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SHORT REVIEW

Natural Carbonated Drink (Kombucha Tea) and its Health Benefits: A Review

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Abstract

Kombucha is a traditional carbonated tea obtained by fermentation with Symbiotic Culture of Bacteria and Yeast(SCOBY). The SCOBY consumes sugar in tea and produces enzymes and organic acids. The process typically takes one or two weeks and changes tea into a fizzy, slightly sour fermented (generally non-alcoholic) beverage. Kombucha contains chemical and biologically active compounds such as polyphenols, antioxidants, and a source of probiotics (*Lactobacillus*) that show health-promoting properties. Kombucha has potential health benefits, but not all have been proven in studies with humans. It is being commercialized in India and can be prepared at home easily with precautions. It can be prepared by fermenting sweetened green or black tea using a Symbiotic Culture of Bacteria and Yeast (SCOBY). As the fermentation proceeds, the yeast in the SCOBY breaks down the sugar present in the tea and releases friendly probiotic bacteria. In this paper, we highlighted various factors affecting fermentation, where the pH shows a significant impact on the tea quality. Herein, we have also discussed the health benefits and toxicity of Kombucha tea along with the recent literature.

Keywords: Antioxidant, Acetobacter, Fermentation, Flavonoids, Health Benefits

1. Introduction

Kombucha is a standard beverage among many traditional fermented foods worldwide. It first appeared in northeast China (Manchuria) and then spread to Russia and the rest of the world. Kombucha is also known as tea fungus. Herein, the fermentation process does not require any external addition of the fungus[1]. The fermented tea is commonly called Kombucha and is known by other names such as Caj Kvas in Russian, Tea Fungus-Indonesia and Japan, Teyi saki in Americian[2], Kargasok Tea-Far North Region (Cameroon), Manchurian Mushroom-China (Meduso)[3]. In the USA, the consumption of Kombucha tea is spread very widely. It has refreshing power and speculative curative effects[4]. Using SCOBY, it can also be prepared at home by fermenting sweetened black tea, and the product formed is slightly sweet and sour with a refreshing taste[5]. Kombucha is a symbiotic growth of bacteria (*Acetobacter xylinum, Acetobacter xylinum, Bacterium gluconicum)* and yeast strains *(Schizosaccharomyces pombe*, *Saccharomyco desludwigii*, *Saccharomyces cerevisiae*, etc.) cultured in a sugared tea[1], which can be considered as a non-alcoholic beverage and amount of ethanol traces may vary with fermentation processes and duration, as it may also be present in the tea. During Kombucha fermentation, indigenous microorganisms produce the biofilm by following a process similar to traditional vinegar production. Fermentation requires a new biofilm layer which can be used for future fermentations as a starter (back-slopping). The end product has a slightly acidic or sour taste and is considered refreshing and typically effervescent[6]. A layer of microbiota may differ and depend on its origin and sometimes includes lactic acid bacteria. The content and activity of starter culture in Kombucha tea and metabolites affect the final product's functionality in all fermented products[7]. The fermentation process is preceded by the microbial activity of yeast and bacteria. The microorganisms

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produced in the fermentation form a thick cellulosic biofilm on the liquid-air interface. The biofilms that have been studied are formed on liquid-solid or air-solid interfaces[8]. The medium for the culture of tea infusions (black, mate, and green) and supplemented by carbon sources; microorganisms can grow without initiation. The refreshing beverage can be produced at home using tea fungus followed by fermentation as sparkling apple cider[9]. Microorganisms of Kombucha can be killed by physical and chemical treatments[10]. A systematic study has been done on cationic minerals present in the tea but not on anionic minerals. Some anions, F^- and I^- are significant micronutrients at low concentrations in tea, but at high concentrations, they become toxic to human health[11]. The consumption of Kombucha tea increases our immunity and prevents many diseases[12]. During fermentation, several compounds such as flavonols (theaflavins and thearubigins), catechins, caffeine, catechin gallates, adenine, theobromine, theophylline, gallic acids, tannins, gallotannin, small amounts of aminophylline and polyphenols are formed[10]. Tea containing catechins has antioxidant, anticancer, antidiabetic, and anti-atherosclerosis properties, acetic acid, and glucuronic acid that show the antidiabetic[12]. Many functional compounds such as polyphenols, flavonoids, and saponins in which polyphenols have been studied in Kombucha as their concentration increases during the fermentation process[13]. Polyphenols can scavenge free radicals (reactive oxygen species) and possess wide-ranging antioxidant properties[10,14]. Kombucha is processed by fermentation, is made from black tea, and contains many bioactive compounds with antidiabetic activity by inhibiting alpha-amylase activity and suppressing blood glucose[15]. A jelly-like membrane formed in the tea when the sugar was added in the presence of oxygen; it increases continuously in the whole solution, and thickens itself at the appropriate temperature. The resulting solution can be sub-cultured every 7 to 10 days by adding 10% old soup with 10% sucrose in tea and can be stored and commercialised[16].

2. Preparation Method of Kombucha

The ideal substrate for Kombucha preparation is black tea sweetened with sucrose, and SCOBY contains traces of carbon dioxide[17,18]. In black tea, oxidation occurs by polyphenol oxidases when exposed to a humid environment. In 1.0 L of boiling water, 5-15 % of sucrose is dissolved, and after that, the addition of 2-3 tablespoons of black tea brews for 5-10 minutes and is removed by filtering the tea solution. The solution is then cooled to room temperature (25°C). The microbial colony (SCOBY) with starter tea (10%) is added to the glass jar and covered with a clean cotton cloth for 7 to 14 days in a dark location at 25 ± 2 °C. After adding starter tea initial pH should be 4.6 or below for brewing Kombucha[19]. If the pH is above 4.6, more starter tea has to be added. If there is no pH change is observed during fermentation it indicates Kombucha tea is not formed. For consumption of Kombucha pH should be in the range of 2.5 to 3.5, as below this it is unfit for drinking. Over-fermented tea is highly acidic (<2.5) and has adverse health effects. To give the Kombucha flavor, taste, and fizziness, it is further fermented with different fruits. Once the fermentation process is complete, the drink is transferred to a one-liter flip-top bottle and four spoonfuls of ginger juice and four spoonfuls of lemon juice are added. It is then allowed to ferment for 3-4 days at room temperature until the desired fizz is obtained. After that, the drink can be stored in the fridge. It is important to note that the alcoholic content of the drink also increases after the second fermentation.

A general preparatory method of tea is shown in Figure 1.

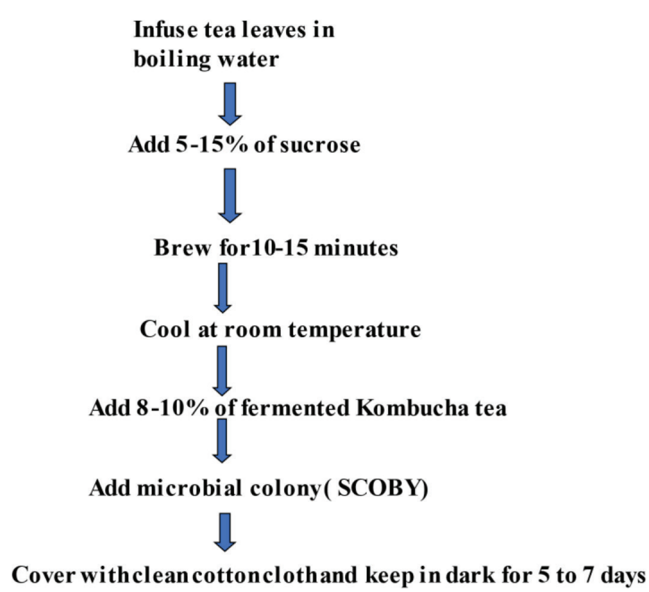

Figure 1. Method of fermentation of Kombucha tea.

2.1 Kombucha at Home

Kombucha tea can be prepared at home easily by maintaining proper hygiene and temperature control throughout the fermentation process[20]. Kombucha involves two-step fermentation.

2.1.1 First Fermentation

2.1.1.1 Ingredients

RO Water (1.2 L)
Green/black tea (2-3 tea bags or 2-2½tsp tea leaves)
Sugar (80-90 gm)
Mother tea (100 ml) and one SCOBY

2.1.1.2 Methodology

Boil 300ml of water and turn off the heat. Infuse the tea for 10-15 minutes. Remove tea bags and dissolve sugar. Now transfer in a clean sanitized 1.5 litre glass jar. Add the remaining water and cool it at room temperature. Now place SCOBY and add starter tea. Cover the jar with a clean sanitized cloth. Keep for fermentation for 6-9 days in a dark place at 25 ± 2 °C (below 30°C). Start tasting after six days. Stop fermentation when a balance of sweet and tart flavors is obtained. Raw Kombucha is yellow orangish or golden in color. pH of tea can be tested by pH strips or a pH meter.

2.1.2 Second Fermentation

It allows for further carbonation and the development of unique flavor profiles in the Kombucha. Additionally, it helps to further break down sugars and increase the probiotic content, making it even more beneficial for gut health.

2.1.2.1 Methodology

Pour raw Kombucha into a flip-top bottle, add the desired flavor (with fruit juices, herbs, and spices), and seal tightly. Keep at room temperature (away from direct sunlight) for 3-5 days to carbonate. Once the desired fizziness level is achieved, refrigerate and consume it within 20-30 days.

3. Factors Influencing Kombucha Tea

Temperature, pH, oxygen content, carbon dioxide dissolution, shear rate in the fermenter, and the nature and composition of the medium[21] are various factors that affect fermentation. The fermentation rate, nutritional quality, organoleptic properties, and other physicochemical properties may vary according to the circumstances.

3.1 Substrate

Many researchers used non-traditional substrates such as Coca-cola, red wine, white wine, vinegar, extract of (Jerusalem) artichoke, milk fresh sweet whey[22], and some sweetened sour cherry juice[23] extract of Jerusalem artichoke tuber is a potential substrate which might be used as a dietary supplement because of its low level of D glucose and D fructose and inulin oligosaccharides which act as dietetic fibers. Molasses from sugar beet is one of the other substrates used by people as it contains many qualities such as vitamins and nitrogen sources like -sweetens echinacea (*Echinacea purpurea* L.) and winter savory (*Satureja montana* L.) and reduces the time of fermentation and is low in cost[24], grape juice is used as a substrate because of its sensorial and functional properties after six days of fermentation[25].

3.2 pH

The most significant environmental factor influencing fermentation is pH. The pH does not show that the fermentation process is finished. The infusion of tea starts at a pH of ≤ 5 which is reduced further to a finishing pH of ≤ 2.5. As long as the pH is below 4.6, no preservative is required, as per FDA's guidelines[19], the beverage is free of microbial contaminants. The crucial limit of pH is ≤ 4.2. At low pH levels, food spoilage bacteria cannot survive. The biological activity of beverages is governed by the acids produced during fermentation, such as acetic acid and gluconic acid, which also link microbial growth and structural changes in photochemical compounds that regulate antioxidant activity[26]. When Titratable Acidity (TA) reaches an ideal concentration of 4 to 5 g/L, fermentation is expected to be stopped. However, depending on the starting culture used and the fermentation conditions, it may take longer or shorter to obtain an optimal TA level[27]. According to the literature, the acetic acid bacteria and tea fungus yeast metabolic activity primarily produces acetic acid and carbon dioxide, which caused the pH values of the black tea to decrease after 30 days of fermentation. The ideal pH for consumption of Kombucha tea is typically between 2.5 and 3.5. This range ensures that the tea has the right balance of acidity, which is significant for its flavor and potential health benefits. Below 3.0 pH strong and above 3.0 minutes flavor of vinegar is developed.

Torre *et al.*,[28] monitored the pH of Kombucha during the storage period and observed pH value of black tea was 5.59 and then dropped to 2.82 after thirty days of fermentation. This pH was used as a control for the Kombucha samples examined over nine months. The value remained stable for two months before dropping dramatically by roughly 0.2 units compared to the control from months four to six. Then there was a sudden increment in pH up to 3.24 and stayed steady next three months.

3.3 Temperature

Enzymatic activity and microbial growth of tea were found best at optimum temperature ranges from 22°C to 30°C[29]. Food originating with plants can change antioxidant activity as their concentration varies with temperature, and the highest value was obtained between 37°C to 42°C. The effect of temperature (50-90 °C) studied by other researchers was on biochemical compounds and free radical scavenging present in tea concludes that heat treatment was not a better way to preserve tea, as time, temperature, and light show the typical transformation in quality and biological activity of tea[28]. Temperature plays a crucial role in effective fermentation. The yeast and bacteria in the SCOBY are affected by temperature. The temperature can affect the balance of these microorganisms in the SCOBY because various strains of bacteria and yeast are more or less tolerant to certain temperature ranges. The growth of bacteria and the SCOBY may be hindered if the brew is dominated by yeast as a result of improper brewing temperature. At low temperatures fermentation becomes slow and SCOBY will not grow properly. At high temperatures, fermentation speeds up and the taste of tea becomes tart and vinegary. The ideal temperature range of 24-29 °C must be maintained during the fermentation process. Aung T and Euan[30] studied the time and temperature effect on physicochemical, microbiological, and nutraceutical properties of laver Kombucha during fermentation at 25°C and 30°C, over 22 days.

3.4 Time Effect

The normal fermentation period for Kombucha is 15 days, however, it may last anywhere from 7 to 60 days. The starter culture and the length of the fermentation, which affect the kinds of metabolites generated, determine the real amount of the activity. biological processes may add intense color to this process. Better results were seen after 15 days when the incubation time increased, and the synergic effect was obtained on the antioxidant activity[30]. Certain organic acids build up, which are harmful if consumed directly. The fruit-flavored refreshing beverage was produced within 6 to 10 days of fermentation which tasted like vinegar. Many studies have concluded that fermented tea in less than ten days is good for human health. Polyphenol content and antioxidant activity during the period (7, 14, 21 days) tends to increase after seven days due to the microbial diversity in Kombucha.

4. Chemical Composition

Knowledge of kinetics is very significant to understanding the properties and composition of Kombucha tea. The composition of metabolite and concentration of organic acids in tea depends on factors like- inoculum source, type of tea, sugar concentration, fermentation time, temperature, and microbial community of starter culture may affect the presence and quantity of chemical compounds[29]. Kombucha beverage has shown the presence of organic acids such as -acetic acid, gluconic acid, glucuronic acid, lactic acid, malic acid, and other organic compounds such as sugars, vitamins, amino acids, and biogenic amine are produced during fermentation. In this tea, some minerals (Mg, Fe, Cu, Zn, Co, etc.), D saccharic acid 1,4-lactone, and metabolite are found as the product of yeast and bacteria[18]. Scientists have proven the presence of usnic acid which plays a role as a natural antibiotic and has an antibacterial and antiviral property that is effective against some bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus*. Organic acid produced in seven days in black tea is found higher than in green tea and oolong tea[31]. In Kombucha tea, the production of acetic acid increases after 15 days and reaches up to 9.5g/L. Whereas the concentration of D-gluconic is formed at 2.5 g/L in twelve days and lactic acid is found in fewer amounts of 0.54 g/L in 3 days. The anionic concentration is low and ranges from 0.04-3.20 g/L as F^- and Cl^- ions are mostly present[29]. Some basic complexes of gallic acid and catechin are theaflavin, theaflavinic acid, thearubigins or theasinisis, and proanthocyanidin. Herein these complexes are formed by the oxidation of polyphenols in black tea. The number of polyphenols and flavonoids increase on 21 days at 28°C in fermented than unfermented tea[32,33]. The following compounds are represented in Figure 2.

4.1 Vitamins

Various chemical components found in green and black tea show a significant effect on the human immune system and metabolic processes. Vitamins such as B1, B2, B6, B9, and B12 are present, and they also have a high amount of vitamin C or ascorbic acid and some vitamins B[34]. Water soluble vitamins B and C are thiamine, riboflavin, niacin, pantothenic acid, B6, biotin, B9, and cobalamin[35].

4.2 Minerals

Kombucha is a complex beverage with many compounds and minerals (F, K, Mn). It is made from green and black tea. Some essential minerals in the form of cations of K, Mg, Mn, Co, Cu, Fe, and anions of F are found in Kombucha[36,37]. The cations of Fe and Cu are found in a free state and consist of pro-oxidant properties which may damage lipid, protein, and nucleic acid. Some researchers have also reported toxic metals like lead and copper in the Kombucha.

4.3 Polyphenols

Polyphenols are very significant phytochemicals. These phytochemicals are bioactive and provide the flavor and aroma of tea. Primary polyphenols are found as flavonoids, flavanols, flavanol gallate, and flavanol glycosides in fresh tea leaves[38]. They also prevent numerous diseases related to oxidative stress as cancer, cardiovascular disease, and neurological disease. They can control the activity of various enzymes and cell receptors to become active, protecting against oxidative stress beginning with reactive oxygen species. Polyphenols produce complexes of catechins and gallic acids such as theaflavin, theaflavins, thearubigins, or theasinisis on oxidation. Bioactive compounds such as catechin are called flavanols. Catechins are also presented in the tea. These are alpha epigallocatechin 3 gallate, alpha epicatechin, alpha epigallocatechin, alpha epicatechin 3 gallate, alpha gallo catechins, and beta catechin[38]. In green tea, catechin is obtained in high concentrations and tastes bitter and astringent. In black tea, it is oxidized to theaflavin and thearubigins, and the

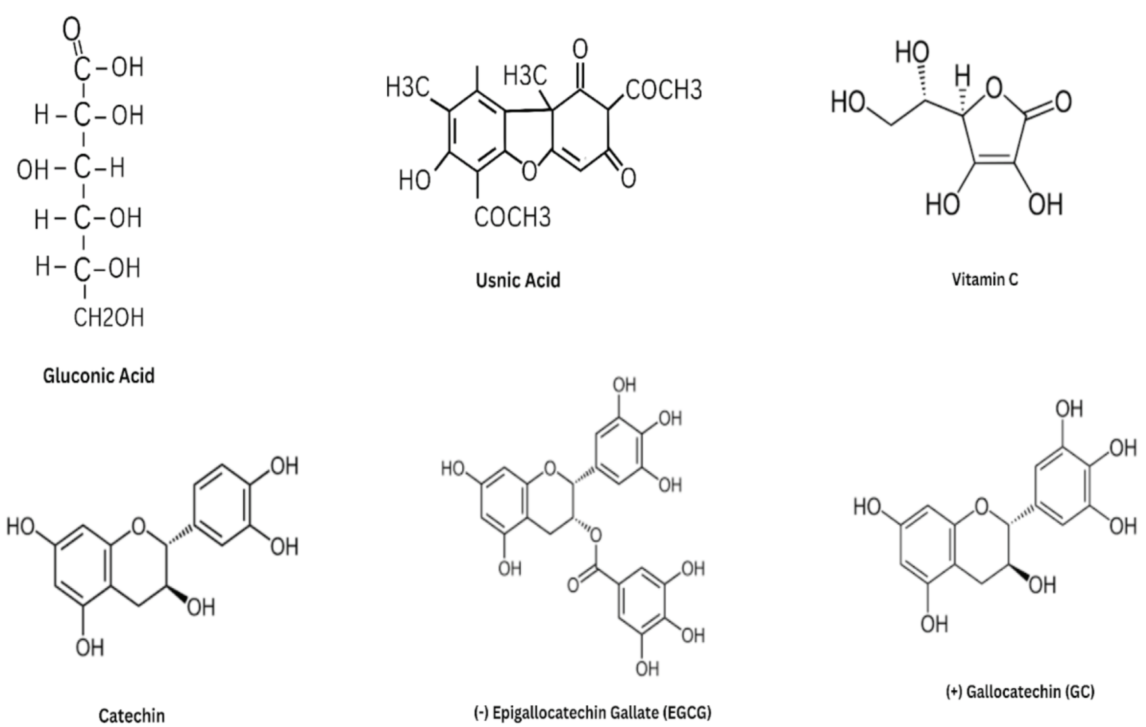

Figure 2. Some basic structure of chemical components presents in tea.

catechin level decreases by 85% and leads darker black color and less bitter taste of tea[39].

4.4 Organic Acid

Several organic acids are present in Kombucha as acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic and usnic, tartaric. It also shows antiseptic and antibacterial properties. Glucose produces glucuronic acid on its oxidation. It is a significant detoxifier in the body and can bind some toxic compounds in the liver. When sucrose is added as a metabolite, acetic acid bacteria form acetic acid. Herein, it is metabolized into glucose and fructose. After completion of one month concentration of sucrose decreased by 11 g/L and left 8.0 g/L after two months as bacteria consume sugar (carbon source). When sugar is replaced by other carbon sources concentration of acetic acid becomes low, and it tastes like vinegar[40]. Glucuronic and gluconic acid play a significant role in xenobacter liver detoxification[41]. It combines with toxic molecules and eliminates them from the organism. Scientists observed the absence of these acids till the 6th day. Scientists also reported these acids' highest values on the 7th and 21st days[42]. D-Saccharic acid 1,4-Lactone (DSL) derived from D-glucaric acid has detoxifying and antioxidant properties. The amount of DSL is 132.72 μ/mL. The highest value of DSL is found on the 8th day and gradually decreases till the end of fermentation, and lactic acid bacteria have a positive effect on the production of DSL[43].

4.5 Amino Acids and Biogenic Amine BAs

Several amino acids are reported in the tea as they are essential for the body. They can also change into Biogenic Amines (BAs) via microbial decarboxylation during fermentation. Certain BAs are necessary for cellular metabolism, and others can be harmful when consumed in large amounts (Table 1). Various chemical components are tabulated in Table 1[33,35,38,44].

5. Microbial Composition in Kombucha Fermentation

Kombucha is a popular fermented beverage made by fermenting black tea and sucrose with SCOBY. Sources, fermentation conditions, cultivation medium, and some geographical regions are some factors that show variations in the composition of SCOBY. At the household level, Kombucha can be prepared using a variety of ingredients and recipes. Commonly found microorganisms in tea are acetic acid bacteria and yeast, and sometimes lactic acid bacteria can occur but not as part of Kombucha.

5.1 Yeast

Alcoholic fermentation begins with a starter culture called *Saccharomyces cerevisiae* due to its high efficiency, nowadays the use of non-*Saccharomyces* in the industry is increased in mixed fermentation (wine, tequila), as yeast species can ferment sugar to ethanol[45]. The various species of yeast are tabulated in Table 2[6,8,21].

Table 1. Chemical components present in Kombucha black tea fermentation

Organic acid	Polyphenols	Vitamins	Amino acids	BA	Minerals
Acetic	Flavonoids	B1	Aspartic	Serotonin	Fe
Gluconic	Flavanol	B2	Threonine	Histamine	Cu
Glucuronic	Flavanol gallate	B6	Glutamic	Cadaverine	Mn
Citric	Flavanol glycosides	B9	Glycine	Putrescine	K
L-lactic	Catechin	B12	Alanine	Agmatine	Co
Malic	Theaflavin	C	Valine	Spermine	Mg
Tartaric	Thearugubin	Thiamine	Methionine	Tyramine	F
Malonic	Theaflavanic	Riboflavin	Isoleucine	Tryptamine	
Oxalic	Thea Sinensis	Niacin	Leucine		
Succinic	Alpha epigallocatechin	Cobalamin	Tyrosine		
Pyruvic	Alpha Epigallocatechin 3 gallate	E	Arginine		
Usnic	Beta catechin	K	Glutamine		

5.2 Bacteria

Acetobacter, are acetic acid bacteria that are present in Kombucha. They produce acetic acid from ethanol. Bacteria need more oxygen for their growth than yeast. There are 17 genera of Acetobacteraceae (AAB), which are gram-negative bacteria and belong to the family *Acetobacteraceae.* A characterized species in AAB is *Komagataeibacter xylinus* is responsible for the production of cellulose pellicle[13,46], which can accumulate 10-20 % of acetic acid, and *Acetobacter* can accumulate 8% acetic acid and is thought to be the most precious species of associated with Kombucha fermentation, due to its superior cellulose–synthesizing ability[6]. Several AABs[47] are present in tea fungus (Table 2). Oxidation of ethanol over alcohol is preferred in *Acetobacter* and *Gluconicacetobacter,* whereas *Gluconobacter* prefers oxidation of glucose, glycerol, gluconic acid, and sorbitol over ethanol[48]. Lactic Acid Bacteria (LAB) are gram-positive bacteria that belong to the phylum *Firmicutes* and produce lactic acid. They also generate other metabolites such as ethanol, acetic acid, carbon dioxide, diacetyl, and mannitol[49]. The systematic isolation of species from genera like *Lactobacillus, Leuconostoc,* and *Bifidobacterium* has been documented, even though LAB are not always present. Since Lactobacilli had been isolated from Kombucha Zygosaccharomyces, which was the dominant yeast in the fermented beverage, considerable abundance of LAB (*Lactobacillus, Bifidobacterium, Lactococcus, Leuconostoc*) was detected. Nine yeast species that had not previously been associated with Kombucha, including *L. fermentati, K. marxianus*, and *Wallemia sebi*, were also detected[50].

Invertase (fructo furanosidase), which yeasts concurrently release, hydrolyzes complex sugars and sucrose into glucose and fructose, which are then converted into ethanol via the glycolysis pathway. Under aerobic circumstances, some yeast species, such as *Brettanomyces bruxellensis* and *Schizosaccharomyces pombe*, can create significant amounts of ethanol and acetic acid. Yeasts under high osmotic pressure produce, sugar, alcohol or glycerol, which can be metabolized by AAB to yield Dihydroxyacetone (DHA). To lower the amount of ethanol and sugar in the Kombucha, AAB also uses the leftover glucose and fructose to produce a variety of organic acids, including acetic acid, gluconic acid, glucuronic acid, ascorbic acid, succinic acid, and other beneficial compounds. Typically, *Acetobacter* and *Komagataeibacter* favor ethanol oxidation as compared to glucose whereas *Gluconobacter* prefers glucose and glycerol. The production of cellulose biofilm and D-saccharide acid-1,4 lactone (DSL) in Kombucha is also associated with the presence of the AAB, particularly *Komagataeibacter*. LAB can convert glucose into lactic acid via the Embden–Meyerhof–Parnas pathway. LAB can utilize glucose via the pentose phosphate pathway and produce lactic acid, ethanol, and carbon dioxide as the primary metabolites. Some LAB species also produce glucuronic acid and a promising detoxifying and antioxidant agent, DSL. It has been reported that ethanol and acetic acid in Kombucha can prevent the growth of pathogenic bacteria, thus protecting the contamination of Kombucha from undesired pathogens[51]. Some common species of bacteria (AAB and LAB) and yeast are represented (Table 2).

Table 2. Common bacteria and yeast present in Kombucha

Bacteria (AAB and LAB)	Yeast
Komagataeibacterxylinus	*Saccharomyces cerevisiae*
Acetobacter xylinoides	*Zygosaccharomyces*
Bacterium gluconium	*Saccharomycodes*
Acetobacter aceti	*Saccharomyces lachancea*
Acetobacter pasteurians	*Schizosaccharomyces*
Gluconobacteroxydians	*Brettanomyces*
Lactobacillus	*Pichia*
Leuconostoc	*Candida*
Lactococcus	*Torulopsis*

6. Mechanism of Kombucha Fermentation

The diagram in Figure 3 illustrates the relationship between microorganisms, fungi, and substrates after fermentation. Kombucha is formed by substrates like black/green tea with the presence of SCOBY, yeast, and sucrose, where sucrose is converted to fructose and glucose in the presence of yeast. *Acetobacter xylinum* is a bacterium that can produce a floating network of cellulose that increases the contact between fungi and bacteria and helps in the oxidation of glucose into gluconic acid and glucuronic acid. Further conversion of fructose into

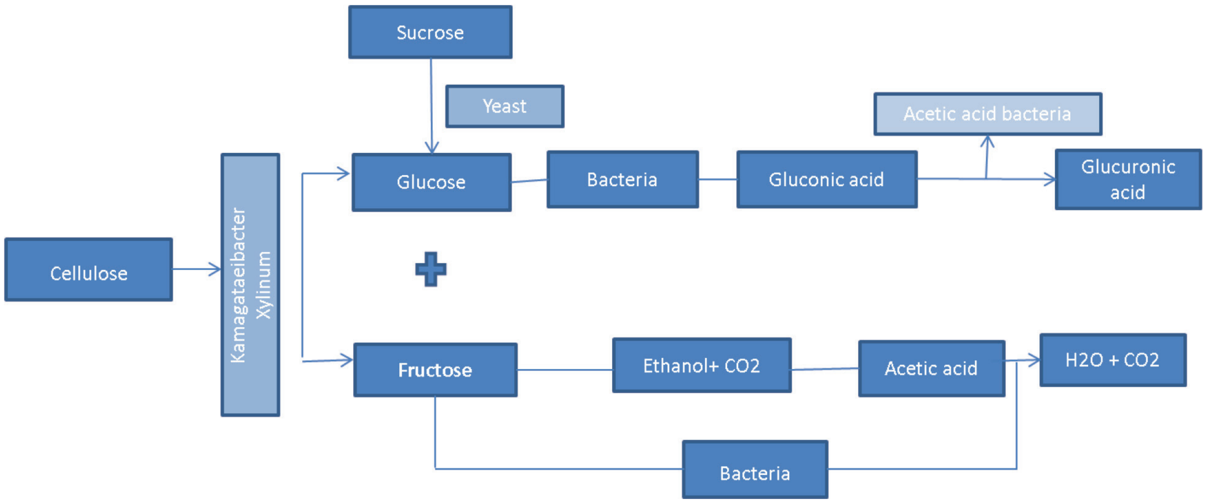

Figure 3. A biological mechanism in Kombucha fermentation[6,8,13,21,46,48].

ethanol and carbon dioxide takes place by yeast. Herein, it continues the conversion of ethanol to acetic acid by bacteria[32,52].

7. Biological Activity

7.1 Antioxidant

Bioactive compounds show interest in some oxidative stress-related diseases that contribute to pathological conditions, such as aging. Antioxidants act as a scavenging agent, binding pro-oxidant, and inhibiting pro-oxidant enzymes. Polyphenols, ascorbic acid, and DSL in Kombucha tea show antioxidant properties. They are found to be higher in fermented tea than unfermented tea. Phytochemicals are present in natural resources as antioxidants. Functional food acts as an antioxidant within the human body[46,53]. Polyphenols act as protecting agents for human beings. They show the scavenging activity on DPPH moieties, and superoxide species, and show inhibitory activity against hydroxyl radical-mediated linoleic acid increases with the fermentation process. Hydroxyl radicles show the scavenging ability (ascorbic acid iron EDTA), and anti-lipid peroxidation ability decreases[54]. Antioxidant properties of tea are affected by tea type, pH, temperature, inoculum size, and starter culture[55].

7.2 Antimicrobial

Fermented tea represses the development of microbes. These microbes possess some human well-being risks, *Shigella sonnei, Escheria coli, Salmonella enteritidis,* and *Salmonella typhimurium*. Tea contains some acetic acid antimicrobial efficacy for *Agrobacterium*, *Bacillus*, and *Salmonella escherichia* but not for *Candida albicans*. Black tea possesses high inhibitory activity as compared to others such as mulberry tea, Japanese green, jasmine tea, and oolong tea[56] the presence of large proteins, catechins, and organic acids (acetic acid) in Kombucha tea is responsible for its microbial activity. Gram-positive and Gram-negative bacteria are inhibited by acetic acid and catechins[57].

7.3 Antibacterial and Antiviral

Gram-positive and Gram-negative bacteria can harm people and are resistant to the antibacterial activity of green tea catechin. Tea extracts contain pathogens like as *Staphylococcus aureus*, *S. epidermis, Plesiomonas shigelloides*[1], and *Salmonella typhi* etc., are ineffective against *E. coli* and *Pseudomonas aeruginosa*. Tea extract from black tea and green tea can kill *Helicobacter pylori* with gastric, peptic, and duodenal ulcers. Polyphenols present in tea can prevent colon cancer by promoting and inhibiting the human large intestine. Tea catechin is an antiviral and antiprotozoal agent and has proven to be effective against HIV enzymes, where polyphenols and sesquiterpenes have a synergic effect on the antibacterial activity and anticarcinogenic of tea.

7.4 Anti-inflammatory

The process of inflammation is essential for defending the body against pathogens. Asthma, arthritis, and cardiovascular diseases are just a few of the illnesses that include inflammation as a contributing factor. In particular, persistent inflammation has been noted to be connected to the precancerous state, which results in cancer, the riskiest disease and the one that kills the most people every year in the world. As a result, anti-inflammatory characteristics are now being targeted for cancer therapy and prevention. Recent studies have shown that Kombucha possesses anti-inflammatory and anticancer properties, which are attributable to the many polyphenols and other crucial metabolites created during fermentation[51]. Some trials have shown that both traditional Kombucha and broths fermented with non-traditional ingredients may have anti-inflammatory effects. Scientists discovered that Kombucha can treat cellular immunological problems in rats with early sepsis, encourage the growth of bacteria that produce butyric acid, and have anti-inflammatory properties. According to Cabral research, Kombucha-fermented oak leaves can successfully lower Nitrogen Oxide (NO) generation as well as macrophage levels of interleukin-1 (IL-1) and Tumor Necrosis Factor-alpha (TNF-α)[58].

7.5 Anticancer

Dietary phytochemicals are used for chemoprevention which means control of different types of cancer with few side effects. Many studies concluded by Russia by the ″Central Oncological Research Unit″ and the Russian Academy of ″Science in Moscow″ in 1951 claimed that tea acts as a protecting agent against cancer cells[59]. Some scientists concluded that Kombucha made from black and savory tea has more anti-proliferative properties. According to them, tea of winter savory has more proliferative activity than black tea and simple water extract of tea winter savory. The presence of polyphenols and degradation products produced during fermentation account for anticancer activity. Studies found that it prevents the angiogenesis or development of new blood vessels and decreases cell growth[60]. Some techniques are listed in Table 3[28,52,61] for determining biological compounds in tea.

8. Health Benefits

Many nutritionists and researchers have concluded Kombucha is effective at easing and promoting digestion and some other health issues such as headache, hemorrhoid, atherosclerosis, metabolic disorder, gout, arthritis, diabetes slugginess of the bowels, fatigue, stress improvement of depression and anxiety, gut health[3,14]. Additionally, it is rich in flavonoids, which can enhance immunity by scavenging free radicals that damage cells. Similar to polyphenols in tea, acetic acid also exhibits antibacterial properties. Kombucha is made from a black and green type of tea that has antibacterial properties against infection-causing bacteria and candida yeast. It shows relief from a variety of health problems like hair loss diseases, AIDS, weight, high blood pressure, and heart disease. Medical studies done by doctors and scientists on Kombucha tea have come up with some primary health benefits in metabolism, boost in the

Table 3. Various techniques to determine biological activity

Biological activity/Compounds	Techniques
Antioxidant Activity	Automated oxygen radical absorbance capacity Spectrophotometric method using synthetic DPPH
Polyphenolic determination	HPLC Thermo scientific Dionex ultimate 3000 pump and UV-150 model detector
Total Phenolic Content (TPC)	Folin-Ciocalteu colorimetric method
Anti-inflammatory	Spectrophotometrically using enzyme 5-LOX
Organic acid	High-Performance Liquid Chromatography (HPLC)
Alcohol	Alcoholometer Ebuliometer Ultra-Performance Liquid Chromatography (UPLC)
Volatile compounds	Gas Chromatography-Mass Spectrometry (GC-MS)
Sugars (sucrose, glucose, fructose)	HPLC equipped with a waters XBridge. light scattering detector

immune system, and support liver and gastrointestinal tract. It is said that Kombucha aids digestion clears the body of toxins, and increases vitality. It is also supposed to strengthen your immune system, aid in weight loss, protect against high blood pressure and heart disease, and prevent cancer. However, there is not much data to back up these statements. Fermented foods are generally beneficial to the microbiota and gut health. Fermentation produces probiotics, which aid in the treatment of diarrhoea, bloating, and Irritable Bowel Syndrome (IBS), and may even enhance your immune system.

The presence of various organic acids and the low pH value of Kombucha products beneficially increase anticancer ability, particularly the toxicity of Caco-2 colorectal cancer cells. The cytotoxic effects of Kombucha on human renal carcinoma, human osteosarcoma cells, and human lung carcinoma have also been reported. The presence of organic acids in the Kombucha products also enhanced health benefits, such as body detoxification, maintaining hormonal balance, and improved bioavailability of phenolic compounds. Glucuronic acid, for example, is considered the most potent detoxifying metabolite, which can bind toxin substances in the liver, allowing their effective excretion. Glucuronic acid is a precursor for vitamin C biosynthesis. It also takes part in glucuronidation, which plays a crucial role in hormonal balances and helps increase the bioavailability of phenolic compounds, which in turn causes the neutralization of free radicals, preventing oxidative damage. Lactic acid has been shown to positively impact human health by improving blood circulation and preventing the formation of blood clots. In addition, the lower pH of Kombucha by lactic acid is also attributed to antimicrobial activity[51].

It has been used as a replacement for hard beverages by persons who want to reduce their alcoholic consumption since it is fermented. It is healthier than most commercial colas and juices since it is free of chemicals and preservatives and has low residual sugar content. Kombucha is low low-calorie drink. Consuming 240 ml of raw Kombucha provides 29 kcal energy as compared to soda/colas (95 kcal). It is a great source of B-complex such as thiamine and niacin[62]. It can be flavored with a variety of spices, herbs, and fruits. Various health benefits are represented in Table 4[55,63].

Table 4. Some basic health effects of fermented Kombucha

Prevention of hair loss and weight loss
Detoxification of body
Maintaining hormonal balance
Hepatoprotective effect against liner contaminants
Gut health
Body resistance to cancer
Boost in the immune system
Gastrointestinal tract
Antibiotic properties
Relief of joint rheumatism, gout, and hemorrhoids
Reduce cholesterol level
Toxic excretion and blood cleansing
Reduce stress, nervousness, and aging problems
Protect against diabetes

9. Toxicity in Kombucha

Kombucha is a traditional drinking beverage that can also be prepared at home. The presence of bacteria in this beverage shows some beneficial health effects, but consuming the contaminated or over-fermented beverage shows harmful side effects on health. A moderate amount of this beverage must be taken in drinking, and the pH should not be less than 2.5. Some cases of disorders were enlightened as dizziness, nausea, severe illness, allergic reactions, and head pain, and were not healthy for pregnant and lactating women as they cause blood clotting and thinner during the trimester of pregnancy. Bacteria and Yeast together make Kombucha, where bacteria multiply in the liquid, and these bacteria are probiotic types that can grow harmful bacteria or molds. Some of the problems include liver problems, lactic acidosis, allergic reactions, and nausea. Some individuals also face gastrointestinal toxicity after 6 months of consuming tea[64,65].

10. Recent Research

- The pH value, Titratable Acidity (TA), total phenolic content, and antioxidant activity against hydroxyl (OH) and 1,1-diphenyl-2-picrylhydrazil (DPPH) radicals were measured throughout the seven-day fermentation process to determine the relationship between the fermentation time and the antioxidant and antibacterial properties of lemon balm Kombucha[66].

- Pure *et al.*, has utilized some herbal infusions such as banana peel and nettle leaves for Kombucha fermentation. He evaluated the total phenolic content and free radical scavenging activity of Kombucha tea, which shows black tea has the highest phenolic content and antioxidant potential as compared to the nettle leaves infusion[67].
- Jakubczyk *et al.*,[37] have reported that Kombucha prepared with black tea has many health benefits. As per the demands of consumers, trial with other types of tea have been increased for producing it. Consequently, health benefits are more affected than the traditionally made Kombucha. Tea mainly shows effects on antioxidant potential, pH, acetic acid content, alcohol, and sugars. Red and Green tea on the 1st and 14th day is a good source of antioxidants and polyphenols. Green tea has the highest antioxidant properties and high reductive potential.
- Danzhou *et al.*,[61] have studied the fermentation of Kombucha and investigated, some basic properties such as its effect on antioxidant activity, total phenolics content, and polyphenols at different stages of fermentation using FRAP (ferric reducing antioxidant power), Trolox equivalent capacity, HPLC.
- In another study Kaashyp *et al.*,[52] identified the microbial community present in the tea by using metagenomic amplicons (16SrRNA and ITS). They identified 34 genera and 200 species as *Acetobacter, Bacillus, and Starmella* produce organic acid. These produced organic acids are healthy for the human gut and have some glucose metabolizing activities that prevent diabetes and obesity.
- In this study, Torre *et al.*,[28] reported the effect of storage of Kombucha 9 months formed by black tea at 4°C for 30 days and analyzed general parameters like pH, total phenolics, flavonoids, and free radicals. Herein, after 4 months pH increases from 2.82 to 3.16, and phenolic content decreases from 234.1 to 202.9 ug GAE mL^{-1} and highlighted that black tea can only be stored for 4 months as after that it loses antioxidant properties.
- Mindani *et al.*,[68] studied the antioxidant property and starch hydrolase inhibitory properties of coconut water. They also analyzed some general parameters like pH, viscosity, ethanol content, ferulic, p-coumaric acid content, and antioxidant properties for 7 days in fermented and unfermented coconut water. In fermented samples, pH decreases, whereas antioxidant properties increase.
- Bacterial cellulose in Kombucha fermentation has been studied by Ahmed *et al.*, Herein, they have studied the applications in medicine, pharmacy, food, agriculture, textile, and electronics. The bacterial cellulose nanofibrils have been produced from black tea and a study has been carried out on the effect of gamma radiations[69].
- Multi-drug resistant enteric pathogens have emerged and awakened scientists to explore the therapeutic potentials of traditional food and beverages. In this study, Debanjana *et al.*, investigated the efficacy of Kombucha black tea against enterotoxigenic *E. coli, Vibrio cholera, Shigella, Flexeril*, and *Salmonella typhimurium* and identified antibacterial components[70].
- Jayabalan *et al.*,[71] have utilized a seawater-based system to produce bioethanol from the waste generated after the first brewing of black tea to reduce the use of fresh water in bioethanol industries.
- Traditional fermentation of wine and tequila by mixing two different yeast species and non-saccharomyces was investigated by Lopez *et al.*,[72] discovered the sensorial characteristics and also determined their biomass and kinetics.
- In this study, Francesca Gaga introduced a new substrate (black and green tea) in Kombucha. These introduced new substrates show less amount of ethanol and acetic acid. In black and green tea, glucuronic acid was the same, and Rooibos leaves affect the recovery of oxidative damage[13].
- Properties such as cytotoxic and anti-invasions have been studied by Rasu Jayabalan *et al*[73]. In this study, chloroform, ethyl acetate, and butanol have been used in fractional amounts with Kombucha. Anticancer properties have been determined by solvent extract and aqueous extract.
- Shaful *et al.*,[74] investigated if Kombucha is fermented with a different substrate like papaya pulp and leaves. Then two different species were highlighted as *Komagataeibacter rhaeticus* MFS1 and *Dekkera bruxellinus*. They show the similarity between the

acetic acid bacterium and yeast, and their tolerance to bile acid has been tested. The results show that *D. bruxellinus* has the highest tolerance capacity in a bile salt environment.

- Rami Rahman *et al.*,[75] investigated the effect of *Brassica tournefortii* leaves in Kombucha when it was fractionated with solvents like ethyl acetate and butane and analyzed on fermented and unfermented tea extract which concluded leaves of *B. tournefortii* showed a reduction in cytotoxicity and xanthine oxidase inhibitory effect.
- Grassi *et al.*,[76] studied temperature effect and storage time on the microbial community, they isolated microbial community (yeast and acetic acid bacteria) from Kombucha as Kombucha was fermented with various herbs at room temperature and 4ºC storage for 90 days using a culture-dependent and independent approach.

The above findings and facts are encouraging but further research on human beings is required.

11. Conclusion

The perceived health benefits of Kombucha have led to an increase in consumption over the last few decades. Due to this, it has turned into a highly commercialized drink that is still made at home but is produced in an industrial setting. Kombucha can be stored at 4°C and commercialized under appropriate temperatures and pressure. Additionally, as a result of the fermentation process, several organic acids, including lactic acid, glucuronic and gluconic acid, are formed. The presence of SCOBY in Kombucha increases the content of alcohol. The pH of initial brewing must be less than 4.6. It further decreases to 2.5 during the fermentation. This is the best pH for tea as it is safe for drinking. This beverage has many health benefits as it is a probiotic. Herein, it also helps in the metabolism and the ability to balance the presence of good bacteria in the gut and relieve some gastrointestinal issues. As it is a drink that originated in European countries, people are very well known and familiar with its health benefits and unique taste. It is easy to brew Kombucha at home. But precautions must be taken as it is sensitive because of the presence of SCOBY and yeast. Nowadays, Kombucha is available at Indian health stores, and grocery shops in a variety of flavors. Despite having a long history of conventional usage, there is no evidence to back up the health benefits of Kombucha tea. Further study can be done by using various substrates, sugar sources, etc.

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