KOMBUCHA – A Nutritional Tea – A Review

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Abstract

Kombucha is a traditional beverage produced by fermenting sweetened tea using a symbiotic culture of bacteria and yeast (SCOBY) . This review explores the complex metabolic cascade where sucrose and tea polyphenols are transformed into potent organic acids—including acetic, gluconic, and D-saccharic acid-1,4-lactone (DSL)—as well as essential vitamins and minerals. While traditional *Camellia sinensis* remains the foundation, recent research highlights the potential of alternative substrates such as fruits, herbs, and grains to diversify and enhance bioactive profiles.

These ferments demonstrate significant antimicrobial, antioxidant, and anti-inflammatory properties in preclinical models, with emerging human clinical trials indicating potential antidiabetic benefits, such as reduced fasting blood glucose levels. Despite these therapeutic promises, product standardization remains a significant challenge due to inherent microbial variability and environmental influences. Furthermore, improper artisanal production poses safety risks, including metabolic acidosis and contamination, necessitating adherence to regulatory frameworks like Brazil's Normative Instruction 41/2019. Future research must focus on precise molecular characterization and rigorous clinical validation to transition kombucha from a functional dietary supplement into a standardized pharmaceutical agent.

Key words: Kombucha , Nutritional drink, Traditional beverage , Fermentation benefits

Introduction

Kombucha is a traditional lightly effervescent beverage , originated about 200 B.C. in Northeast China. It is prepared by fermenting sweetened tea (typically from Camellia sinensis) with a symbiotic culture of bacteria and yeasts (SCOBY), also known as a “tea fungus” or pellicle. “The fermentation process is a combination of alcoholic, lactic, and acetic fermentation initiated by osmotolerant microorganisms and ultimately dominated by acid-tolerant species.” [1,2]. It has gained popularity as a functional drink due to its probiotic-like properties, though much evidence remains from in vitro, animal, or limited human studies.

Historical Origin

Historical literatures highlight the emergence of name "Kombucha" around 414 A D when a Korean physician named Dr. Kombu reportedly brought the drink called "cha" nothing but Tea to Japan to treat Emperor Inyo. From Japan it spread through Russia and eastern Europe, gaining worldwide popularity during World War II [3,4]. Then, it broke into western societies and, more recently, into Europe and the United States of America (USA). In the sources, it is frequently referred to by its microbial descriptor, the SCOBY, a cellulose-rich biofilm. The movement started with health-conscious individuals who discovered kombucha during ultra-marathon training or international travels. What began as small batches for personal recovery or as a natural alternative to soda quickly expanded into family and friend circles, eventually leading to commercial bottling and sales. [5,7]

current trends of Kombucha

The Kombucha market is becoming quite famous and growing globally. It is estimated that there are approximately 235 companies of Kombucha distributed through Europe, North America, and Asia [6]. As a result, kombucha businesses in these areas have hiked to almost 2 billion euros in 2019 (Kombucha Brewers International, 2019). The market should reach USD 3.5–5 billion by 2025 [**6**]. There is a wide price gap in the Indian market based on how the

product is positioned, it ranges from Rs.100 to Rs.400(premium) [7]

Manufacturing Processes of kombucha

Kombucha production can be divided into traditional artisanal methods and modern industrial scaling.

- **Traditional Manufacturing Steps are as follows [2]**
  - **Preparation:** Water is boiled, and tea leaves (black or green, ~5–10 g/L) are infused for 10–15 minutes.
  - **Sweetening:** Sucrose is added (50–150 g/L, or 5–10% w/v) and the mixture is cooled to room temperature.
  - **Inoculation:** The tea is inoculated with the **SCOBY** and **10–30% mature kombucha broth** (starter) to lower the initial pH and deter spoilage.
  - **Fermentation:** The mixture ferments statically in a breathable vessel at ambient temperature for **7–14 days**. A fully fermented kombucha often exhibits a **lighter colour**, which is attributed to the microbial conversion of complex tea polyphenols like theaflavins into theobromine.
  - **Finishing:** The beverage is filtered and bottled, often undergoing a **secondary fermentation** (1–3 days) for natural carbonation before being refrigerated.

Production challenges: Primary concerns include

- **Contamination:** Poor sanitation can introduce **pathogenic microorganisms** or non-beneficial bacteria. Manufacturers must manage microbial variability and use **HACCP (risk analysis and critical control points)** principles to prevent contamination.
- **Incorrect Fermentation:** Over-fermentation leads to a dangerously acidic product, while inaccurate **SCOBY selection** can compromise the drink's safety profile.

- **Chemical Stability:** pH levels must be strictly monitored; guidelines recommend a range between **2.5 and 4.2** to prevent both contamination and the risk of acidosis.

SYMBIOTIC CULTURE OF BACTERIA AND YEAST (SCOBY)

Kombucha fermentation relies on **SCOBY**, which exists in a static, aerobic environment. Core Microbes involved are as follows [8]:

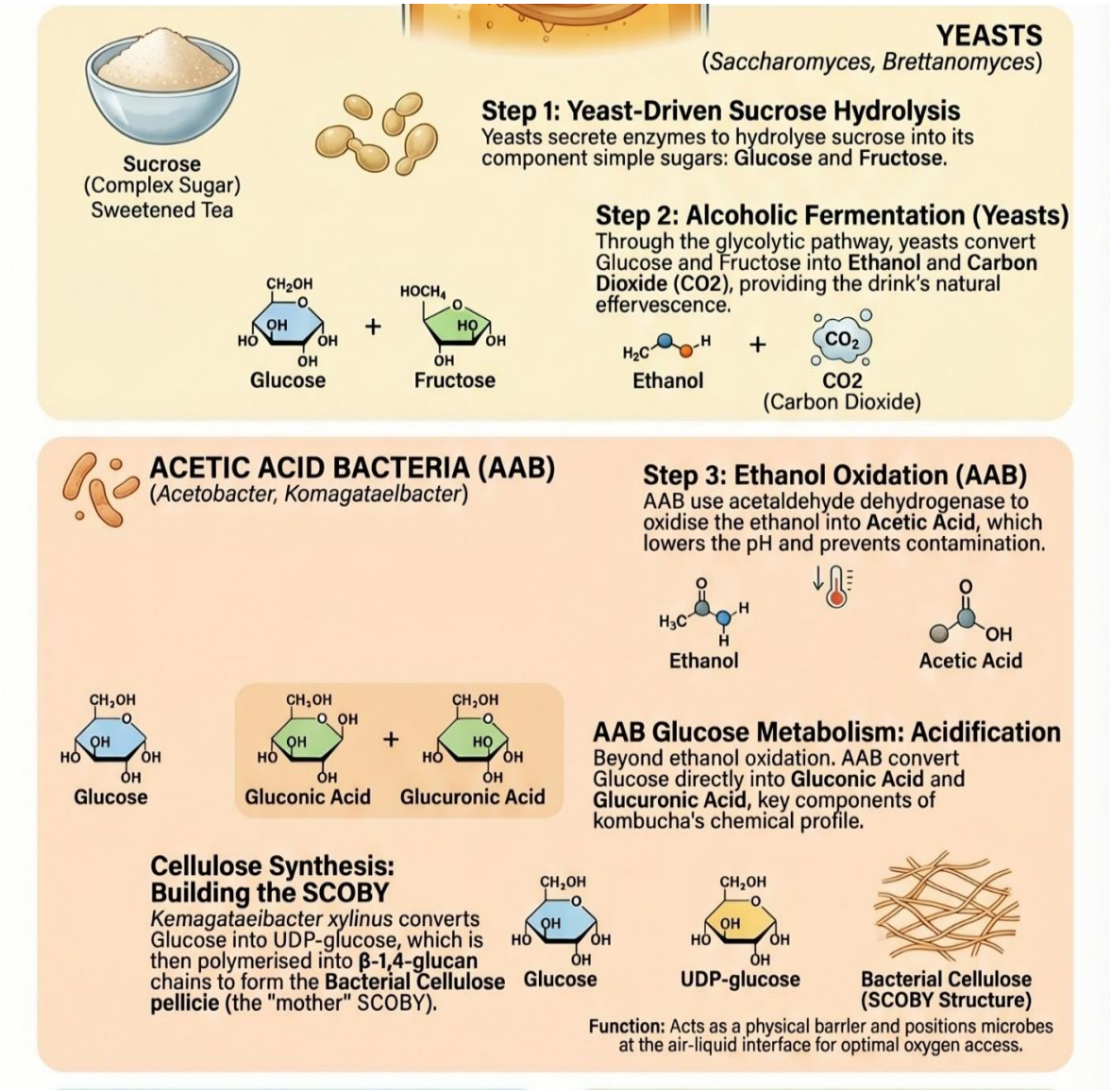
Acetic Acid Bacteria (AAB): Predominant species include *Komagataeibacter* (formerly *Gluconacetobacter*), *Acetobacter*, and *Gluconobacter*. These are Gram-negative, aerobic rods

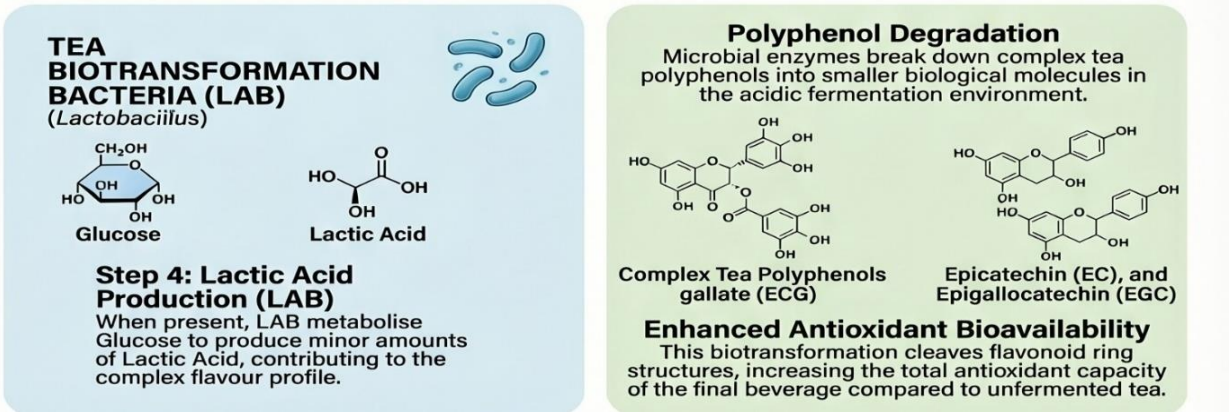
Yeasts: Common genera include *Saccharomyces*, *Brettanomyces*, *Zygosaccharomyces*, and *Schizosaccharomyces*. They are often dominant initially.

Lactic Acid Bacteria (LAB): Species such as *Lactobacillus* may contribute minor amounts of **lactic acid**.

- **Microbial Distribution:** Microbial composition varies by origin and substrate; high-throughput sequencing shows that AAB typically dominate the surface pellicle, while yeasts are more prevalent in the broth

Fig. 1 The metabolic cascade involved in fermentation process [9]


YEASTS
(Saccharomyces, Brettanomyces)
Sucrose
(Complex Sugar)
Sweetened Tea
Step 1: Yeast-Driven Sucrose Hydrolysis
Yeasts secrete enzymes to hydrolyee sucrose into its component simple sugars: Glucose and Fructose.
Step 2: Alcoholic Fermentation (Yeasts)
Through the glycolytic pathway, yeasts convert Glucose and Fructose into Ethanol and Carbon Dioxide (CO2), providing the drink's natural effervescence.
Glucose
Fructose
Ethanol
CO2
(Carbon Dioxide)
ACETIC ACID BACTERIA (AAB)
(Acetobacter, Komagataelbacter)
Step 3: Ethanol Oxidation (AAB)
AAB use acetaldehyde dehydrogenase to oxidise the ethanol into Acetic Acid, which lowers the pH and prevents contamination.
Ethanol
Acetic Acid
Glucose
Gluconic Acid
Glucuronic Acid
AAB Glucose Metabolism: Acidification
Beyond ethanol oxidation. AAB convert Glucose directly into Gluconic Acid and Glucuronic Acid, key components of kombucha's chemical profile.
Cellulose Synthesis:
Building the SCOBY
Kemagataeibacter xylinus converts Glucose into UDP-glucose, which is then polymerised into β-1,4-glucan chains to form the Bacterial Cellulose pellicie (the "mother" SCOBY).
Glucose
UDP-glucose
Bacterial Cellulose
(SCOBY Structure)
Function: Acts as a physical barrler and positions microbes at the air-liquid interface for optimal oxygen access.


TEA
BIOTRANSFORMATION
BACTERIA (LAB)
(Lactobaciilus)
Glucose
Lactic Acid
Step 4: Lactic Acid
Production (LAB)
When present, LAB metabolise Glucose to produce minor amounts of Lactic Acid, contributing to the complex flavour profile.
Polyphenol Degradation
Microbial enzymes break down complex tea polyphenols into smaller biological molecules in the acidic fermentation environment.
Complex Tea Polyphenols
gallate (ECG)
Epicatechin (EC), and
Epigallocatechin (EGC)
Enhanced Antioxidant Bioavailability
This biotransformation cleaves flavonoid ring structures, increasing the total antioxidant capacity of the final beverage compared to unfermented tea.

Note: Industrial Production: Industrial scaling emphasises **consistency and reproducibility** through controlled consortia and batch systems that maintain a high interfacial area.

4. Chemical Composition of kombucha [1]:

The final fermented product is a complex mixture of raw material residues and metabolites generated during the metabolic cascade of yeast and bacteria. The **chemical composition** of traditional kombucha includes:

Table 1. key chemical compounds involved in fermentation

Compound	Fermentation Time (Days)	Content (approx.)
Sucrose	13–14	35–40 g/L
Glucose	10–60	12–37.7 g/L
Fructose	10–60	30.9–55 g/L
Acetic Acid	10–60	1.65–8.36 g/L
Gluconic Acid	21–60	0.016–39 g/L
DSL	4–21	0.39–2.24 g/L
Ethanol	10–20	5.5–11 g/L
Tea Polyphenols	14	67.2 mg/g (dry weight)

Other components in the final product are:

- Transformed catechins like **epigallocatechin-3-gallate (EGCG), epicatechin gallate(ECG), and EC**, which provide significant antioxidant properties.

- **Vitamins and Minerals:** It contains essential **B-vitamins** (B1, B2, B6, B12), **Vitamin C** (L-ascorbic acid), and minerals including **Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), and Nickel (Ni)**.
- Dissolved **CO_2** for effervescence, and various **hydrolytic enzymes**, proteins, and amino acids. Trace amounts of citric, oxalic, and pyruvic acids.
- **Purine Bases:** Inherited from the tea substrate, the final drink contains **caffeine, theobromine, and theophylline**.

Note: The exact concentrations of these components are highly dynamic and fluctuate depending on the **initial substrate**, **fermentation time** (typically peaking at 7–14 days), and **temperature**.

STORAGE of Kombucha [6, 10]:

Temperature is the primary tool used to control probiotic levels once the desired fermentation stage is reached. At room temperature, "live" probiotics remain active. If not cooled, the yeast will continue to consume residual sugars, leading to excessive carbonation (which can cause bottles to burst), increased alcohol levels, and a shift toward an unpalatably acidic, vinegary taste. After the initial 7–14 days of fermentation, the beverage is refrigerated specifically to "halt activity".

- **Cold chain storage**: Traditional unpasteurized kombucha requires a continuous cold chain to stay safe and "live".
- **Room Temperature Storage**: Some manufacturers use filtration or pasteurization to remove or kill the live yeast and bacteria, allowing the drink to be stored at room temperature for up to 12 months.

However, this process effectively eliminates the "live" probiotic benefit, sparking an **industry debate** over whether such products should still be considered true kombucha. Industry experts argue that once these live cultures are destroyed, the beverage becomes

functionally equivalent to a standard "iced tea" rather than a true probiotic ferment. On the other hand, researches also says that the probiotic benefits are not solely derived from the microorganisms themselves but from their ongoing metabolic activity. For this reason, some researchers classify **kombucha as a "postbiotic"**.

Key factors influencing fermentation technology include:

fermentation parameters that strongly influence outcomes are discussed below [8,11,12]:

1. **Influence of Fermentation time**

- Fermentation time significantly influences both the **quantity and composition of the probiotic flora** within kombucha, driving a dynamic shift between the various microbial populations.
- **Optimal Window:** Microbial changes and bioactivity typically **peak or stabilise between 7 and 14 days**. Most traditional manufacturing processes target this 7- to 14-day range to achieve a balanced flavour and effective probiotic levels.
- While longer fermentation can enhance certain properties, it can also lead to a decline in quality. Extending the fermentation time (up to 15–21 days) can **increase the concentration of Bio actives** (organic acids, B-complex vitamins, and phenolic compounds), which are often associated with the beverage's health-promoting "postbiotic" properties , whereas fermenting beyond the optimal window may eventually lead to **nutrient depletion** within the broth and creates a highly acidic environment (lowering the pH), which may not only affect the taste but can also become a safety concern, potentially leading to **acidosis** in consumers. (Srihari & Satyanarayana, 2012) (Aung & Eun, 2022)

2. Influence of Fermentation Temperature

Temperature influences how quickly microbes grow, the types of metabolites they produce, and how long they remain viable in the beverage.

The standard temperature range for active fermentation is 20–30°C, with an ideal window typically cited between 22°C and 26°C.

Higher temperatures within this range accelerate the metabolic processes of the bacteria and yeast, particularly **increasing the rate of organic acid pro**duction. While heat speeds up the process, temperatures at the higher end of the spectrum risk creating a microbial imbalance, where certain species may outcompete others, potentially **altering the intended flavour** and probiotic profile.

High-throughput sequencing shows that the distribution of Acetic Acid Bacteria (AAB) and Yeasts fluctuates based on temperature and time. Because different strains have different thermal tolerances, the specific "flora" that survives and thrives in a batch is directly dictated by the temperature maintained throughout the fermentation cycle.

3. Influence of SCOBY:

SCOBY flora affects fermentation consistency. The specific types and ratios of microbes within a SCOBY are not fixed; they vary significantly based on geographical origin, the substrate used, ambient temperature, and fermentation time. Because different strains of yeast and bacteria have different metabolic rates and produce varying levels of metabolites, these shifts directly result in an **inconsistent chemical profile** in the finished beverage. Hence it is difficult to achieve **consistent inhibitory activity** or standardized levels of bioactive compounds.

4. Influence of Infusion methods of the extracts in the preparation

The technique used to extract bioactive compounds from substrates directly impacts the

concentration of organic acids and the beverage's antioxidant capacity.

Standard manufacturing involves infusing tea leaves in boiling water for approximately **10–15 minutes** whereas **Ultrasound-Assisted Extraction** has been shown to enhance the final product's profile. Specifically, higher organic acid content, particularly α-ketoglutaric acid and acetic acid, with higher titratable acidity, lower pH, and increased DPPH scavenging capacity (Aung & Eun, 2021).

5. Influence of Bioreactors and Culture Methods

The environment in which the SCOBY resides determines the speed and reproducibility of the fermentation.

- **Static vs. Shake Cultures:** Traditional fermentation is static and aerobic. However, the use of **shake cultures** in bioreactors allows for a more controlled fermentation strategy. This method improves the **safety of the fermentation process** by allowing for better management of starter cultures.
- **Oxygen Transfer and Agitation:** Aerobic conditions are essential for Acetic Acid Bacteria (AAB), and in static cultures, the rate of fermentation is often limited by oxygen transfer. While bioreactors can improve oxygenation, **agitation** must be managed carefully as it can **disrupt the cellulose pellicle** (the SCOBY biofilm).
- **Industrial Scaling and Consistency:** Modern industrial manufacturing utilizes **batches systems and bioreactors** designed to maintain a high **interfacial area**. This is critical for Reproducibility and consistency.

Surface Area-to-Volume Ratio (interfacial area): The design of the vessel or bioreactor affects the yield. The ratio of the inoculum to the volume of the vessel affects the overall yield of metabolites. The interfacial area of the vessel affects how the Acetic Acid Bacteria (AAB) access oxygen to produce the cellulose

pellicle and acetic acid. If the flora is disrupted by agitation or if the vessel shape changes, the oxygen diffusion rates shift, leading to **inconsistent metabolite production.** It is noted that Higher interfacial area improves **oxygen diffusion and there by metabolite production**. (Sharifudin et al., 2021)

6. **Influence of Sugar concentrations:**

affect Inhibition of α-glucosidase enzyme for antidiabetic activity. The optimal treatment for inhibition was at a sugar concentration of 10% and a fermentation time of 14 days.

7. **Influence of Alternative Substrates**

When alternative materials like herbs or fruits are used, the chemical composition shifts to include specific markers from those plants, such as **anthocyanins** from berries, **gingerol** from ginger, or **curcuminoids** from turmeric. These alternative substrates can significantly alter the antioxidant and phenolic profile of the final ferment

Table 2. Studies on Alternative substrates and its proven medicinal activities:

alternative substrate	**Bio-actives responsible**	**Medicinal activities proven**
Yerba mate	Phenolic acids (chlorogenic, caffeic), flavonoids (quercetin, rutin), xanthin	Antioxidant (DPPH, ABTS scavenging), anti-inflammatory (LOX inhibition), skincare (anti-aging, moisturizing, collagenase and elastase inhibition) [13] [14]

	(caffeine, theobromine), saponins, tannins	
Oak leaves	Polyphenols (catechin, quercetin, kaempferol, naringin, ellagic acid), tannins	Anti-inflammatory (downregulation of NO, TNF-, and IL-6), antioxidant, protection against oxidative stress [15]
Cactus pear juice	Acetic acid, betalains (betacyanin), polyphenols, Indica xanthin	Antibacterial (inhibits S. aureus, B. cereus, E. coli), antioxidant (DPPH, ABTS) [16]
Snake fruit (Salacca zalacca)	Natural bioactive compounds, phenolic compounds, flavonoids, tannins, organic acids	Antibacterial (S. aureus, E. coli), antidiabetic (mitigates oxidative stress, stabilizes fasting blood glucose), antioxidant (DPPH) [17] [18]
Pomegranate juice	Phenol-carboxylic acids, anthocyanins, tannins, glucuronic acid	High antioxidant activity, total acidity enhancement [19]
Ginger	Gingerols, shogaols, zingerone, paradols	Anti-cancer (inhibits tumour proliferation, stimulates apoptosis), anti-inflammatory, antioxidant. [20]
Turmeric (Curcuma longa)	Curcuminoids, terpene derivatives (sesquiterpenes, monoterpenes)	Antibacterial (E. coli), immunomodulation (increased CD4+, TNF-, IFN-; decreased IL-6) [21,22]

Green coffee	Phenols, flavonoids, caffeine, methylxanthines	Skincare (inhibits collagenase and elastase, improves hydration, sunscreen effect), antioxidant [23]
King coconut water	Phenolic compounds (ferulic, p-coumaric acid), flavonoids, Vitamin B complex, Vitamin C	Antidiabetic (-amylase and -glucosidase inhibition), antioxidant (DPPH, ABTS, FRAP, ORAC) [24]
Skim milk	Polyphenols (flavonoids, catechins), Vitamin E, organic acids, bioactive peptides	Antihypertensive (ACE inhibition), antidiabetic (reduced blood glucose, ALT, AST, ALP), liver health support [25]

The table highlights that while traditional tea-based kombucha provides a foundation of antioxidant and antimicrobial activity—primarily through the transformation of catechins and the production of acetic acid—the use of alternative substrates such as ginger, turmeric, and yerba mate significantly broadens its therapeutic spectrum to include anti-inflammatory, antidiabetic, and even anti-cancer potential.

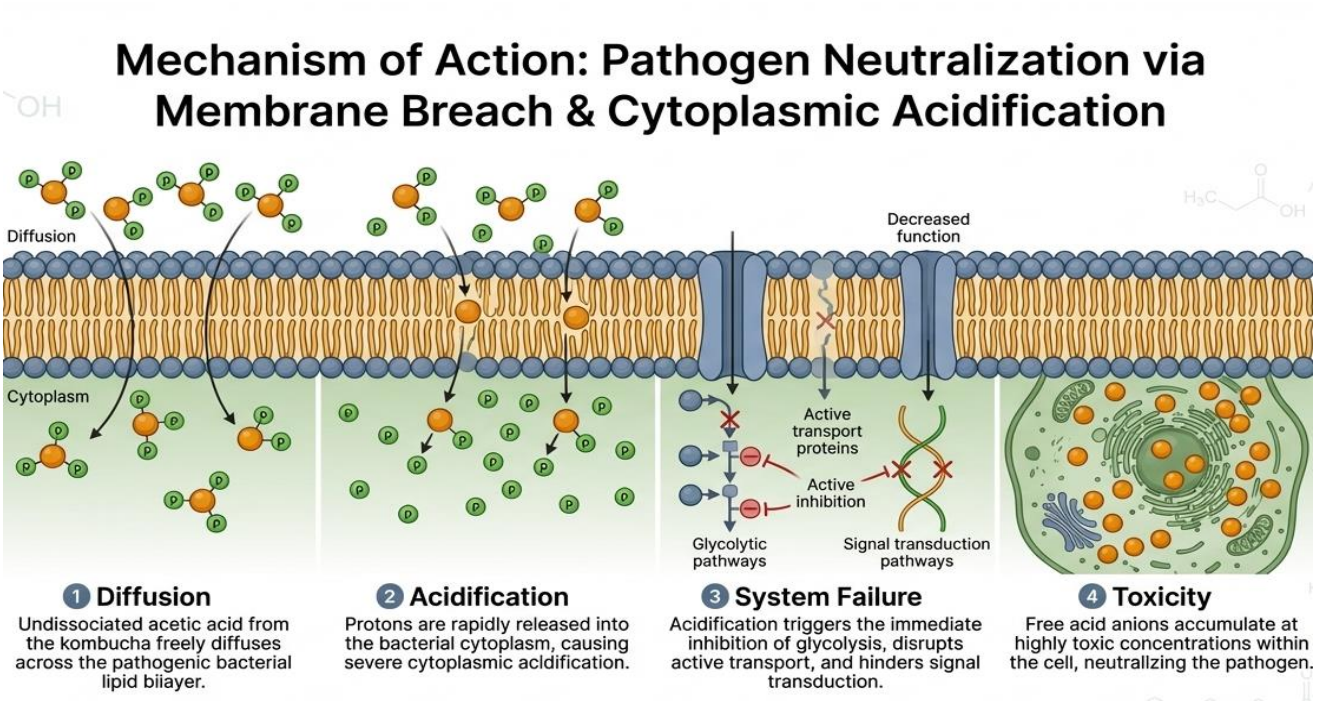
Applications of kombucha

Kombucha proven to have **probiotic potential** (though often classified more as postbiotic due to metabolites). Claimed benefits, primarily from in vitro/animal models, include:

1. Antimicrobial Activity

The primary antimicrobial activity is driven by organic acids (specifically acetic acid) and plant-derived phenolic compounds.

Fig. 2 The mechanism of action behind kombucha's antimicrobial activity [1]



- **Alternative Pathway (Phenolics/Terpenes):** Active compounds (like those from turmeric or Lycium barbarum) disrupt the cell membrane integrity, impeding the passage of nutrients and modifying bacterial physiology.
- **Result:** Inhibition of growth or cellular proliferation of pathogenic bacteria.

2. Anti-inflammatory effect

The anti-inflammatory effects are attributed to flavonoids and phenolic acids produced during biotransformation [13,14,16]

- **Step 1:** Bioactive components (e.g., theaflavins, naringin, quercetin) are generated through microbial metabolism.
- **Step 2:** These components **downregulate the production of Nitric Oxide (NO)** and pro-inflammatory mediators.
- **Step 3:** They inhibit key inflammatory enzymes: **Lipoxygenase (LOX)** and **Cyclooxygenase-2 (COX-2)**.
- **Step 4:** Expression of inflammatory genes and cytokines, such as **TNF-α**

and Interleukin-6 (IL-6), is significantly decreased.

- **Result:** Resolution of inflammation and potential protection against conditions like gastric ulcers.

3. Antioxidant activity

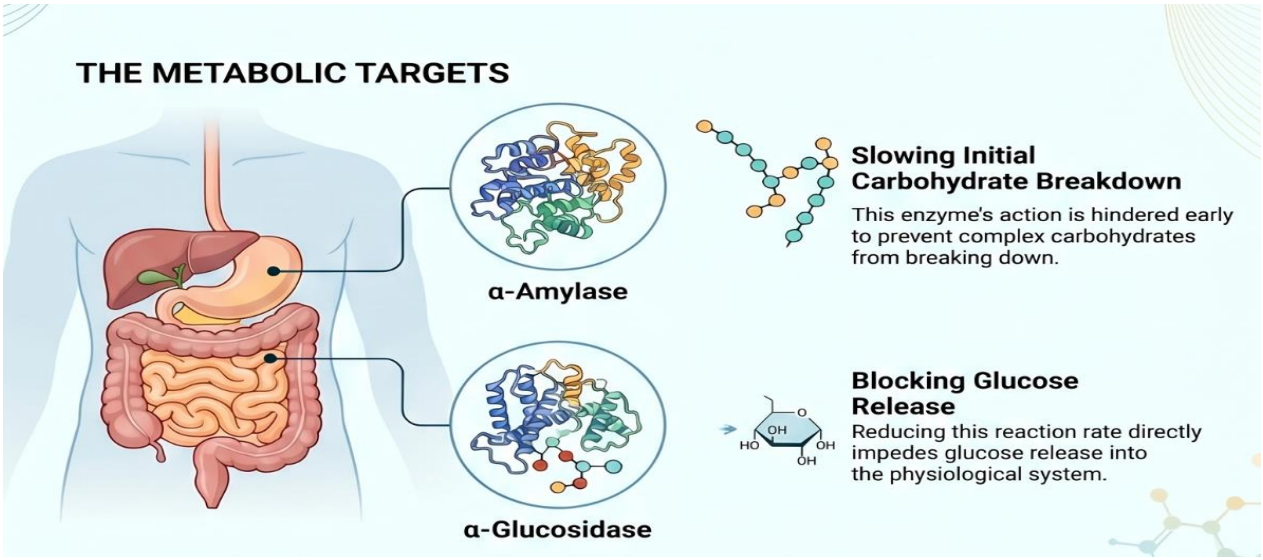
Kombucha acts as a potent source of antioxidants through the transformation of polyphenols [26,27].

- **Step 1:** During fermentation, microbial enzymes (secreted by Acetobacter and Saccharomyces) break down complex polyphenols into smaller biological molecules.
- **Step 2:** Flavonoid ring structures are cleaved, and theaflavins are depolymerised into compounds like **gallic acid, epicatechin (EC), and epigallocatechin gallate (ECG)**.
- **Step 3:** These phenolic compounds readily **donate hydroxyl hydrogen** due to their resonance stabilization.
- **Step 4:** The donated hydrogen supply enhances the **scavenging of free radicals** (such as DPPH and NO).
- **Step 5:** Lipid peroxidation is effectively inhibited.
- **Result:** Enhanced total antioxidant capacity and radical absorption.

4. Anti-diabetic effect:

The mechanism behind anti diabetic effect focuses on the regulation of glucose metabolism and enzymatic inhibition [18,24,25]. Polyphenols, organic acids, and flavonoids in the fermentation broth act on digestive enzymes.

Fig.3 Antidiabetic Potential of Kombucha



- Oxidative stress is mitigated, and markers like Liver enzymes are lowered, supporting liver health and glucose stability.
- **Result:** Mitigation of hyperglycaemia and stabilization of fasting blood glucose levels.

5. Skincare application:

Alternative substrates like yerba mate and coffee provide specific dermatological benefits such as **Anti-aging and Moisturizing effect [14,23].**

- **Step 1:** Fermentation increases the concentration of phenolic acids, methylxanthines, and flavonoids.
- **Step 2: Enzyme Inhibition**: These substances strongly inhibit **collagenase, elastase, and lipoxygenase (LOX)**, which are responsible for skin aging and structural breakdown.
- **Step 3: Moisturizing Effect**: Hydroxyl groups in antioxidants and carbohydrates form hydrogen bonds with water, providing sustained hydration.
- **Step 4: Sunscreen Effect**: Anthocyanins, polyphenols, and

vitamins absorb UV radiation, offering protection against sun damage.

- **Result:** Improved skin hydration, reduced aging markers, and nourishment.

6. Anti-cancer activity

Kombucha enhances the body's ability to fight cancer cells through apoptosis and enzyme inhibition [20].

- **Step 1:** Active metabolites like **D-saccharic acid-1,4-lactone (DSL)** are produced during fermentation.
- **Step 2:** DSL acts as an effective **inhibitor of β-glucosidase**, an enzyme closely linked to carcinogenesis.
- **Step 3:** Bioactive components from alternative substrates (e.g., gingerol and shogaol from ginger) **inhibit tumour proliferation**.
- **Step 4:** These compounds **stimulate apoptosis** (programmed cell death) in various cancer cell lines, such as HeLa and MCF-7.
- **Step 5:** Peroxidase and malondialdehyde activities in tumour homogenates are reduced.
- **Result:** Suppression of tumour growth and enhanced immune surveillance.

7. Other Biological Activities

The sources also highlight several secondary mechanisms:

- **Antihypertensive:** Fermentation liberates peptides that inhibit the **Angiotensin-Converting Enzyme (ACE) [25,26]**.
- **Immunomodulation:** Turmeric kombucha enhances the **adaptive immune response** (increasing CD4+ and IFN-γ) while modulating the innate response (decreasing IL-6) [22].
- **Neuroprotection:** Fermentation results in **anti-acetylcholinesterase (Anti-**

AChE) activity, which may have implications for cognitive health[1].

- **Gut health:** Supports microbiota balance [33,35].
- **Anti hyperlipidaemic activity**: showed potential cholesterol reduction,

Note: SCOBY cellulose applications extend beyond beverage production to **biomedical** (wound dressings, tissue scaffolds, artificial vessels), food (thickener), **cosmetics**, textiles, and environmental (heavy metal adsorption).

Evaluation of Kombucha

Evaluation encompasses physicochemical, microbiological, sensory, and bioactivity assessments for quality, safety, and functionality.

Perform analysis in triplicate, use calibrated instruments, and include appropriate standards/controls. Changes typically peak or stabilize around 7–14 days of fermentation. Sample preparation usually involves filtering or centrifuging the liquid kombucha.

I. Sensory Evaluation

Trained panels assess appearance, aroma (fruity, vinegary, tea-like), taste (sweet-sour balance, astringency), flavour, and overall acceptance using hedonic or descriptive scales. Electronic tongue or GC-MS for volatiles aids objective correlation with sensory data. [12]

II. Table 3. Physicochemical evaluation of Kombucha [8,11]

Test Parameter	Methodology	Significance
pH	Calibrated glass electrode pH meter. Ideal pH is **2.5 to 4.2**	Safety monitoring to inhibit pathogens and prevent acidosis

Ethanol content % (v/v) or g/L	Headspace Gas Chromatography (HS-GC-FID) or enzymatic kits. Ideal limit is **≤0.5%** (non-alcoholic)	Regulatory compliance for non-alcoholic beverages.[44,45] Up to 1% (CDC Canada) [37]
Titratable Acidity (TA)	Titration with 0.1 M NaOH to endpoint pH. Max. limit is **130 milliequivalents per Liter.**	Measures total acid production and flavour balance
Total Soluble Solids (TSS)	Digital or handheld refractometry. TSS levels **decrease** throughout the fermentation cycle	Tracks sugar consumption and residual sweetness to ensure the desired sweet-sour balance is achieved before bottling
Organic Acids (Acetic/Gluconic) (g/L)	High-Performance Liquid Chromatography (HPLC) with UV detection. Ideal limit is Acetic acid:5-16 g/L; Gluconic: up to 39 g/L	Quantifies individual bioactive acids versus total acidity
Viscosity	Measured using a **viscometer** (either rotational or capillary) under strictly controlled temperature. It should be low.	helps ensure the liquid profile rather than becoming overly thick or syrupy, which could indicate an imbalance

		in the microbial flora or over-fermentation

II. Microbial Test [8]

This tests tracks population shifts (yeasts early, AAB later). Serially dilute sample in sterile saline/peptone water. Plate on selective media such as:

- GYCA (Glucose-Yeast Extract-Calcium carbonate-Agar) or similar for Acetic Acid Bacteria (AAB): aerobic incubation at 25–30°C.
- YPD (Yeast Extract-Peptone-Dextrose) for yeasts: often with antibiotics to suppress bacteria, incubate at 25–28°C.

Count colonies (CFU/mL) after 3–7 days.

III. **Phenolic Profile [27]**

Evaluating the **phenolic profile** of kombucha involves both a broad estimation of the total phenolic content and a detailed identification of individual bioactive molecules. This procedure is critical because fermentation dynamically transforms complex tea polyphenols into smaller, more bioavailable biological molecules.

1. Total Phenolic Content (TPC) Assay

The standard method for a rapid, simple estimation of the overall concentration of phenolic compounds is the **Folin-Ciocalteu colorimetric assay**.

Sample Preparation: The liquid kombucha is typically **filtered or centrifuged** to remove microbial cells before analysis.

Procedure: The sample is mixed with the Folin-Ciocalteu reagent and incubated. Subsequently, **sodium carbonate** is added to the mixture.

Measurement: After a specified incubation period, the **absorbance is measured at 765 nm** using a spectrophotometer or microplate reader.

Reporting: Results are calculated based on a calibration curve and expressed as **mg gallic acid equivalents (GAE)/L**.

2. Detailed Profiling via LC-MS

To reveal specific shifts during fermentation—such as the increase in phenolic acids and the degradation of flavonoids—researchers use more advanced chromatographic techniques.

Instrumentation: The preferred methods are **UPLC-QTOF-MS** (Ultra Performance Liquid Chromatography-Quadrupole Time of Flight-Mass Spectroscopy) or **HPLC-DAD-MS** (High-Performance Liquid Chromatography-Diode Array Detector-Mass Spectrometry).

Stationary Phase: A **C18 column** is commonly used for the separation of compounds.

Mobile Phase: This typically consists of a gradient of **acidified water** (e.g., 0.1% formic acid or TFA) and **acetonitrile** over a period of 30–40 minutes.

Detection: Identification is performed via a diode-array detector (scanning UV wavelengths between **200–400 nm**) and mass spectrometry using **electrospray ionization (ESI)** in both positive and negative modes.

Identification and Quantification: Compounds are identified by comparing their **retention time, UV spectra, exact mass, and MS/MS fragments** against established standards such as gallic acid, catechins, and quercetin.

IV. Bioactivity evaluation:

Always perform tests in triplicate, use appropriate controls (e.g., ascorbic acid/Trolox for antioxidants, acarbose for antidiabetic, positive/negative controls for antimicrobial/cytotoxicity), and calculate results as % inhibition, IC50 (half-maximal inhibitory concentration), or equivalents (e.g., mg GAE/mL or μmol TE/mL). Sample preparation typically involves filtering kombucha, diluting it (often in water, buffer, or ethanol/methanol), and adjusting pH if needed.

1. Antioxidant Assays

These measure free radical scavenging or reducing power, often expressed as % inhibition or Trolox/Gallic acid equivalents.

There are three types of assays. Different studies choose specific assays based on the substrates being tested and the specific properties they wish to measure. While not mandatory, using different assays allows researchers to capture different types of antioxidant behaviour:

- **DPPH:** A very common, rapid colorimetric assay used to measure general free-radical scavenging ability.
- **ABTS:** Noted for being effective for measuring **both hydrophilic and lipophilic** (water- and fat-soluble) antioxidants.
- **FRAP:** Unlike DPPH and ABTS, which measure radical scavenging, FRAP specifically measures **reducing capacity** (the ability to convert Fe^{3+} to Fe^{2+})

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay [28,29]:

Fermentation often increases DPPH activity in kombucha due to enhanced polyphenols and organic acids.

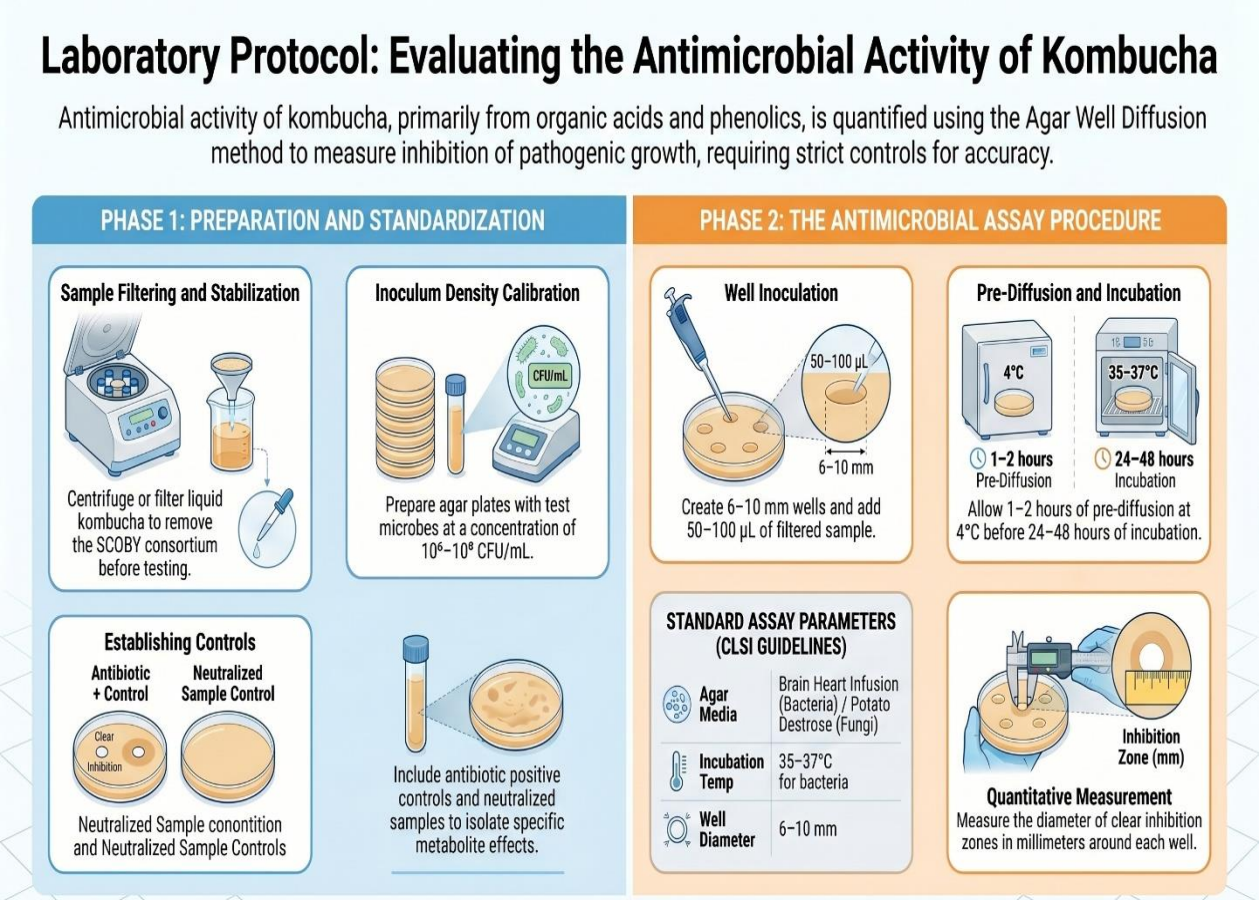
Method: Prepare a 0.1–0.3 mM DPPH solution in ethanol or methanol. Mix sample (e.g., 20–50 µL diluted kombucha) with DPPH solution (150–180 µL or more). Incubate in the dark for 15–30 min at room temperature. Measure absorbance at 517–518 nm using a spectrophotometer or microplate reader.

Calculation: % Scavenging = [1 – (A sample – A blank) / A control] × 100.

Interpretation: Higher % inhibition or lower IC50 indicates stronger activity. (Brand-Williams)

2.Antimicrobial activity[29]

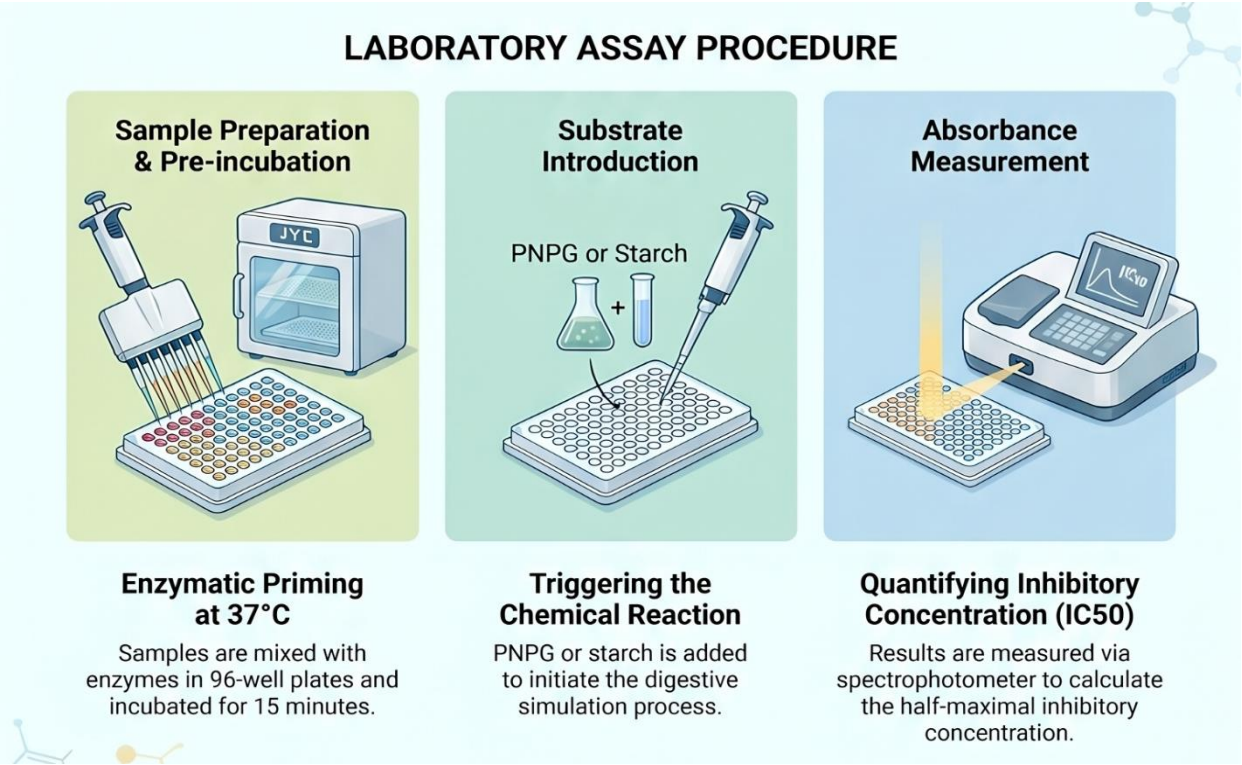
Fig. 4 Zone inhibition assay



3. Antidiabetic activity [28]

This test focus on conducting assay for carbohydrate-digesting enzymes linked to glucose metabolism

Fig.5 Enzyme inhibition assay



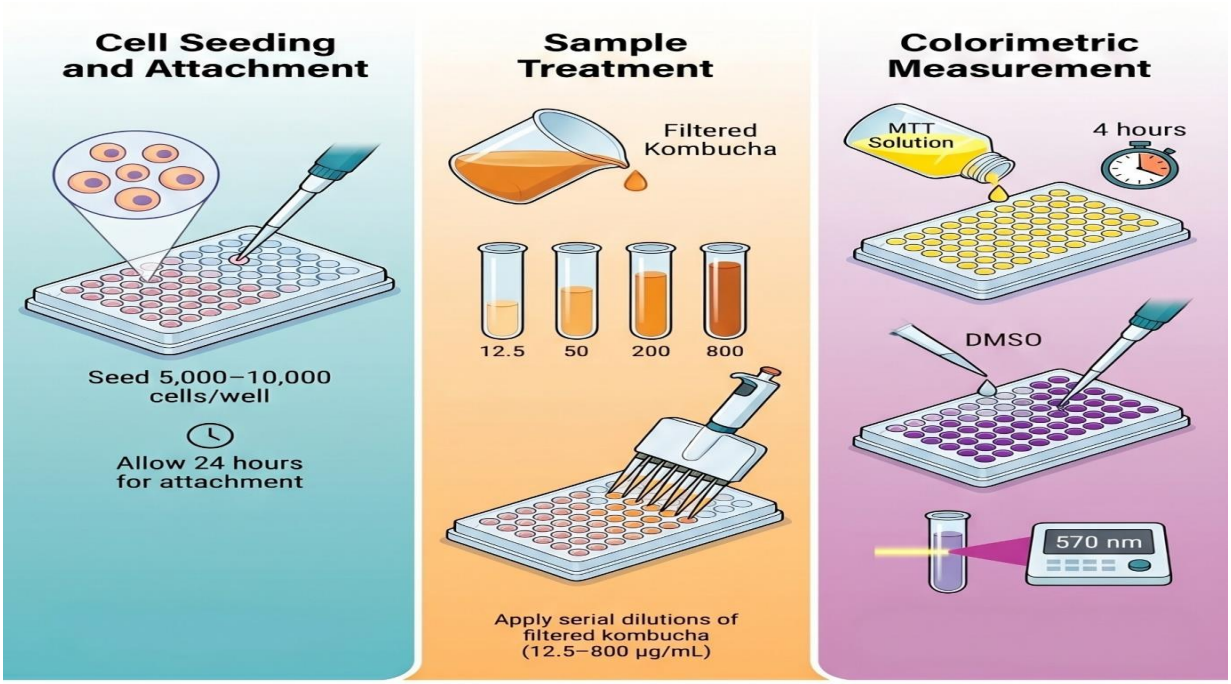
Standard Assay Parameters

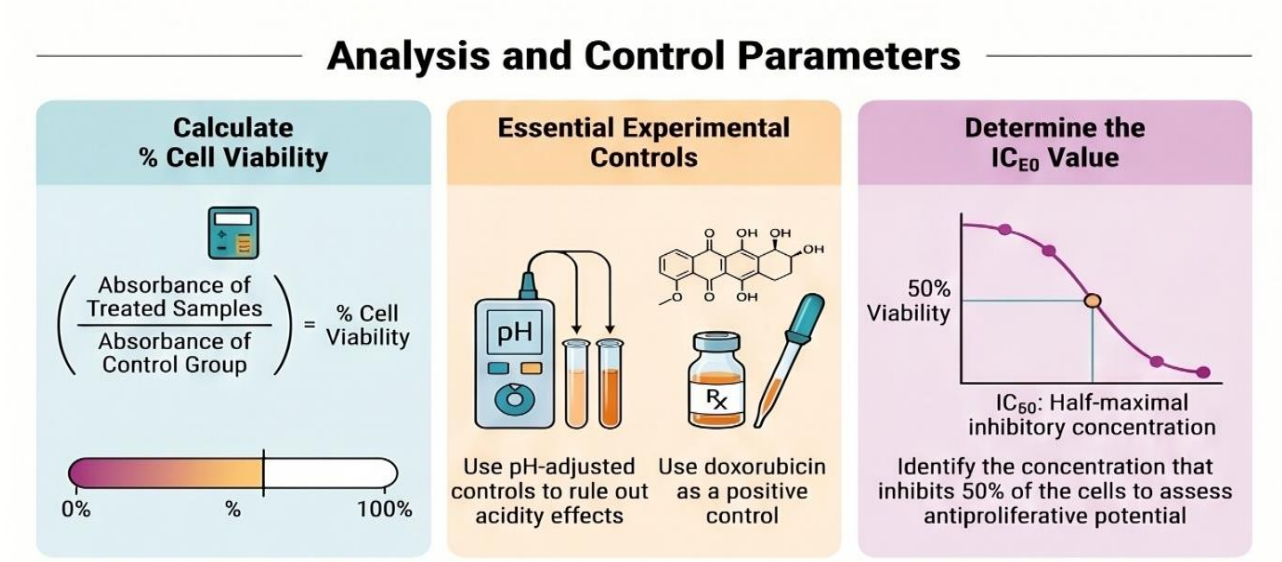
Assay Type	Substrate Used	Detection Wavelength
α-Glucosidase	PNPG (5 mM)	405 nm
α-Amylase	Starch	540 nm
Control Standard	Acarbose	N/A

4. **Cytotoxicity Tests [29]**

This test assesses antiproliferative potential of kombucha. Include pH-adjusted controls to rule out acidity effects and doxorubicin as positive control.

Fig.6 MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay for cell viability





Notes: green tea kombucha sometimes shows stronger effects due to catechins.

Table 4. Key clinical studies on Kombucha

Study	Design/Method	Results of the study
Human Clinical	Randomized crossover trial; 240 mL daily vs. placebo for 4 weeks; Adults with Type 2 Diabetes [30].	Significantly lowered average fasting blood glucose from 164 to 116 mg/dL; no safety issues noted.
Human Clinical	8-week RCT; 4-week daily supplementation in healthy adults [31].	Modest microbiome shifts (enrichment of *Weizmannia coagulans*); minimal changes in overall inflammatory or biochemical markers.
Human Clinical	RCT (n=60); healthy adults; 250 mL daily of fibre-enriched kombucha for 6 weeks [32].	Significant triglyceride reduction; increased *Bifidobacterium* abundance and reduced pro-inflammatory *Ruminococcus torques*.
Human Clinical	Systematic review of 8 trials (healthy or cardiometabolic issues) [33].	Potential relief for gastrointestinal symptoms (e.g., constipation); subtle microbiota shifts; inconsistent glucose metabolism results.
Pre-clinical	In vitro; Black and green tea residues added to fermentation [34].	Fermentation with residue increased antioxidant activity by 3.25 times and polyphenols by 5.68 times compared to standard methods.
Pre-clinical	In vitro; Oolong, green, and black tea with fruit juices [35].	Oolong tea with apple juice showed the highest antioxidant activity; pomegranate juice variants had highest total phenolics.

Pre-clinical	Systematic review of 15 animal studies (obesity/high-fat diet models) [36].	Attenuated oxidative stress/inflammation; supported liver detoxification and reduced intestinal dysbiosis.
Pre-clinical	Rodent models alloxan/streptozotocin -induced diabetes [37].	Consistent reductions in blood glucose and HbA1c; protection and improvement of pancreatic beta-cells.
Pre-clinical	In vitro; Herbal ferments (Rubus, Vaccinum, etc.) on skin cell lines [14].	Positive effect on viability of fibroblasts and keratinocytes; ferments showed stronger antioxidant properties than unfermented plants.

General Observations of the above quoted studies :

- Human Evidence: While emerging studies show benefits for blood sugar and gut microbiome modulation, review note that sample sizes remain small and results for glucose metabolism can be inconsistent.
- Functional Ingredients: The benefits observed in preclinical studies are largely attributed to D-saccharic acid-1,4-lactone (DSL), organic acids, and transformed polyphenols like gallic acid and catechins.

Health Risks and Consumption Limits

While generally considered safe by the FDA, excessive or improper consumption of kombucha poses several health risks. Documented adverse effects range from **allergic reactions, nausea, and jaundice** to severe conditions such as **lactic acidosis, acute renal failure, and hyperthermia**. Specifically,

massive **liver necrosis** has been observed in individuals with a history of alcohol abuse after excessive intake. To mitigate these risks, health authorities like the CDC suggest that daily consumption should not exceed approximately **100 to 120 ml** for healthy individuals [1][38].

High-Risk Populations

Certain groups must exercise extreme caution or avoid the beverage entirely [37][38]:

- **Pregnant Women:** Avoided due to the beverage's **ethanol content, high acidity, and potential heparin** presence.
- **Immunocompromised Individuals:** Highly susceptible to complications from microbial complexity or potential contaminants.
- **Sensitive Individuals:** Those with **Mold allergies, alcohol sensitivity, or a predisposition to acidosis** are at higher risk.

case studies and clinical reports regarding kombucha consumption:

- **90-day animal study on rats** :

  found no toxic signs, with all haematological and biochemical variables remaining within clinical limits [39].

- **Severe Systemic Reaction in an HIV-Positive Patient**:

  In a 2009 case study, a 22-year-old man recently diagnosed with HIV experienced a severe reaction within 12 hours of drinking kombucha tea. He developed shortness of breath and a high fever (39.4 °C), and within 15 hours, he exhibited symptoms of hyperthermia, lactic acidosis, and acute renal failure [40].

- **Case Series of Allergic and Gastrointestinal Reactions**:

  A report involving four separate patients detailed a variety of side effects likely linked to the beverage. Two

patients suffered from allergic reactions, a third developed jaundice, and a fourth experienced nausea, vomiting, headaches, and neck pain. In all four instances, the symptoms began near the start of kombucha consumption and resolved once the patients stopped drinking it [41].

- **Massive Hepatic Necrosis and Alcohol History**:

  Clinical literature has documented cases where individuals with a history of excessive alcohol consumption suffered from massive liver necrosis after heavy kombucha intake. Pathological examinations in these cases showed significantly elevated levels of liver enzymes (AST and ALT), which suggested a direct correlation between the ingestion of kombucha and severe liver damage beyond what would be expected from alcohol use alone [42].

Regulatory Requirements and Standards [1, 43]

The global regulatory landscape for kombucha is largely unstandardized, though efforts are increasing to ensure consumer safety.

- **Brazil:** Currently the only nation with **specific legislation** (Norm Instruction n° 41) that strictly defines the identity, quality standards, and mandatory composition of kombucha [44].
- **United States:** No specific national law exists, but manufacturers must often implement a **HACCP (risk analysis and critical control points) plan**. Regional guidelines, such as those in Pennsylvania, prohibit health claims and mandate warnings regarding alcohol content and contraindications [44].
- **Canada:** Focuses on safety plans that limit **ethanol content to 1%** or less and require pH levels to remain above 2.5.
- **Industry Advocacy:** The **Kombucha Brewers International (KBI)** was

established in 2014 to promote modern legislation, research, and standardized manufacturing practices across the industry.

Research Gaps identified:

The review highlights several critical research gaps regarding the study of kombucha, particularly as it relates to its potential transition from a dietary supplement to a standardized pharmaceutical agent. The specific gaps mentioned include:

- **Lack of Molecular Clarity and Detailed Analysis**: There is a significant lack of qualitative and quantitative analysis regarding specific substances produced during fermentation, such as terpenoids or specific polyphenols. While the general benefits of total acids and phenols are recognized, the exact metabolic reaction pathways and the pharmacological mechanisms of action for specific substances remain understudied.
- **Insufficient Data on Alternative Substrates**: The review note a lack of comprehensive literature reviews focusing specifically on functional active substances, and their efficacy mechanisms of alternative kombucha substrates. There is also limited research into the characterisation and purification of active components generated from these non-traditional materials.
- **Standardization and Consistency Challenges**: Achieving a consistent product is a major hurdle due to high microbial variability across different SCOBY strains, substrate compositions, and environmental factors. This inconsistency makes it difficult to ensure standardized levels of bioactive compounds or consistent inhibitory activity in the finished beverage.
- **Preliminary Nature of Clinical Evidence**: Human clinical evidence is described as "emerging and modest in

scale/scope". Most research on anti-inflammatory and anti-diabetic effects is still in preliminary stages, relying on homogeneous experimental models (in vitro or animal studies) rather than rigorous human validation. There is a cited need for more rigorous in vivo and ex vivo experiments and formal clinical value assessments.

- **Narrow Antimicrobial and Pathogenic Focus**: Few studies have explored the inhibitory effects of kombucha on drug-resistant, lethal, and pathogenic microbiota. Research is needed to refine the antimicrobial spectrum and identify the specific compounds responsible for these effects.
- **Exploration of Mixed-Strain Processes**: There is insufficient investigation into the reaction processes of specific substances when subjected to mixed-strain fermentation. Further research is required to supplement our understanding of metabolic reaction pathways during the fermentation of specific pharmaceutical-grade fermenters.

Conclusion

In summary, kombucha represents a biotechnologically interesting fermentation system with scalable potential, though standardization of microbiota, parameters, and evidence-based claims remains an active research area. Proper manufacturing and evaluation ensure safety and quality. The conclusion of the review highlights both the significant potential for these functional beverages and the substantial gaps. Various researches have successfully demonstrated that using alternative substrates (such as herbs, fruits, and grains) can produce beverages with a wide range of biological activities.

Future work must focus on optimizing and standardising the **"SCOBY" flora** to create reliable, consistent product. **Clinical value assessments** and more rigorous *in vivo* and *ex*

vivo experiments are required to establish a solid foundation for human health applications.

Kombucha currently competes with traditional, cheaper Indian gut-health staples like **buttermilk, kanji, and yogurt**. Ultimately, the goal is to transform kombucha from a dietary supplement into a standardised pharmaceutical agent through more in-depth research.

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