

Review

Chemical Composition of Kombucha

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Abstract: Kombucha is a fermented sweetened tea with a mixed fermenting culture of yeast and acetic acid bacteria. While the history of kombucha is not completely clear, it is now available around the world and has shown an increase in availability and demand in the United States market. The proponents of kombucha consumption tout the varied health benefits it can provide. The final kombucha flavor and composition is a function of both the initial tea used and the fermentation process. While the ascribed benefits are varied and numerous, the number of direct studies has been limited. This review focuses on the current state of understanding of the chemical composition and the potential health effects both positive and negative reported in the literature.

Keywords: kombucha; fermentation; tea; health benefits; chemical composition

1. Introduction

Kombucha is sweetened tea that has been fermented by an inoculation of symbiotic culture of bacteria and yeast (SCOBY) [1]. The origins of kombucha are not well understood; however, evidence suggests that it originated in the Manchuria region, which is located in Northern China [2]. There is also evidence to suggest that kombucha has a long history of consumption in China, Russia, and Germany [3]. In addition, kombucha is brewed in several Asian countries, such as Japan, India, Korea, Java, and the Philippines. While kombucha is an old beverage it has become increasingly popular in Western society recently. Now it is possible to find kombucha in a large portion of the world, including Eastern Europe, the United Kingdom, the United States, Canada, and Brazil [4].

The fermented tea and kombucha market is globally worth USD 1.84 and the leading consumer of kombucha is the North American market, with the United States consuming about half of all the kombucha produced [5]. Another leading consumer of kombucha is the European market, with the United Kingdom and Russia leading the consumption within that market share. Probiotic drink consumption has increased in Asian countries, most prominently in China. Additionally, markets in the Middle East and North Africa have increased the consumption of kombucha because of the beverage's health benefits and non-alcoholic nature. The global kombucha market is forecast to grow by 500% over the next 7 years [5].

The popularity and availability of Kombucha in the United States has been, in part, driven by social media, highlighting the positive health benefits that can be achieved from regular consumption. It has been suggested that kombucha can increase vitality, combat acne, eliminate wrinkles, purify the gall bladder, improve constipation, increase weight-loss, relieve arthritis, increase immune response, reduce blood pressure and cholesterol, reduce kidney calcification, and even inhibit cancer proliferation and cure aids [2,3,6–8]. These ascribed associated health effects have certainly caused an increased interest in this product, yet there is limited published scientific research attributing these effects to the consumption of kombucha. However, it should be noted that there are some indications that



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the consumption of kombucha may indeed provide some health prophylaxis and recovery through detoxification, antioxidants, energizing, and immune-stimulating effects [9]. A recent review focused on the biochemical and microbiological aspects of the fermentation process [10]. This review will focus on the connection between the initial tea and the resulting chemical composition and the current understanding of the potential health effects of kombucha.

2. Tea

Tea is the second most consumed beverage in the world behind water [11]. Tea comes from the leafy perineal plant known as *Camellia sinensis*, from the *Thaectae* family. Although tea can and has historically been grown as far north as the 42nd parallel in the country of Georgia and as far south as the 35th parallel of Argentina, most tea is cultivated in the tropical and subtropical regions of the world. Tea is currently cultivated in approximately thirty-five countries worldwide [12]. The five primary countries that produce and export tea are China, India, Sri Lanka, Kenya, and Indonesia, producing a combined total of 5 million metric tons out of 6.15 million metric tons produced worldwide [13,14]. China produced nearly 46% of the global tea crop in 2019 and produced more than 2.79 million metric tons of tea [11].

In addition to China, India is the second largest producer of tea [14]. That said, the majority of the tea produced in these countries is not exported and is consumed domestically. The leading exporters of tea are Kenya and Sri Lanka, exporting about 90% of their tea crop. The largest tea consumers worldwide are Turkey, Ireland, the United Kingdom, Russia, and Morocco who all consume over 2.6 pounds (1.17 kg) of tea per capita per annum [15].

2.1. Types of Tea

The tea plant is both harvested and processed differently throughout the world. These differences in harvesting and processing lead to different styles of tea (Table 1). These styles include oolong tea, which makes up about 2% of global production; green tea, at about 20% of production; and black tea, at about 78% of production. There are six varieties of *Camellia sinensis* tea consumed: black, dark (including Pu-erh), green, white, yellow, and oolong. The differences between the different teas are determined by the growth stage when the leaves are plucked and how they are processed. All teas are partially dried and then allowed to age in oxygen causing the tea to oxidize. The varying degrees of oxidization known as fermenting is what gives tea its distinctive flavor [4]. Green tea is made from fresh leaves and is widely consumed in Japan and China. Western countries tend to favor black tea, which is made by oxidizing the tea leaves, curing them through a maceration process, and exposing the leaves to air [16]. Oolong tea is mostly consumed in China and Taiwan, while roasted teas are primarily consumed in Japan [16]. Green tea and roasted teas are steamed to prevent enzymatic oxidation. Oolong tea is a semi-fermented tea to allow for a moderate level of enzymatic oxidation to occur during the processing of the leaves [16,17]. Black and green tea are the most popular of the six varieties and two of the most commonly used varieties for making kombucha [11].

Table 1. Processing methods for different types of tea used to brew kombucha.

Tea Type	Process	Reference
Black	Withered, rolled, fully oxidized (fermented), and dried	[4,13,18–20]
Green	Withered (heated or steamed), rolled/shaped, and dried	[4,13,20,21]
Oolong	Solar withered, rolled/bruised, partially oxidized (semi-fermentation), fixed and dried	[4,13,20]
White	Drying	[4,13,20]

2.1.1. Black Tea

Black tea originated in Mongolia/China and is currently cultivated in several other countries throughout the world. The highest quality of black tea comes from countries, such as India and Sri Lanka [22]. Black tea is the most popular form of tea around the world and is the result of oxidation associated with the polyphenol compounds through a multi-stage enzymatic process. A number of traditional kombucha recipes call for the use of black tea because it is fully oxidized, which in turn enhances the tea's flavor and potency [4]. Black tea can range in taste and flavor from savory to sweet, depending on the level of oxidation and processing [18]. When making kombucha using black tea, black tea is generally steeped at relatively high temperature (90–100 °C) for a moderate amount of time (2–5 min). By steeping tea using these methods, the flavor extracted from the tea is maximized without increasing the bitterness [4].

2.1.2. Green Tea

Green tea is primarily associated with the inactivation of polyphenol oxidase by heating or applying steam to the leaves to ensure that the catechins remain unoxidized [21]. Green tea has a lighter flavor, color, and smoother finish than black tea. Due to the delicate nature of green tea, a lower brewing temperature (66–85 °C) is utilized to prevent excessive amounts of tannins from being extracted from the leaves [4]. The primary component of green tea levels belongs to the chemical group polyphenols, which make up approximately 25–35% of the dry weight basis [17,23]. Green tea is rich in catechin polyphenols, including epigallocatechin gallate (EGCG). EGCG has been shown to have antibacterial properties as well as the ability to lower low-density lipoprotein (LDL) cholesterol and boost immunity [4].

2.1.3. Oolong Tea

Oolong is a combination of black (fully oxidized) and green (partially oxidized) tea [4]. The degree of oxidation can range between 10% to 80%. This variation of oxidation is what gives oolong tea its diverse flavor profile, from light, sweet, and fruity to rich, dark, and smokey [24]. The word oolong comes from the Chinese word for “Black Dragon.” [4] Similar to other teas, oolong tea originated in China. Along with China being one of the primary producers, Taiwan also contributes to the production of oolong tea [24].

2.1.4. White Tea

Once only cultivated in China, this tea is now grown in India and Sri Lanka. The Chinese define white tea based upon the subspecies that it is manufactured from: *C. sinensis* var *bhengkhe* bai hao and *C. sinensis* var *fudin* bai hao [20]. White tea is the youngest of all the teas and has the most delicate buds and leaves which are covered in thin white hairs, hence the name. Unlike other types of tea that are oxidized, the leaves for white tea undergo a delicate drying process. The drying process protects the delicate flavor and ensures the highest concentration of antioxidants. White tea produces a kombucha that has a milder taste and is high in catechins [4].

3. Chemical Composition

Kombucha is composed of a number of organic acids, sugars, vitamins, amino acids, biogenic amines, purines, pigments, lipids, proteins, some hydrolytic enzymes, ethanol, caffeine, carbon dioxide, polyphenols, anions, minerals, D-saccharic acid-1, 4-lactone (DSL), bacterial metabolites [2]. Several examples are shown in Figure 1. The chemical composition of the tea leaves used to produce kombucha has been well studied and will impact the concentration of the compounds within the kombucha [4]. The presence and quantities of certain chemical compounds are dependent upon the microorganisms found with the SCOBY, fermentation parameters (time and temperature), sucrose concentration, type of tea used, and the analytical method used for quantification [25]. If sucrose is used as the primary carbon source for the fermentation, acetic acid will be the predominate metabolite

produced. Other organic acids like gluconic and glucuronic are also produced during the fermentation process. If the fermentation process is allowed to go on for too long the pH will drop to low and it becomes undrinkable [26,27].

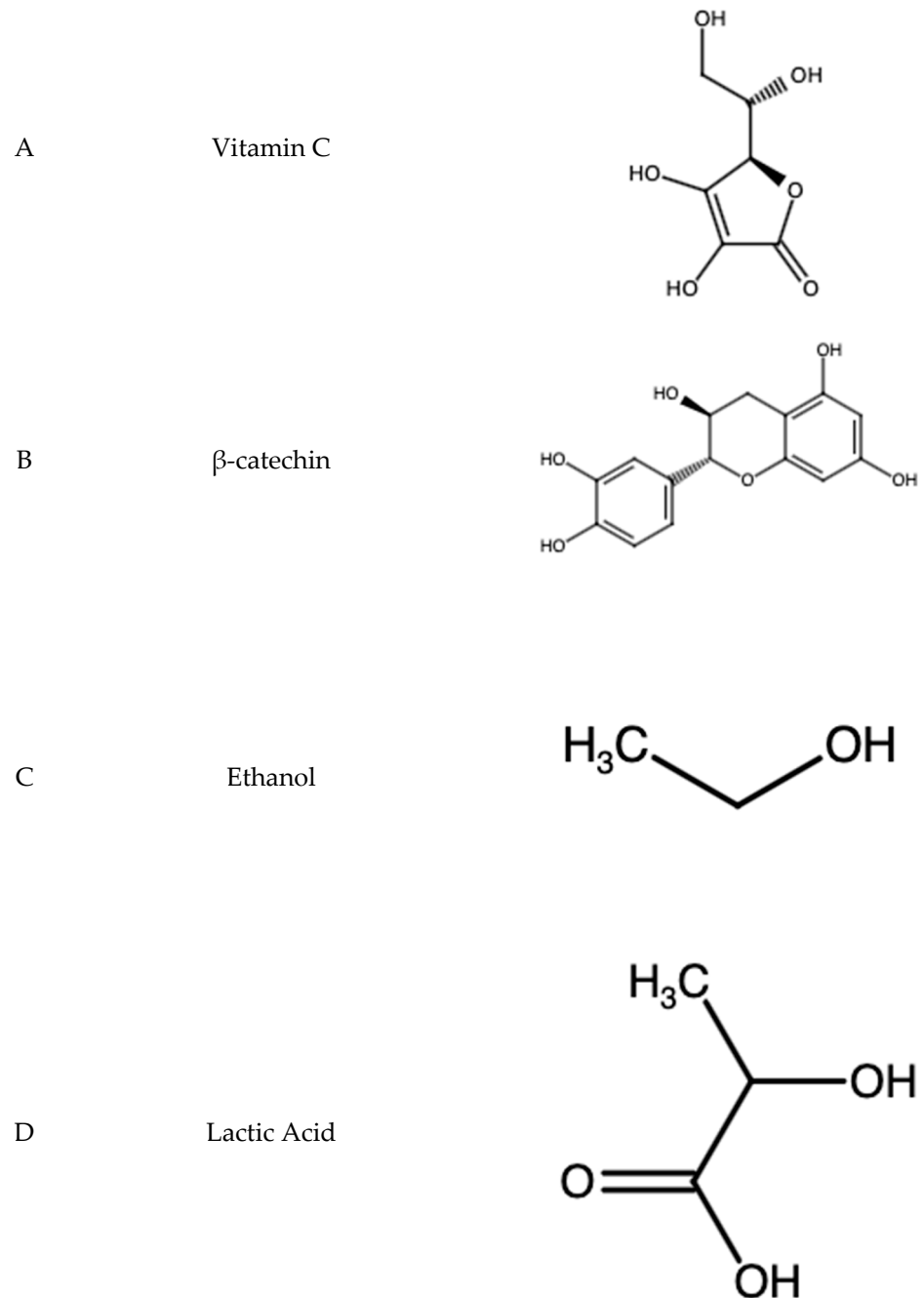


Figure 1. Cont.

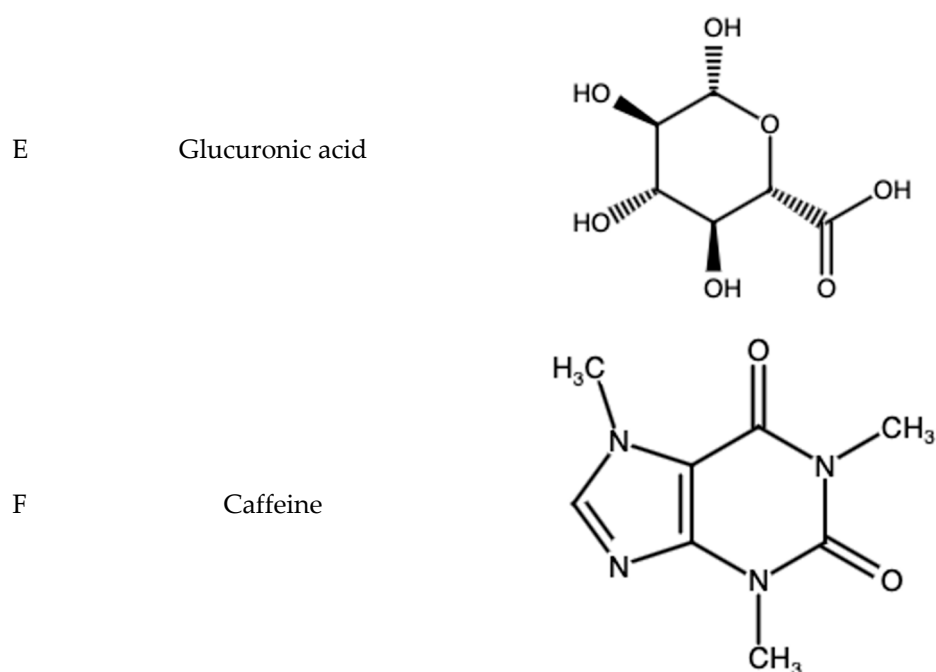


Figure 1. Several chemical structures for example compounds found in kombucha including: (A) vitamin C, (B) β -catechin, (C) ethanol, (D) lactic acid, (E) glucuronic acid, and (F) caffeine.

3.1. Compositions

3.1.1. Vitamins

Although the clinical evidence regarding the benefits of kombucha on health is lacking, scientists have confirmed through research that kombucha is comprised of a wide array of chemical components that come from both green and black tea. Some of these components are known to positively affect the human immune system and metabolic processes in the body. Kombucha fermented with green or black tea contains high levels of Vitamin C or ascorbic acid, and trace amounts of some B vitamins. Vitamins are necessary components for numerous biochemical and physiological processes that take place in the body. Vitamins cannot be synthesized within the body; therefore, they must be supplemented in the diet to obtain healthy levels [28]. The water-soluble Vitamin C and Vitamin B (thiamine, riboflavin, niacin, pantothenic acid, B6, biotin, B9, and cobalamin) have been reported in kombucha [29].

Water-soluble vitamins are less likely to be stored within the body like fat-soluble vitamins (Vitamins A, D, E or K) because they are quickly transported through the blood stream following consumption [29]. Vitamin C supports human health by using defensive antioxidants, the formation of collagen to aid in connective tissue, and during an immune response. At the height of an infection, levels of Vitamin C are quickly depleted [29]. The mechanism behind this action can be explained by the fact that Vitamin C does not require a coenzyme, although it acts as a cofactor for an enzyme called “prolyl hydrolase”, which aids in the formation of collagen [30].

3.1.2. Minerals

Kombucha is a complex beverage composed of a number of compounds, some of which are minerals (F, K Mn) that come from the tea itself [31]. Vitamins and minerals are used by the body for a number of metabolic pathways along with physiological functions [32]. Minerals are inorganic substances in which play an important role in the human body. Small amounts are required by the body for normal function, growth, and maintenance [33]. Essential minerals, such as potassium (K^+), cobalt (Co^{2+}), manganese (Mn^{4+}), copper (Cu^{2+}), iron (Fe^{2+}), magnesium (Mg^{2+}), and fluoride ions (F^-) [33,34], can be found in kombucha made from green and black tea. Bauer-Petrovska and Petrushevska-Tozi (2000)

quantified the content of manganese, iron, nickel, copper, zinc, lead, cobalt, chromium, and cadmium in kombucha [33]. Mineral concentration can range from 0.004 µg/mL for cobalt to 0.462 µg/mL for magnesium [33]. The authors also analyzed known toxic metals. Lead (0.005 µg/mL) and chromium (0.001 µg/mL) were detected in kombucha, while cadmium was not. Studies have shown that certain essential minerals (Cu, Fe, Mn, Ni, and Zn) increase due to the fermentation process, while others, such as cobalt, did not.

Tea, and ultimately kombucha, contain various minerals including fluoride (F). Fluorine plays an important role in the hard tissue mineralization process. However, there is a fine line between enough and too much fluorine. Approximately 85% of fluoride comes from food and beverages (tea, herbal infusions alcoholic beverages, and coffee), while the remaining 15% comes from toothpaste and drinking water [31]. It is well known that certain compounds will decrease sugar concentration, while others will increase it (ethanol and acetic acid). Little is known about what happens to certain ions, such as fluoride, during the fermentation process. Jakubczyk et al. (2021) examined the fluoride content during the fermentation process. Four different tea (white, red, green, black) infusions were analyzed, and the fluoride content ranged from 0.42–0.93 mg/L [31]. The authors saw that the white tea kombucha had the lowest levels, while green had the highest. It should be noted that the authors used distilled water during their experiment; however, if a kombucha brewery uses a municipal water source that will also contribute to the overall concentration of fluoride in the finished product [31]. The recommended dietary allowance (RDA) for adults is 3 mg/L (women)–4 mg/L (men) [35]. A single glass of kombucha has the ability to contribute significantly to an individual's RDA for fluoride [31].

3.1.3. Polyphenols

Polyphenols are bioactive substances that contain more than one phenol structure per molecule. Polyphenols represent the largest group of phytochemicals and are the most abundant antioxidants in the diet. People are estimated to ingest upwards of 1 g/day of polyphenols [36]. Green, black, and many other teas are rich in water soluble polyphenols; these components make up the aroma and taste of the tea. These compounds may account for up to 30% of the dry weight of the tea leaves, according to the literature. The primary polyphenols found in fresh tea leaves are flavonoids flavanols, flavanol gallate, and flavanol glycosides [12]. Flavonoids are a group of bioactive compounds produced during plant metabolism. Flavonoids can be found in fruits and vegetables, such as raspberries, blueberries, and spinach, along with beverages, such as wine and tea [37]. Flavonoids are composed of two six figure rings linked together by a three chalcone structure.

Catechins, often referred to as flavanols, are a type of bioactive compound that is a subclass of flavonoids. These compounds are also the primary secondary metabolites found in tea [12,37]. The major catechins are: α -epigallocatechin-3-gallate (EGCG), α -epigallocatechin (EGC), α -epicatechin-3-gallate (ECG), α -epicatechin (EC), α -epicatechin-3-gallate (ECG), α -gallocatechin, and β -catechin [12]. It should be noted that the concentration of catechins can vary based on the tea type and style. Catechin levels in green tea are relatively stable, unlike black tea, because in contrast to black tea, green tea does not undergo any sort of oxidative process during manufacturing. Due to the higher concentration of catechins, this is the main reason that green tea's characteristic flavor profile is often described as being bitter and astringent. The catechins in black tea are oxidized to form theaflavins and thearubigins, which causes the catechins levels to drop by 85% when compared to green tea. This is the reason black tea is darker and less bitter [37]. The antioxidant properties of polyphenols are responsible for the health benefits associated with tea and kombucha, such as the prevention of cancer, increased immunity, reduced inflammation, and arthritis [38,39].

The total polyphenols and individual concentrations of specific polyphenols in kombucha can vary based on the type of tea leaves used. Cardoso et al. (2020) analyzed kombucha brewed with green and black tea leaves and found that the total polyphenol concentrations varied from 0.70 mg GAE/mL (green)–1.09 mg GAE/mL (black) [40]. Yang

et al. (2022) analyzed nine (black, green, and mixture black/green) kombucha samples for total polyphenols and four tea catechins (catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG)). The total polyphenols measured ranged from 120 µg/L GAE–380 µg/L GAE. Of the samples analyzed, the black tea had the highest concentration of total polyphenols, while a mixed kombucha (black/green) had the lowest [41]. Ozyurt (2020) found similar results to both Cardoso and Yang when analyzing the total polyphenols for green (312 µgGAE/mL) and black (422 µgGAE/mL) [42]. These results are consistent with previously reported results from other research groups [6,39].

3.1.4. Ethanol

Ethanol, a byproduct of yeast fermentation, can also be found in kombucha. The concentration of ethanol in kombucha will continue to increase as the fermentation progresses. Chen and Liu (2000) found in their study that the ethanol concentration reached its maximum value of 5.5 g/L on day 20 of the fermentation, followed by a slow decrease [43]. The entire chemical profile (sugars, carbon dioxide, organic acids, etc.) of kombucha plays a role in the final flavor and aroma profile of the finished beverage, which is why it is important to control ones fermentation parameters to obtain the desired characteristics of the final product [43]. Traditional kombucha does contain ethanol. The Food and Drug Administration (FDA) has investigated the range of kombucha to be between 0.7–1.3% alcohol by volume (ABV). However, craft breweries are starting to make what is known as “hard kombucha”. Kombucha with higher amounts of alcohol. Hard kombucha is known to have an alcohol content of around 3.5–5.5% ABV or higher [44].

3.1.5. Organic Acids

Kombucha is made up of a number of organic acids, such as acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, and usnic [2]. The composition and metabolite concentration within kombucha can vary greatly due to the starter culture used [45], sugar and tea concentration [46], fermentation time [43], and the fermentation temperature [39].

Yeast and bacteria hydrolyze sucrose into glucose and fructose using the enzyme invertase. Yeast within the matrix then produces ethanol via glycolysis, using fructose as the primary substrate. Acetic acid bacteria uses the glucose to make gluconic acid and also utilizes the ethanol produced by the yeast and turns it into acetic acid [47]. Acetic acid is the organic compound responsible for the vinegary flavor and aroma commonly associated with kombucha. The concentration of this acid can vary; however, it tends to reach its peak at 11 g/L on day 30 of the fermentation process and will drop to 8 g/L by day 60. The reason for the reduction in acetic acid is due to the microorganisms within the kombucha that utilize acetic acid as a carbon source after they have depleted all the sugar and ethanol within the fermentation matrix [2]. The ethanol and the acetic acid found in kombucha have been reported to provide antiseptic properties, inhibiting the growth of pathogenic microbes [47].

Lactic acid is primarily found in kombucha made from green tea, rather than other teas, for example, black. Microorganisms produce glucuronic acid from the oxidation of glucose during the fermentation process. Glucuronic acid is the most significant detoxifier in the body because of its ability to bind with toxic compounds in the liver. Once those toxic compounds are bonded the body is able to excrete them via the kidneys [45].

Glucuronic acid (GlcUA) plays a significant role in detoxifying the liver because of its ability to combine toxic molecules, which are eliminated by the organisms. This acid is very involved with endobiotic elimination. Bilirubin is a well-known endobiotic, which is eliminated by GlcUA (glucuronidation) to prevent toxic pigments from harming the organism. Most bilirubin is excreted through bile, while a smaller portion is excreted in the urine, which is why high concentrations of bilirubin are found in the urine are an indication of damage somewhere in the process [48]. GlcUA also plays an important role in increasing the bioavailability of polyphenols. Phenols will conjugate with GlcUA, thus improving its

ability to transport, along with its bioavailability [9]. Glucuronic acid is also a precursor for the biosynthesis of vitamin C [45].

3.1.6. Caffeine

Caffeine is a naturally occurring xanthine alkaloid found in a number of plants, such as coffee, tea, and cocoa [4]. Caffeine, and to a lesser extent theobromine and theophylline, are well known components of tea [23]. The caffeine within the plant is used as a pesticide to protect the plant from insects. Humans tend to consume products with caffeine in them for the stimulating effect on the nervous system that they provide, along with an increase in energy. However, some people are sensitive to caffeine and watch their daily consumption of caffeine [4]. Caffeine makes up approximately 3% to 6% of the tea leaves. The concentration of caffeine within the tea leaves varies based upon cultivation conditions and the further processing of the tea leaves [12]. When it comes to kombucha, caffeine plays an important role during the fermentation process, by providing the yeast and bacteria with the nitrogen necessary for metabolic processes and building new cells, as well providing energy for the yeast and bacteria so they can undergo the fermentation process [4].

3.1.7. Amino Acids and Biogenic Amine (BAs)

Amino acids found in foods and beverages are essential for the human body. There are a number of amino acids that have been identified in tea, such as aspartic acid, threonine, glutamic acid, glycine, α -alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, glutamine, asparagine, tryptophan, and theanine [19,23]. Theanine is the most abundant amino acid found in tea, accounting for 50% of the total amino acids and 1% of the dry weight of the tea [34].

While amino acids play an important role in the human body, they can transform into biogenic amines (BAs). BAs are the result of microbial decarboxylation during the fermentation process [49]. Some BAs are essential for cellular metabolism, while others can be harmful if consumed in high concentrations, such as histamine tyramine, putrescine, cadaverine, β -phenylethylamine, agmatine, tryptamine, serotonin (SRT), spermidine, and spermine [50]. Typically, consuming low amounts of BAs tends to not have an impact on the human body; however, toxic elevated levels of BAs can be found in fermented foods [51]. Several factors can influence the formation of BAs, such as starting materials, starter culture, processing, and storage conditions [49,51].

BAs are an important group of compounds to control and monitor because of the potential toxicological and health implications on the human body but are also used as quality, safety, and product freshness indicators for various types of foods (meat and fish) and beverages (wine) [49,50]. The number of articles and reviews that discuss the concentration of BAs in kombucha are limited. Researchers have focused on analyzing the individual amino acids and the BAs in the SCOBY (tea fungus) itself; however, no researcher has discussed the concentration of the kombucha itself (broth) that is typically consumed. Researchers have found that the SCOBY contained higher concentrations of lysine, isoleucine, and leucine and lower levels of phenylalanine, valine, methionine, threonine, tryptophan, glutamic acid, alanine, aspartic acid, and proline [25]. Of the amino acids identified in the SCOBY only three (lysine, phenylalanine, and tryptophan) are precursors for potentially harmful BAs [51]. Ethylamine, choline, and adenine are the only identifiable BAs found in kombucha that have been discussed in the literature, none of which are harmful [52]. Bromley (2021) examined the BAs concentration in kombucha broth and found there were no biogenic amines in the sample [52].

4. Health Implications

Based strictly off the chemical composition, kombucha has potential for various health benefits. Researchers have concluded that fermented foods are higher in probiotics, which support a healthy gut microbiome. A number of nutritionists and medical researchers have suggested that kombucha may have a positive effect on gut health; however, further

research is required. Studies based on tea, the predecessor of kombucha, as it relates to cancer treatment and prevention, are generally inconclusive.

Studies have also been conducted to demonstrate connections between tea drinking and heart disease [53]. Tea is rich in flavonoids, which have potential antioxidant activities that boost immunity. However, there have been no distinct conclusions on whether or not flavanol consumption can significantly reduce the risk of cardiovascular diseases. There are claims that kombucha, the fermented product, has been shown to help with the function of the gastrointestinal (GI) tract and liver, boost in the immune system, and improve metabolism. The benefits associated with kombucha are likely due to the fermentation process itself, although there is no current study concluding a direct correlation.

4.1. Positive

Kombucha has been adopted into a category of functional super-foods due to its high demand in the Western market. The therapeutic benefits associated with kombucha have been linked to the health components found in tea and compounds produced during the fermentation process [8]. Kombucha has been shown to provide therapeutic and prophylactic properties when consumed regularly [3,13]. Studies have shown that kombucha has antioxidant, antibacterial, and antidiabetic properties along with its ability to reduce cholesterol, support the immune system, and stimulate the detoxification of the liver. However, it should be noted that while some of these properties have been shown to be true through scientific and experimental studies, limited scientific evidence is based on human models. Further research is still required [3,13]. It should be noted that biological activities of kombucha have been studied in several animal models: rats [9], mice [9], rabbit [9,54], ducks [9], dogs [9], pigs [55], cattle [55], broiler chickens [56], and human peripheral blood lymphocytes [9].

4.1.1. Antioxidants

Oxidative stress is a major contributor to a number of pathological conditions, such as aging. Bioactive compounds that have been shown to have antioxidant properties have been gaining interest as potential therapeutic tool to combat oxidative-stress-related diseases [13]. The most popular definition of an antioxidant is that of any substance, when present in low concentrations of an oxidative substrate will significantly delay or inhibit oxidation of the substrate [34]. The bioactivity of antioxidants are broadly classified in the form of (1) scavenging properties, (2) binding prooxidant metals, and (3) the inhibition of prooxidant enzymes. Numerous studies have proven the benefits of antioxidants against disease [57].

Polyphenols found in kombucha are a result of the type of tea leaves used [34]. Kombucha made from green or black tea contains three types of polyphenols that were listed earlier in this review: flavonoids flavanols, flavanol gallate, and flavanol glycosides. Catechins is a flavan-3-ol, which belongs to the subgroup of polyphenols called flavonoids [58–60]. Polyphenols play a vital role in human diet and have been connected to wellness because of their health-promoting properties, such as certain antioxidant effects [34]. Polyphenols are considered to have the highest level of antioxidant properties because of their ability to scavenge free radicals and reactive oxygen species [61]. They are able to reduce free radical chain reactions by obtaining peroxide anions, lipid radicals, hydroxyl radicals, and reactive oxygen species [34]. Various parameters can influence the antioxidant concentration found in kombucha, such as the type of tea leaves used, fermentation duration [34] carbon source, pH, temperature, inoculum size, and starter culture [62]. Antioxidants are predominantly found in natural plant sources, such as tea leaves, which can be found in kombucha. Polyphenols make up approximately 30% of the total dry weight of fresh tea leaves. Epigallocatechin, epigallocatechin-3-gallate, epicatechin-3-gallate, and epicatechin are the most predominate types of polyphenols found in tea leaves [38].

Plant-derived polyphenols have a beneficial impact on slowing down the ageing process and reducing the risk of age-related neurodegenerative conditions, such as Alzheimer's disease, Parkinson's disease, or ischemic brain injury [34].

Jayabalan et al. (2008) have been investigating the antioxidant properties of kombucha and reported that black, green, and tea waste material all had radical scavenging activities. These compounds were able to demonstrate their ability to scavenge α , α -diphenyl- β -picrylhydrazyl (DPPH) radicals, superoxide radicals, and hydroxyl radicals [38]. More recently, Gramza-Michalowska et al., 2016, conducted a study comparing concentrations of antioxidants within kombucha made from black, green, white, and yellow tea. They determined that yellow tea had the highest concentrations of total phenols and DPPH radical scavenging capability [63]. Chakraborty et al. (2016) showed that kombucha tea has the ability to scavenge NO (nitric oxide radicals), suggesting that antioxidants within kombucha might be able to combat nitrosative stressors as well [6]. Jakubczyk et al. (2020) analyzed the chemical profile and the antioxidant activity of kombucha made from different types of tea. They found that fermentation time and the type of tea leaves used had an influence on the antioxidant found in the kombucha. Green tea was found to have the highest concentration of antioxidant potential on the first day; however, as the number of free radicals increased the antioxidants would decrease over time [34]. As the fermentation progresses the accumulation of organic acids could potentially be harmful when consumed [61].

In most cases, the antioxidant concentration in kombucha is higher than the originating tea. It is assumed that a few of the lower molecular weight phenolic compounds are structurally modified by enzymes during the fermentation process. It has also been shown that antioxidant activity increases with fermentation time. It should be noted that the increase in antioxidant activity is based upon the type of tea utilized, the microbiota of the SCOBY, and the fermentation time, all of which determine the metabolites formed during and after the fermentation process [38]. Therefore, it should be noted that kombucha could be a potential source for antioxidants [13].

4.1.2. Antimicrobial

It has been shown that kombucha, in addition to probiotic characteristics, has antimicrobial qualities as well. Kombucha has been shown to inhibit the growth of several microbes known to pose a health risk to humans, such as *Shigella sonnei*, *Escherichia coli*, *Salmonella enteritidis*, and *Salmonella typhimurium* [64]. Acetic acid and catechins are known for their ability to inhibit a wide range of Gram-positive and Gram-negative bacteria [37]. The kombucha liquid itself has also been shown to contain some antibiotic like properties along with its antibacterial and antifungal properties. The antimicrobial activity is predominantly due to the kombucha's low pH and the presence of acetic acid, as well as other organic acids and catechins [65].

4.1.3. Probiotic Effects

Probiotics are defined as live microorganisms that, when consumed in adequate amounts, can confer health benefits onto the host [66]. Changes in an individual's diet can result in modifications to a person's gut microbiota [67]. *Bifidobacterium* and *Lactobacillus* are two of the most common strains of bacteria associated with probiotics [68]. These microorganisms tend to belong to the same bacteria already present within the host and have the ability to tolerate acidified environments [69]. Benefits associated with the continual consumption of probiotics are the inhibition of pathogenic microbes; lowering blood cholesterol; reduction in the incidences of constipation, diarrhea and bowel cancer; improvement of lactose intolerance, calcium absorption, vitamin synthesis; and the stimulation of the immune system [70].

Kombucha is well known as a probiotic drink due to the live and active cultures found within it [71]. Similar to other probiotic foods/beverages, some kombucha manufacturers are adding labels to their product to inform the consumer that their product is "raw",

“natural”, and contains “living cultures’ and “non-dairy probiotics”, along with other health claims [72]. Kombucha is a unique beverage that lacks the traditional standardized production processes that typical fermented beverages have. The end product is dependent upon the type and concentration of the tea and sugar used [73–75], fermentation time, temperature [76,77], and the composition of the starter culture [45,78]. Fermented foods and beverages can be an important source of nutrition for some. Due to the lack of standardization, inconsistencies in the presence of probiotic strains found in kombucha have been found [7,79–81]. To be classified as a probiotic food or beverage enough microbes must be capable of colonizing the gastrointestinal tract of the host [8]. It should also be noted that in order for the health benefits associated with probiotics to be conferred to the host, a large enough dose must be present in the beverages. Plus, the amount of probiotics found within the product should also be noted on the label [82].

Kombucha is a complex beverage, in that the microbes used to produce this product contain a number of microbes from multiple taxa and can fluctuate, which could impact the flavor and aroma of the final product [73,83,84]. The variability associated with the microbes within the SCOBY can impact the final product greatly. Commercial breweries cannot depend on untested or inconsistent microbial loads to produce a high quality product on a consistent basis [85], which is why the industry has had to standardize the process using a set of specific microbes [83,86]. Researchers have been characterizing essential microbes so they can come up with the best mixture of microbes to improve upon the yield of kombucha [84,86]. Wang et al. (2020) developed a synthetic microbial community that could be used to produce kombucha. The three microorganisms utilized were *Acetobacter pasteurianus*, *Komagataeibacter xylinus* (previously named *Gluconacetobacter xylinus*), and *Zygosaccharomyces bailii*. The authors determined that they could obtain satisfactory results. By manipulating the microbe used in the production of kombucha the functional properties of the beverage could potentially be improved as well [84].

Al-dulaimi et al. (2018) studied *Lactobacillus delbrueckii* and *Lactobacillus fermentum*, two well-known probiotic strains. Their research showed that probiotic black tea kombucha had a positive impact on the total lipid profile, ALT (alanine transaminase), AST (aspartate aminotransferase), ALP (Alkaline phosphatase) hepatic enzymes, and serum glucose when compared to the control animal group [87]. In another study conducted by Bueno et al. (2021), *Lactobacillus rhamnosus* (LR) and *Lactobacillus casei* (LC), which are two probiotic strains incorporated into a coffee kombucha, the research team evaluated the microbial diversity of the kombucha following storage at 4°C for fifteen days. They showed that LC remained the same, whereas a slight reduction in LR was seen. Based on the counts following the storage study it was suggested that both LC and LR would be able to survive in both the gastric and intestinal tract. It should also be noted that there was a shift seen in the microbial diversity by the introduction of LR and LC. There was a shift in the predominance of the Acetobacteraceae to the Lactobacillaceae family [88].

A number of kombucha breweries do not pasteurize their product in order to ensure that the microbes found within it have the ability to continue to potentially be classified as a probiotic or prebiotic beverage. While there are other kombucha breweries that will pasteurize their product and add recognized probiotic strains back in (*Bacillus coagulans*, *Saccharomyces boulardii*, and *Lactobacillus rhamnosus*) [72]. The addition of probiotics can be useful depending on the country of sale [71].

4.1.4. Other Therapeutic Benefits

It has been suggested that kombucha has a number of other therapeutic properties, such as anticancer, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic, and antistress, to name just a few. A number of these health benefits are linked to the antioxidant activity.

The antioxidant capacity of kombucha is due to the polyphenols produced during the fermentation process and the synergistic activity of other compounds [2]. The polyphenol content increases linearly during the fermentation process. However, prolonged fermenta-

tion is not ideal due to the excessive production of organic acids [38]. Researchers have proposed several anticancer mechanisms associated with tea polyphenols, including (1) the inhibition of genetic mutation; (2) the inhibition of cancer cell proliferation; (3) the induction of apoptosis of cancer cells; and (4) the end of metastasis [89,90].

Kombucha has also been attributed to having hepatoprotective properties against several toxic and carcinogenic agents in the liver, which has been studied in several animal models using rats and albino mice. Again, the detoxification and the hepatoprotective properties of kombucha are likely linked to the antioxidant activity within this beverage. The compound responsible for this protective effect is d-saccharic acid 1,4 lactone (DSL), which is produced by the *Gluconacetobacter* sp. during the fermentation process [91].

4.2. Contradictions to the Health Benefits

The general consumption of kombucha tends to present no adverse side effects; however, there have been a few cases of adverse effects being reported. Consumers have reported an upset stomach; allergic reactions, particularly if someone is sensitive to acid; dizziness; nausea; and renal insufficiencies, which usually improve after decreasing or stopping consumption [92]. Srinivasan et al. (1995) reported four cases of toxic reactions and two cases of unexplained metabolic acidosis which were supposedly associated with the consumption of kombucha [93]. Perron et al. (1995) reported a case of possible hepatotoxicity [94], while Sadjadi et al. (1998) reported a case of skin disease [95]. The cause and mechanism for adverse reactions have still eluded researchers [47]. The few individuals who suffered from adverse side effects associated with the consumption of kombucha had underlying conditions, such as HIV and acute kidney failure [96].

Although brewing kombucha at home can be fun and fulfilling, precautions have to be taken, especially when it comes to preparing the tea used for making kombucha. If made using unsanitary practices, it has the potential to become contaminated with pathogenic microbes [97]. One telling sign that kombucha is contaminated is when a questionable brown fungus grows on top of a cloudy brown liquid. Although the fermentation process itself occurs in a non-sterile environment, each time a SCOBY is shared with someone else, the potential for contamination increases each time [47]. It should be noted that while contamination is possible, the microbiota within the SCOBY has the ability to protect itself against foreign microorganisms [98]. Another risk of home brewing is that the longer a brew has been fermenting, the more acidic the batch becomes [99].

There are concerns for pregnant women wishing to consume kombucha. Tea contains the chemical compound heparin (glycosaminoglycan), which inhibits the blood-clotting system protein in the body. High concentrations of heparin for a pregnant woman can be incredibly dangerous, especially in the third trimester. While heparin has not been successfully identified in analyzed samples, heavy consumption could cause production within the organisms, which is why caution is recommended [25].

Although kombucha is generally considered a healthy drink, especially when consumed in moderation [27], kombucha does pose some negative risks to human wellbeing. One area of negative impacts, especially when consumed in high amounts, are the effects to dental health. Since the beverage is made from fermented tea, kombucha contains a lot of tannins [100]. Excessive and prolonged consumption of tannin-rich beverages can lead to tannin deposition and cause the staining or darkening of the enamel of the tooth.

Additionally, due to the low pH and high acid content, further tooth enamel degradation can be caused by excessive consumption of kombucha [101]. The typical pH range for kombucha is between 2.5–3.5, which is much more acidic than its precursor, black tea. This is why researchers and dentists alike have concerns about the potential negative effects it may have on tooth enamel. By the very nature of the fermentation process, it has been well established that a low pH is required to ward off any unwanted or harmful bacteria from a kombucha batch. Researchers have followed the drop in pH during the fermentation process and discovered that similarly to other fermentation products, a large decrease in the pH occurs, dropping from 6.7 to 2.9. A final pH was reached by day ten at a pH of

2.9 [99]. By comparison, soft drinks have a pH of 2.6, while vinegar is approximately 2.2, followed by lemon juice at 2.0. Thus, drinking kombucha day in and day out may be more harmful than the proclaimed benefits of the brew. While drinking kombucha, the mouth is constantly flushed with acidic fluids, which creates a consistently acidic environment. This oral environment can lead to halitosis (bad breath) and weakened enamel, which eventually leads to increased cavities. When taking into account the sugars added to aid the fermentation process, kombucha looks less than appealing to dental researchers [102].

Although traditional kombucha has a relatively low residual sugar content and, typically, the added sugar to the tea is consumed during fermentation [78], between 60–70% of kombucha is flavored [5]. These flavorings often come as fruit juices and post-fermentation-added sugars [103]. It has been shown that beverages with added sugars have a negative effect on dental health and can lead to cavities and tooth erosion [104].

The added sugar in beverages, such as kombucha, can also pose other negative health impacts, including obesity, cardiovascular issues, and diabetes [105,106]. Studies have shown that the chronic consumption of kombucha can impede weight loss, especially in individuals who are diabetic [107].

5. Conclusions

The popularity of Kombucha has increased in the last several decades, in part because of its purported health-promoting properties. The increase in popularity means kombucha can now be purchased at the local supermarket and is no longer restricted to local health food stores or home production. A wide range of compounds found in kombucha, including organic acids, polyphenols, vitamins, and other bioactive compounds can be altered by adjusting the starting tea matrix and the fermentation environment. The starting tea has been shown to be the source of several bioactive compounds with known health-promoting properties. However, the lack of human studies in the literature limits the clarity of the benefits of consuming kombucha. Although kombucha is well known for its overall health benefits, the low pH concentration could be potentially harmful to oral health, causing enamel erosion.

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