	Reference
HPLC-UV	Polyphenols, organic acids, catechins	High accuracy, good separation, widely available	Requires sample preparation and standards	Jayabalan et al., 2014
HPLC-MS / LC-MS	Phenolic compounds, metabolites	Very high sensitivity and selectivity; structural identification possible	Expensive instrumentation and complex operation	Villarreal-Soto et al., 2018
GC-MS	Volatile compounds, ethanol, organic acids	Excellent for volatile metabolite profiling; high sensitivity	Requires derivatization for non-volatile compounds	Chakravorty et al., 2016
UV–Vis Spectrophotometry	Total phenolic content, antioxidant assays	Simple, rapid, low cost	Limited specificity; interference from other compounds	Jayabalan et al., 2014
FTIR Spectroscopy	Functional groups, overall metabolite fingerprint	Rapid, non-destructive, minimal sample preparation	Limited quantitative accuracy for individual compounds	Coelho et al., 2020
NMR Spectroscopy	Metabolites, organic acids, sugars	Detailed structural information; reproducible	High cost and lower sensitivity compared with MS	Villarreal-Soto et al., 2018

CHALLENGES, STANDARDIZATION, AND RESEARCH GAPS IN KOMBUCHA ANALYTICAL STUDIES[19-21]

Kombucha is a complex fermented beverage whose chemical composition varies greatly depending on a number of factors, including the type of tea substrate, sugar concentration, fermentation time, microbial community composition, and environmental conditions. These variations can significantly influence the concentration and profile of bioactive compounds, making analytical comparisons between different studies difficult. One major challenge in kombucha research is the lack of standardized analytical protocols; different studies frequently use different sample preparation methods, extraction techniques, and analytical conditions, which can result in inconsistent reported concentrations of bioactive compounds.

For instance, the quantification of organic acids and phenolic substances may be impacted by variations in detection wavelengths, chromatographic columns, and solvent systems. Therefore, increasing the reproducibility and comparability of results among laboratories requires the creation of standardized analytical processes.

The complexity of the kombucha matrix, which includes a variety of metabolites such polyphenols, organic acids, carbohydrates, vitamins, amino acids, and microbial byproducts, is another significant problem. This complicated makeup can make it more difficult to accurately identify specific molecules and interfere with analytical results. Although chemical identification has improved thanks to advanced analytical techniques like high-resolution mass spectrometry and metabolomics methodologies, it is still difficult to fully profile all of the metabolites in kombucha.

Analytical challenges are also brought forth by the fermentation process' dynamic character. Microbial activity constantly modifies the beverage's chemical makeup throughout fermentation, resulting in the creation of new metabolites and the breakdown of preexisting molecules. Kombucha's chemical profile can therefore alter dramatically over time. Therefore, to comprehend these metabolic alterations and their effects on product quality and bioactivity, ongoing monitoring utilizing trustworthy analytical techniques is required.

Furthermore, nothing is known about the relationship between particular bioactive components and the health advantages of consuming kombucha. While antioxidant, antibacterial, and anti-inflammatory qualities have been documented in a number of studies, the precise chemicals causing these effects are not always well understood.

To elucidate these interactions, further focused analytical research combining chemical analysis with biological activity assessments is needed.

In order to gain a more thorough understanding of the composition of kombucha, future study should also concentrate on integrating various analytical approaches. Combining spectroscopic, metabolomic, and chromatographic methods can yield complementary data and increase the precision of compound identification. Furthermore, complicated datasets produced by contemporary analytical instruments can be analyzed with the use of chemometric and machine learning methods.

“The major research gaps in the analytical characterization of kombucha are summarized in Figure 3”.

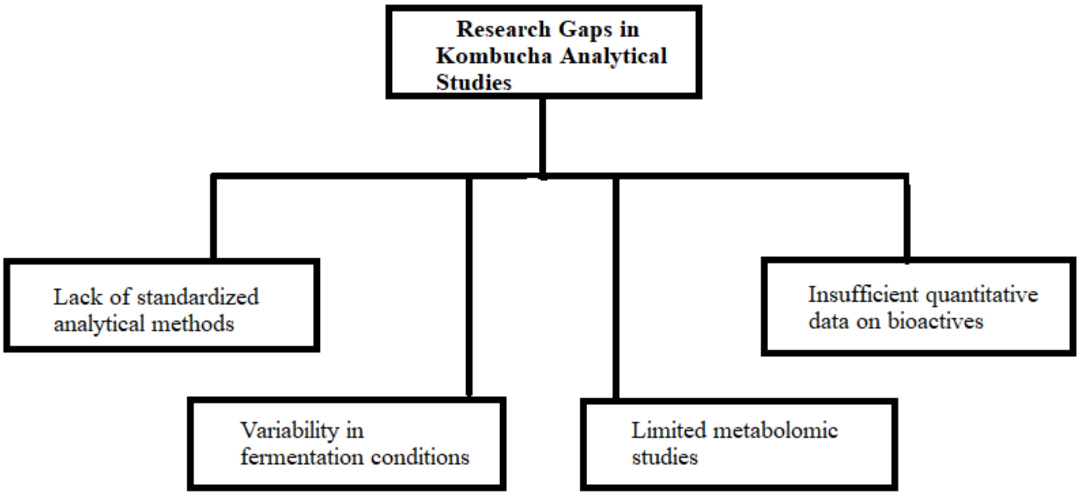

Figure No. 3: Tree diagram illustrating major research gaps in analytical studies of kombucha, including lack of standardized analytical methods, limited metabolomic investigations, variability in fermentation conditions, and insufficient quantitative data on bioactive compounds.

FUTURE PERSPECTIVES[22-25]

- Advanced analytical technologies: To enhance the identification and detection of low-abundance bioactive chemicals in kombucha, future research should make greater use of high-resolution methods like UHPLC–HRMS and advanced mass spectrometry.
- Integration of multi-omics approaches: The link between microbial populations and metabolite production during kombucha fermentation can be better understood by combining metabolomics, metagenomics, and proteomics.
- Quick and non-destructive analytical techniques: New approaches like Raman spectroscopy, hyperspectral imaging, and near-infrared spectroscopy (NIR) may provide quick and real-time monitoring of fermentation processes without requiring a lot of sample preparation.
- Development of portable analytical tools: Biosensors, microfluidic devices, and smartphone-based analytical platforms could be used to improve quality control in commercial kombucha production and enable on-site detection of important metabolites.
- Data science and chemometrics application: Sophisticated machine learning and chemometric technologies can help analyze intricate analytical datasets and enhance metabolomic profile interpretation.

- Standardization of analytical protocols: Reproducibility and comparability of various kombucha investigations will be enhanced by establishing consistent analytical techniques and reference metabolite databases.
- Linking bioactive chemicals with health effects: To further understand the functional and therapeutic potential of kombucha, future research should concentrate on linking certain metabolites with biological activities.

Table No. 6: Emerging Analytical Techniques for Kombucha Research.

Technique	**Application**	**Future potential**
Metabolomics (LC-MS based)	Comprehensive metabolic profiling	Understanding fermentation pathways
Biosensors	Rapid detection of phenolics	Portable analysis
Electrochemical sensors	Antioxidant measurement	Real-time monitoring
Chemometrics	Data analysis of complex datasets	Improved compound identification

"To examine the chemical makeup of kombucha, recent research has used sophisticated chromatographic, spectroscopic, and metabolomic techniques. Table No. 6 summarizes a few research that emphasize the analytical characterisation of kombucha."

Table No. 7: Summary of Recent Studies on Analytical Characterization of Kombucha.[26-31]

Year	**Study Focus**	**Analytical Techniques**	**Major Compounds Identified**	**Key Findings**	**Reference**
2026	Bioactive profiling of enriched kombucha	HPLC-DAD, LC–MS/MS	Phenolic acids, flavonoids, organic acids	Advanced LC–MS methods revealed diverse phenolic compounds and bioactive metabolites in enriched kombucha formulations	Popović et al., 2026
2026	Flavor and metabolite analysis during fermentation	HPLC, GC–MS, GC-IMS	Organic acids, esters, volatile compounds	Kombucha microbial fermentation significantly alters volatile flavor compounds and metabolic profiles	Qiao et al., 2026 (PubMed)
2024	Chemical characterization of kombucha fermentation	LC-ESI-MS, GNPS, metabolomics software	Polyphenols, organic acids, fermentation metabolites	Metabolomic analysis revealed dynamic changes in bioactive compounds during fermentation	Costa et al., 2024 (RSC Publishing)

2024	Phenolic content and biological activity of kombucha	HPLC, spectrophotometric assays	Catechins, flavonoids, phenolic acids	Tea substrate significantly affects phenolic composition and antioxidant activity	Mihai et al., 2024 (MDPI)
2024	Identification of metabolites in fermented kombucha	GC–MS	Organic acids, hydrocarbons, antimicrobial compounds	GC–MS analysis identified several metabolites with antibacterial properties	Jothilakshmi et al., 2024 (Biochemistry Journal)
2024	Nutritional and metabolomic analysis of kombucha	Metabolomics, biochemical assays	Organic acids, sugars, antioxidants	Kombucha fermentation produces diverse bioactive compounds with potential health benefits	Selvaraj et al., 2024 (PubMed)

CONCLUSION

The complex blend of bioactive substances found in kombucha, a fermented beverage, includes polyphenols, flavonoids, organic acids, and metabolites created during microbial fermentation. These substances are crucial to the beverage's nutritional content and possible health benefits. Analytical methods for studying kombucha have changed throughout time, moving from simple spectroscopic and chromatographic procedures to more sophisticated instruments including metabolomics analysis and high-resolution mass spectrometry. There are still difficulties even if these methods have significantly enhanced the identification and measurement of kombucha ingredients. Variability in reported results can be caused by variations in microbial cultures, tea types, fermentation conditions, and analytical techniques. In order to guarantee accurate comparisons between researches, more focus must be placed on standardized analytical techniques and regulated fermentation conditions. Further investigation utilizing sophisticated and integrated analytical methods will enhance our comprehension of kombucha composition and aid in the creation of reliable, superior kombucha goods.

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ABBREVIATIONS

Table No. 8: Abbreviations.

Abbreviation	Full Form
CC	Correlation Coefficient
FTIR	Fourier Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalent
GC–MS	Gas Chromatography–Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
LC–MS	Liquid Chromatography–Mass Spectrometry
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
ppm	Parts Per Million
SIR	Single-Image Reference
UV–Vis	Ultraviolet–Visible Spectroscopy
ΔE*	Color Difference Metric (CIE 1976 L*a*b*)

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REFERENCE

1. Jayabalan R, Malbaša RV, Lončar ES, Vitas JS, Sathishkumar M. A review on kombucha tea—Microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Compr Rev Food Sci Food Saf., 2014; 13(4): 538-550.
2. Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P. Understanding kombucha tea fermentation: A review. J Food Sci., 2018; 83(3): 580-588.
3. Laavanya D, Shirkole S, Balasubramanian P. Current challenges, applications and future perspectives of SCOBY cellulose of kombucha fermentation. J Clean Prod., 2021; 295: 126454.
4. Marsh AJ, O’Sullivan O, Hill C, Ross RP, Cotter PD. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha samples. Food Microbiol., 2014; 38: 171-178.
5. Dufresne C, Farnworth E. Tea, kombucha, and health: A review. Food Res Int., 2000; 33(6): 409-421.
6. Chen C, Liu BY. Changes in major components of tea fungus metabolites during prolonged fermentation. J Appl Microbiol., 2000; 89(5): 834–839.

7. Kallel L, Desseaux V, Hamdi M, Stocker P, Ajandouz EH. Insights into the fermentation biochemistry of kombucha teas and potential impacts of kombucha drinking on starch digestion. Food Res Int., 2012; 49(1): 226-232.
8. Zhu Y, Li F, Zeng Y, et al. Characterization of metabolites in kombucha tea during fermentation using LC–MS techniques**.** Food Chem**.**, 2020; 320: 126631.
9. Dutta H, Paul SK. Kombucha drink: Production, quality, and safety aspects. Food Rev Int., 2019; 35(5): 384-401.
10. Gaggìa F, Baffoni L, Galiano M, Nielsen DS, Jakobsen RR, Castro-Mejía JL, et al. Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity**.** Nutrients., 2019; 11(1): 1-20.
11. Wang B, Shao Y, Chen F. Overview on mechanisms of kombucha fermentation and analytical characterization of its metabolites. Food Chem., 2022; 377: 131885.
12. Jayabalan R, Malbaša RV, Lončar ES, Sathishkumar M. A review on kombucha tea—Microbiology, composition and beneficial effects. Compr Rev Food Sci Food Saf., 2014; 13: 538-550.
13. Coton M, Pawtowski A, Taminiau B, Burgaud G, Deniel F, Coulloumme-Labarthe L, et al. Unraveling microbial ecology of industrial-scale kombucha fermentations by metabarcoding and metabolomics. Food Microbiol., 2017; 67: 1-10.
14. Kapp JM, Sumner W. Kombucha: A systematic review of the empirical evidence of human health benefit. **Ann Epidemiol.**, 2019; 30: 66-70.
15. Chakravorty S, Bhattacharya S, Chatzinotas A, Chakraborty W, Bhattacharya D, Gachhui R. Kombucha tea fermentation: Microbial and biochemical dynamics. **Int J Food Microbiol.**, 2016; 220: 63-72.
16. Coelho RM, Almeida AL, Amaral RQ, da Mota RN, de Sousa PH. Kombucha: Review of substrates, regulation, composition, and biological properties. **J Food Sci.**, 2020; 85: 3543-3553.
17. Vázquez-Cabral BD, Rocha-Guzmán NE, Gallegos-Infante JA, Moreno-Jiménez MR, González-Laredo RF. Advances in analytical techniques for phenolic compound determination in fermented beverages**.** Food Chem**.**, 2021; 346: 128914.
18. De Filippis F, Troise AD, Vitaglione P, Ercolini D. Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during kombucha tea fermentation. Food Microbiol., 2018; 73: 11-16.

19. Coelho RM, Almeida AL, Amaral RQ, da Mota RN, de Sousa PH. Kombucha: Review of substrates, regulation, composition, and biological properties**.** J Food Sci., 2020; 85: 3543-3553.
20. Wang B, Shao Y, Chen F. Overview on mechanisms of kombucha fermentation and analytical characterization of its metabolites. Food Chem., 2022; 377: 131885.
21. Chakravorty S, Bhattacharya S, Chatzinotas A, Chakraborty W, Bhattacharya D, Gachhui R. Kombucha tea fermentation: Microbial and biochemical dynamics. Int J Food Microbiol., 2016; 220: 63-72.
22. Marsh AJ, O'Sullivan O, Hill C, Ross RP, Cotter PD. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. Food Microbiol., 2014; 38: 171-178.
23. Dufresne C, Farnworth E. Tea, kombucha, and health: A review. Food Res Int., 2000; 33: 409-421.
24. Laavanya D, Shirkole S, Balasubramanian P. Current challenges, applications and future perspectives of SCOBY cellulose of kombucha fermentation. J Clean Prod., 2021; 295: 126454.
25. Mousavi SM, Hashemi SA, Zarei M, Bahrani S, Yousefi K. Data on the chemical composition and biological activity of kombucha beverages. Data Brief., 2020; 30: 105372.
26. Popović D, et al. HPLC-DAD/LC-MS/MS characterization of bioactive compounds in enriched kombucha beverages. ACS Omega, 2026.
27. Qiao C, Wang Y, Xia S, Ji Q, Huo L, Zhang H, et al. Effects of kombucha-derived microbial fermentation on the flavor profile of grape fruit pulp**.** J Sci Food Agric., 2026.
28. Costa GO, Pontes CLM, Parize AL, Sandjo LP. Chemical characterization of kombucha fermentation using LC-ESI-MS-based metabolomics. Food Funct., 2024.
29. Mihai RA, Cubi-Insuaste NS, Catana RD. Biological activity and phenolic content of kombucha beverages under different tea substrates. Fermentation., 2024.
30. Jothilakshmi K, Gayathry G, Jayalakshmi T. GC-MS elucidation of bioactive metabolites from fermented kombucha tea. Int J Adv Biochem Res., 2024.
31. Selvaraj S, Gurumurthy K. Metagenomic and nutritional characterization of fermented kombucha tea. Front Microbiol**.**, 2024.