

A critical review of purported health benefits of kombucha consumption

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UNIVERSITY OF RIJEKA
DEPARTMENT OF BIOTECHNOLOGY
University undergraduate program
"Biotechnology and Drug Research"

Inge Ladavac

**A CRITICAL REVIEW OF PURPORTED HEALTH
BENEFITS OF KOMBUCHA CONSUMPTION**

Bachelor thesis

Rijeka, September 2023

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SVEUČILIŠTE U RIJECI
ODJEL ZA BIOTEHNOLOGIJU
Preddiplomski sveučilišni studij
„Biotehnologija i istraživanje lijekova“

Inge Ladavac

**KRITIČKI PREGLED ZDRAVSTVENIH PREDNOSTI
KONZUMIRANJA KOMBUČE**

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SUMMARY

Kombucha, a fermented beverage originating from the Manchuria region in Northeast Asia over 2000 years ago, is produced by fermenting sweetened tea with a symbiotic consortium of bacteria and yeasts known as SCOBY. The resultant kombucha tea is rich in organic acids, polyphenols, sugars, vitamins, minerals, etc. Organic acids, such as acetic, gluconic, glucuronic and lactic acids, contribute to the beverage's acidity and purported health properties. The beverage's antioxidant, antimicrobial, anticancer, hepatoprotective and anti-inflammatory effects have been documented, backed by *in vitro* and *in vivo* studies on rodents. Phenolic compounds, vitamins and microbial enzymes are thought to contribute to these effects. Kombucha's microorganisms include yeasts and bacteria (acetic and lactic acid bacteria). However, potential health risks like excessive acidity, lead contamination and contraindications for certain individuals may exist. The potential benefits of kombucha's various organic compounds, though promising, require further research and clinical trials for confirmation.

KEY WORDS:

Kombucha tea, fermentation, SCOBY, antioxidants, antimicrobials, probiotics

SAŽETAK

Kombucha je fermentirano piće koje potječe iz Kine prije više od 2000 godina. Proizvodi se fermentacijom zaslađenog čaja uz SCOBY. Rezultirajući čaj od kombuche bogat je organskim kiselinama, polifenolima, šećerima, vitaminima, mineralima, itd. Organske kiseline doprinose njegovoj kiselosti i zdravstvenim svojstvima. Dokazani su antioksidativni, antimikrobni, antikancerogeni, hepatoprotektivni i protuupalni učinci ovog pića, poduprti *in vitro* i *in vivo* istraživanjima na glodavcima. Polifenoli, vitamini i ostali spojevi doprinose tim učincima. Mikroorganizmi prisutni u kombuchi uključuju kvasce i bakterije. Međutim, postoje rizici kao što su pretjerana kiselost, trovanje olovom i kontraindikacije za određene rizične osobe. Daljnja istraživanja u svrhu testiranja svojstava kombuche na ljudsko zdravlje su potrebna.

KLJUČNE RIJEČI:

Kombucha, fermentacija, SCOBY, antioksidasni, antimikrobi, probiotici

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INTRODUCTION

Kombucha is a low alcoholic beverage resulting from the fermentation of sweetened tea (*Camellia sinensis*) and a culture containing a **symbiotic consortium of bacteria and yeasts** (SCOBY). The SCOBY is also known as tea fungus, cellulosic pellicle or consortium [1].

The beverage originated in China, specifically in the Manchuria region, more than 2000 years ago. It became popular during the Tsin Dynasty and by that time was known for its healing properties. Doctor Kombu introduced the beverage to Japan as he was using it to treat digestive troubles of the emperor at the time. When it started to become popular in Japan, around 415 AD, it was named "kombucha" in honor of doctor Kombu but also as a combination of words "kombu" and "cha", meaning algae and tea, respectively. It was further introduced to Russia and other eastern European countries through trade routes. After World War II kombucha was widely spread around Europe and France - dominated North Africa [2]. In the late 20th century scientists started conducting more detailed research of kombucha and its health benefits. However, clinical trials on humans are still lacking and most of the research papers are done *in vitro* or *in vivo* on rodents.

Kombucha is usually fermented using black or green tea and sweetened with sucrose. The standard procedure is: 1 liter of water is boiled and 50 grams of sucrose is added. In the boiling water 5 grams of tea leaves are added and left to infuse. After 5 - 10 minutes tea leaves are removed by filtration. The liquid must be cooled before adding the SCOBY inside so that the microorganisms aren't harmed with heat. Optimal temperature range for fermentation is 18 – 30 °C [3]. The vessel containing cooled tea and SCOBY must be covered with cheesecloth or similar cover to enable air flow but prevent insects and dirt from entering the liquid. It is also recommended to add 0.2 liter of previously fermented kombucha to lower the pH and start the

fermentation process. This also helps to inhibit the growth of unwanted microorganisms [2]. It is best to leave the kombucha to ferment for 6 – 14 days since longer fermentation periods could produce higher acid levels. New tea fungus is created during the fermentation process and is removed with a little extra liquid once the fermentation is over. The remaining liquid is kombucha tea that is stored in capped bottles at temperature of 4°C.

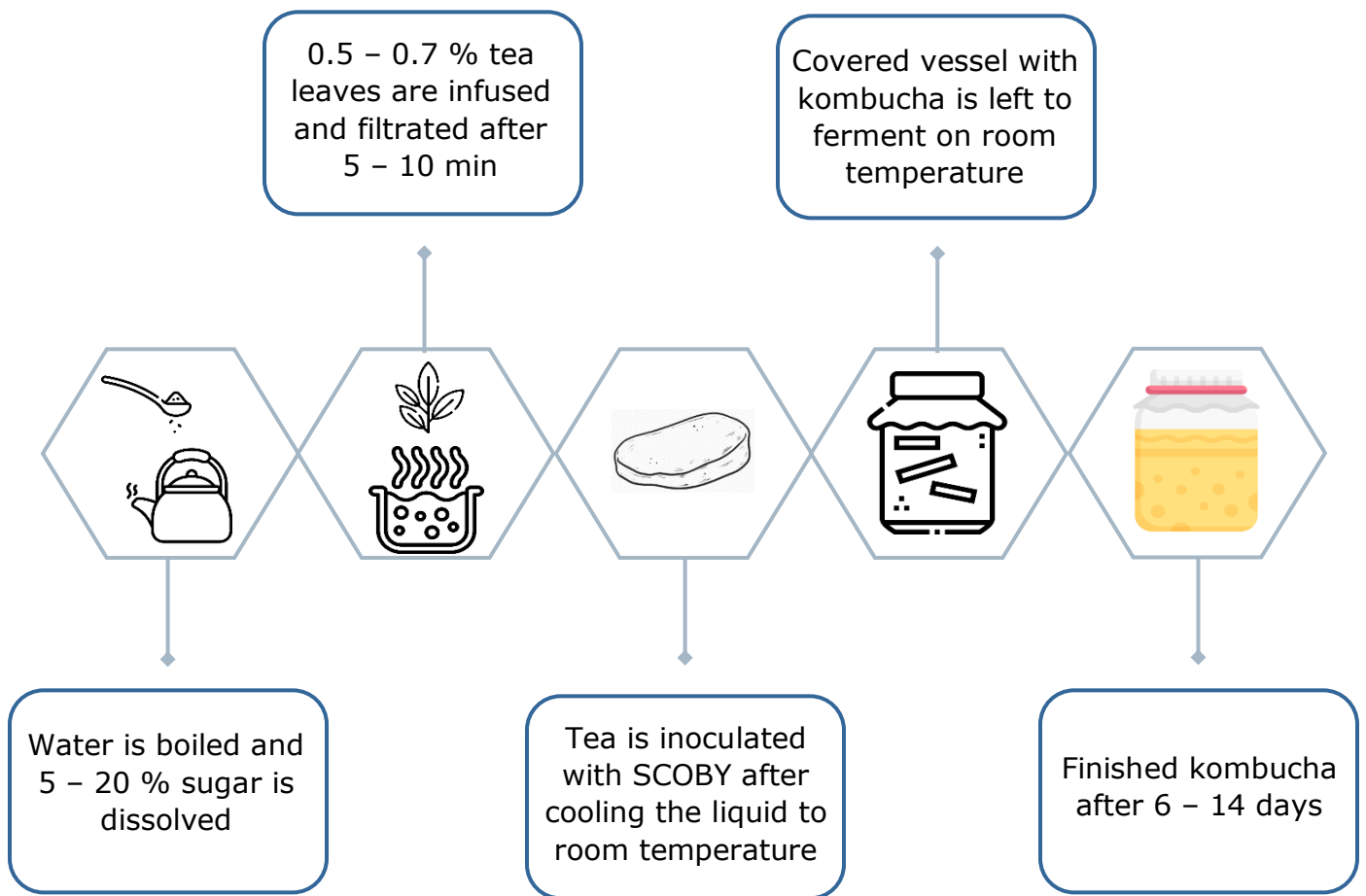


Figure 1: Production of traditional kombucha

Scientists have tried making kombucha with different substrates and sources of sugar such as molasses [2] or coconut palm sugar [4]. Changing the certain component of the beverage can change its health properties. Nontraditional

substrates such as Coca - Cola, red wine, white wine, vinegar, extract of Jerusalem artichoke, milk, fresh sweet whey, Echinacea, mint, and more have been tested in fermentation of kombucha. Few scientists also tested different types of tea such as mulberry tea, Japanese green tea, jasmine tea, oolong tea and sage, thyme, and peppermint teas [2]. However, it was concluded that black tea has the best properties for kombucha fermentation [5]. Possible alternatives with stimulating effect are green tea and lemon balm tea or Jerusalem artichoke tuber extract which showed great potential as a dietetic product. Additional metabolites are recognized when fermenting kombucha using Jerusalem artichoke tuber extracts such as fructooligosaccharides and inulin which are prebiotics and help regulating bacteria in the human intestinal flora [2]. Using molasses is considered an attractive option because it is a lower cost carbon source and there is a presence of a number of components, including minerals, organic compounds, and vitamins which are very useful in the fermentation process. Different sources of sugar can affect the overall antioxidant activity of kombucha. D. Morales (2020) explained in his work that coconut palm sugar showed higher antioxidant activity than both sucrose and molasses, tested *in vitro*.

Variables such as the type of tea, sugar content, range of temperatures at which the fermentation is conducted and the overall fermentation time can significantly affect the characteristics of the final product, including health properties [3].

PURPOSE STATEMENT

The primary purpose of this study is to comprehensively examine and summarize existing literature concerning the impact of kombucha consumption on human health. Kombucha, a relatively modern functional food, has been gaining attention from the scientific community due to its potential health - enhancing properties and associated risks. Made through the fermentation of black or green tea in the presence of a symbiotic culture of bacteria and yeast (SCOBY), kombucha undergoes transformation in both its chemical and microbial composition, resulting in an improved physiological effect on the human body. Although numerous health - promoting properties have been documented over time, the empirical evidence is still lacking and more clinical trials should be conducted. This paper assembles reported health benefits and *in vivo* experiments targeting these purported health benefits.

MAIN PART

Various compounds were found in kombucha by chemical analysis. Some of those are organic acids, sugars, vitamins, minerals, ethanol and some tea polyphenols. Yeasts and bacteria that are present in the beverage are involved in metabolic activities that make new metabolites which are responsible for kombucha's health properties.

Carbon source that is the most commonly used is sucrose and using enzyme invertase (β -fructofuranosidase) yeasts hydrolyze it in order to release smaller compounds – glucose and fructose. Both are used for further reactions, each on a separate pathway. Fructose is again used by yeasts to produce ethanol in the process of glycolysis. In some reviews it is mentioned that different kind of yeasts can prefer glucose to fructose to produce ethanol [3]. However, gluconic acid is produced from glucose by oxidation with the help of acetic acid bacteria (AAB). Depending on the genera of AAB used, glucuronic acid can also be obtained. AAB also use ethanol to produce another metabolite which is acetic acid. The overall pH value of kombucha tea decreases due to production of organic acids and the expected range of the pH value is 2.5 – 3.5 [2, 6].

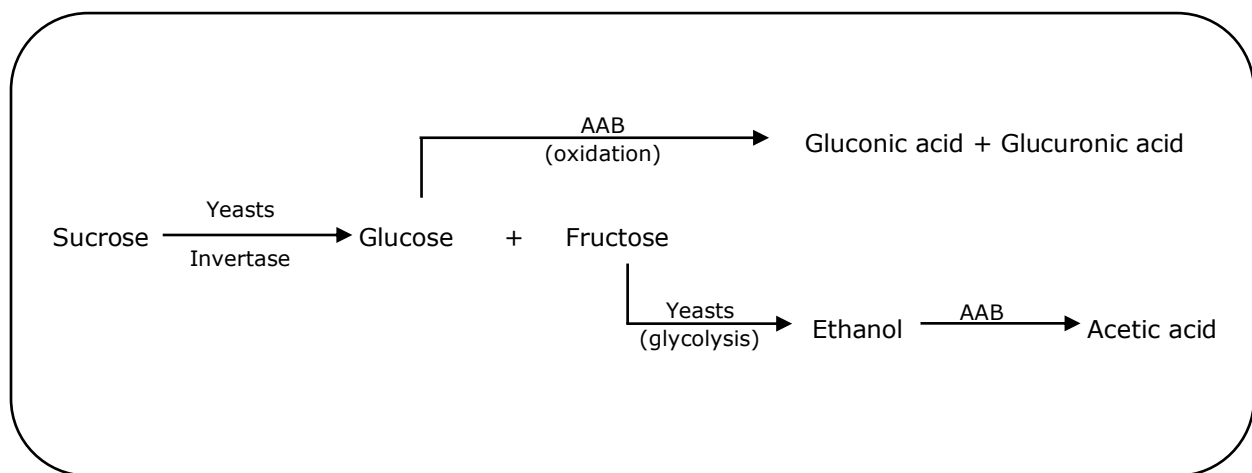


Figure 2: Metabolic activity during kombucha fermentation, adapted from [1]

Microorganisms found in kombucha tea

The microorganisms that are found in kombucha tea are yeasts, acetic acid bacteria (AAB) and small amounts of lactic acid bacteria (LAB). The microbial community in kombucha varies between fermentations and some scientists specifically tested different samples of kombucha made in different parts of the world to find similarities or differences between them [1]. Species vary due to environmental factors (geographic and climatic conditions) and even contamination between starter cultures.

Most common yeasts found in all tested kombucha are *Zygosaccharomyces (Z.) bailii*, *Zygosaccharomyces (Z.) rouxii*, *Schizosaccharomyces (S.) pombe*, *Saccharomyces (S.) ludwigi*, *Brettanomyces (B.) bruxellensis* and *Saccharomyces cerevisiae* also known as baker's yeast.

Acetic acid bacteria are widely used in fermentation in the food industry, they can be found in both sugary and alcoholic environments. Genera that are mostly used are *Acetobacter*, *Gluconobacter*, *Gluconacetobacter* and *Komagataeibacter*. *Acetobacter* and *Gluconacetobacter* are found in alcoholic environments and are used more to oxidase ethanol whereas *Gluconobacter* is rather found in sugar abundant environments and they oxidase glucose and glycerol [1].

Lactic acid bacteria that were found in kombucha are *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus nagelii*, *Lactobacillus rhamnosus*, *Lactobacillus mali* and *Pediococcus (P.) pentosaceus*. Currently LAB are not considered essential for kombucha fermentation but they are used to achieve unique flavor and deliver health benefits. Despite not being essential, it has been shown that LAB genera do enhance antioxidant and antimicrobial health properties.

Acetic acid bacteria	Yeasts	Lactic acid bacteria
<i>Acetobacter (A.) aceti</i>	<i>Zygosaccharomyces (Z.) bailii</i>	<i>Lacticaseibacillus casei</i>
<i>Acetobacter (A.) pasteurianus</i>	<i>Zygosaccharomyces (Z.) rouxii</i>	<i>Lactiplantibacillus plantarum</i>
<i>Acetobacter (A.) musti</i>	<i>Schizosaccharomyces (S.) pombe</i>	<i>Lactobacillus nagelii</i>
<i>Bacterium (B.) gluconium</i>	<i>Saccharomyces (S.) ludwigi</i>	<i>Lactobacillus rhamnosus</i>
<i>Glucobacter (G.) oxygedans</i>	<i>Brettanomyces (B.) bruxellensis</i>	<i>Lactobacillus mali</i>
<i>Gluconobacter (G.) potus</i>	<i>Saccharomyces (S.) cerevisiae</i>	<i>Pediococcus (P.) pentosaceus</i>
<i>Komagaeibacter (K.) kombuchae</i>		

Table 1: Most common microorganisms found in kombucha tea [1]

Compounds present in kombucha tea

Compounds that are found in kombucha tea are the following: organic acids (acetic, gluconic, glucuronic, citric, L-lactic, malic, malonic, oxalic, succinic, pyruvic, etc.), sugars that are used as a main source of carbon (sucrose, fructose and glucose), amino acids, tea polyphenols (theaflavins, thearubin, thearubigins) and flavonoids, vitamins (B1, B2, B6, B12, and C), ethanol, minerals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn), DSL (D – saccharic acid – 1,4 – lactone) and many enzymes (cellulase, glucanase, xylanase, pectinase, and glucosidase etc.) [2, 7].

ORGANIC ACIDS

Most common organic acids that are found in kombucha tea are acetic, lactic, gluconic and glucuronic acid. Another metabolite that is created is D – saccharic acid – 1,4 – lactone (DSL) that is known as glucuronidase inhibitor.

It is an enzyme related with cancers and DSL has shown properties in reducing oxidative stress and damage and inhibition of apoptosis of pancreatic cells.

Antolak et al. (2021) mentioned how higher percentages of organic acids were measured from black than green or oolong tea. Overall amount of organic acids present in kombucha tea influences the final pH value of the beverage, therefore it is expected for black tea kombucha to be more acidic. The health benefits associated with the presence of organic acids are antimicrobial activity, increased bioavailability of phenolic compounds, body detoxification and hormonal balance.

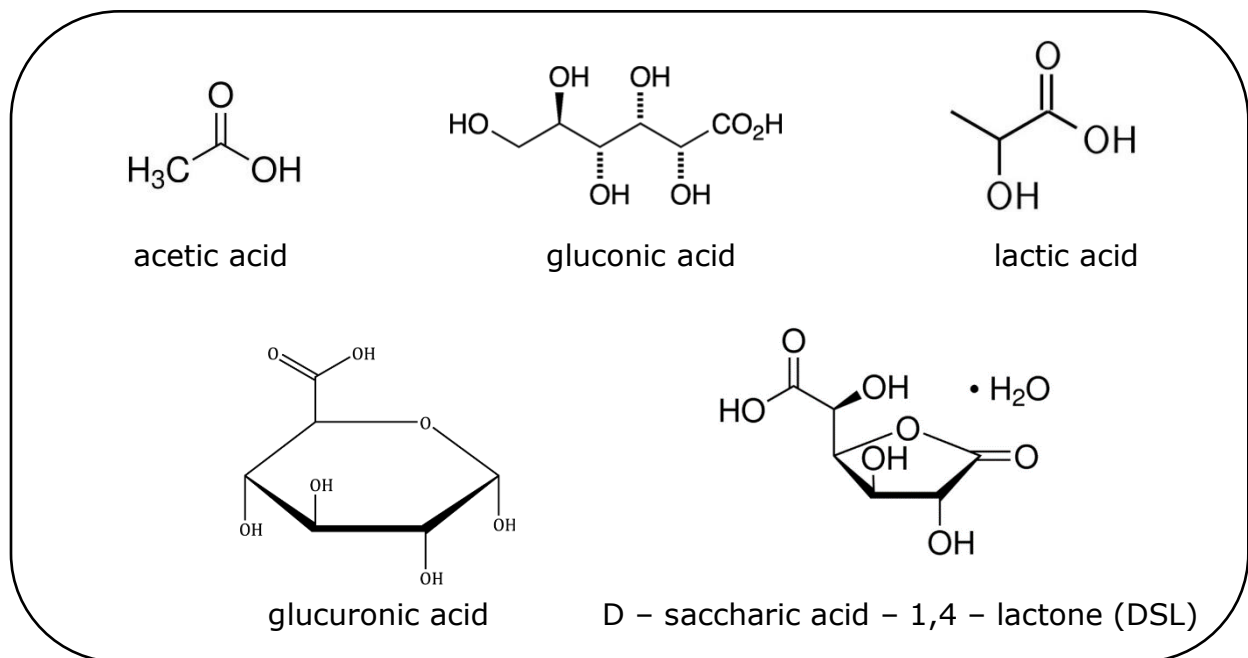


Figure 3: Most common organic acids present in kombucha tea

Concentration of acetic acid is directly dependent on the concentration of ethanol. If lower amounts of sucrose are used to ferment kombucha, low- or non-alcoholic beverage is made and it influences the concentration of acetic acid obtained. Depending on the genera of acetic acid bacteria that are used or present in the consortium, both gluconic and glucuronic acid can be

produced from glucose. To produce gluconic acid *Gluconobacter oxydans* and *Komagataeibacter xylinus* strains are used and to produce glucuronic acid mainly *Komagataeibacter* genera are used. Glucuronic acid had shown the highest detoxifying properties and it takes part in glucuronization which produces more phenolic compounds that have further health benefits. Glucuronic acid is also a precursor of vitamin C which is important for immune system, antioxidant activity, functioning and production of some enzymes and absorption of iron in the body. Lastly, lactic acid is also one of the organic acids that lowers the overall pH value of the beverage which we can relate with antimicrobial properties of kombucha as well as controls blood circulation and prevents formation of blood clots [3].

PHENOLIC COMPOUNDS

Phenolic compounds present in kombucha tea are tea polyphenols, flavonoids and phenolic acids. Most abundant compounds are catechins which have strong antioxidant properties. They account for more than 70% of TPC (total phenolic content) [3]. Some of those are: gallic acid (GC), catechin gallate (CG), gallic acid gallate (GCG), epigallocatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (ECG) and epigallocatechin gallate (EGCG). Gallic and ellagic acid were most detected in several samples – anti - inflammatory and anticarcinogenic health benefits.

Concentration of catechins was found higher in green and yellow teas than black and oolong teas, specifically for EGCG which was most concentrated in yellow tea. Expected health benefits are similar to other phenolic compounds as well as prevention of brain and heart disease. Stronger antioxidant properties were measured when fermenting black tea compared to green tea [3].

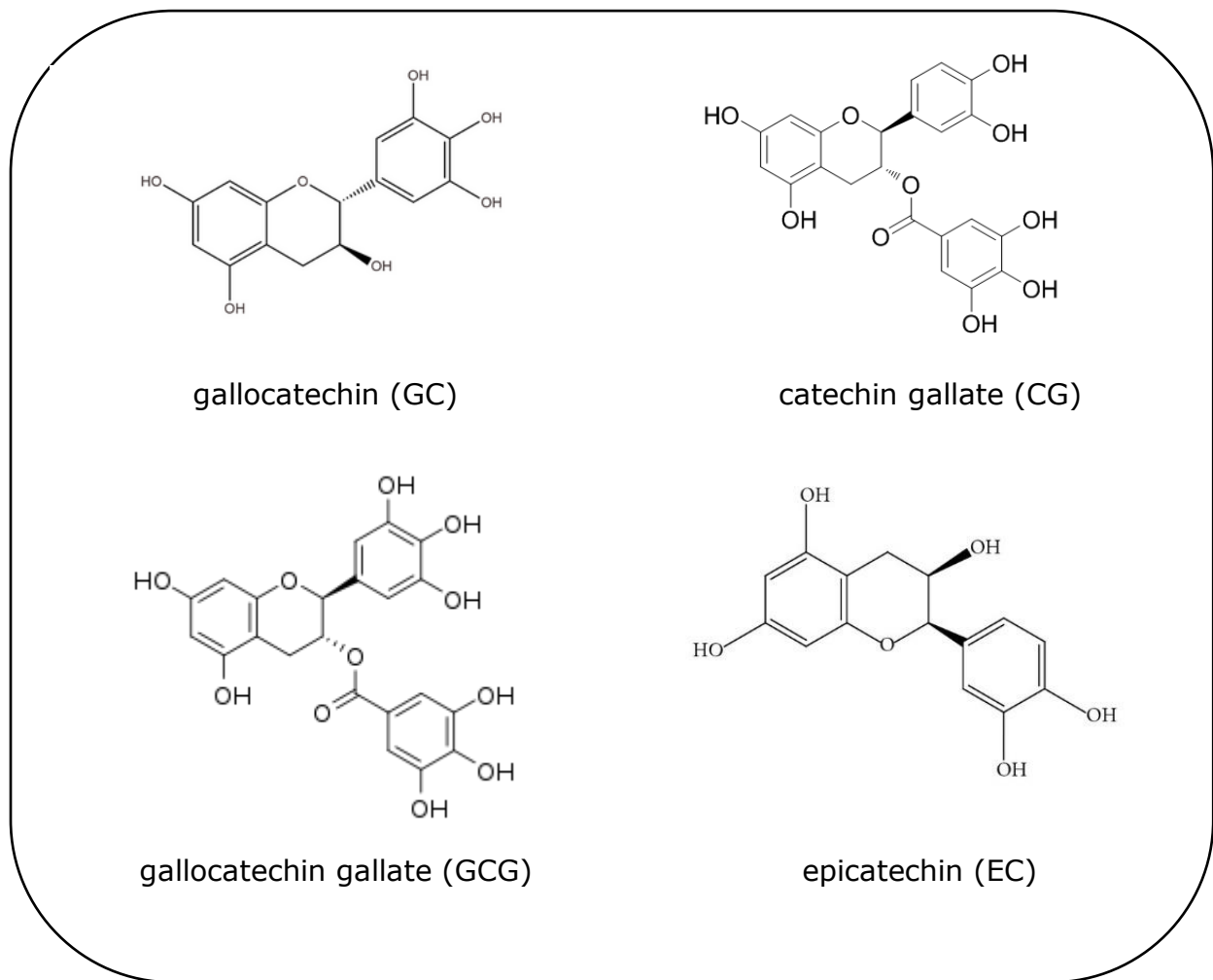
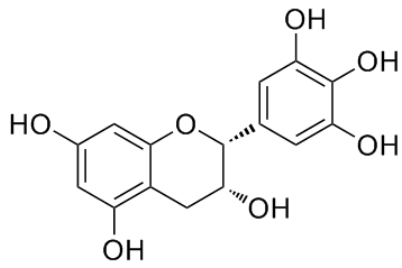
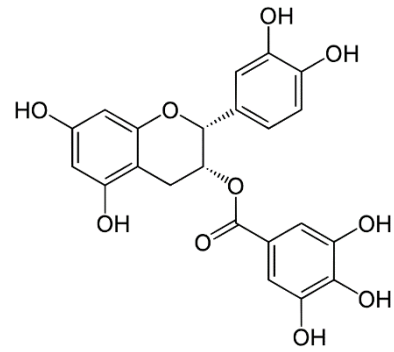


Figure 4.1: Most common phenolic compounds present in kombucha tea

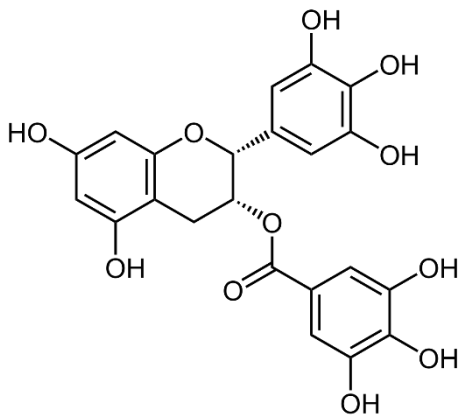
Tea polyphenols are present in teas before fermentation, for example theaflavin and its derivatives in black tea but after fermentation they can change and undergo oxidation which results in new compounds such as thearubigins or theabrownins. Total content of polyphenols and flavonoids is increased in kombucha after fermentation as opposed to sweetened tea.



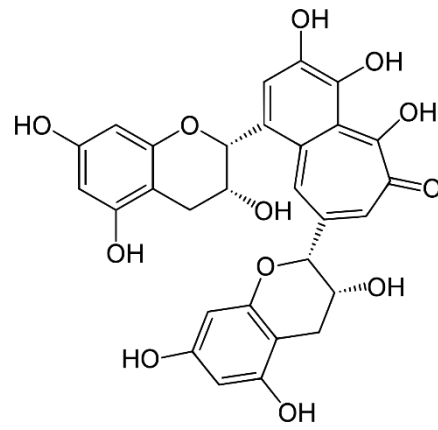
epigallocatechin (EGC)



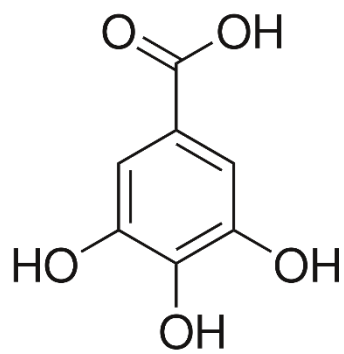
epicatechin gallate (ECG)



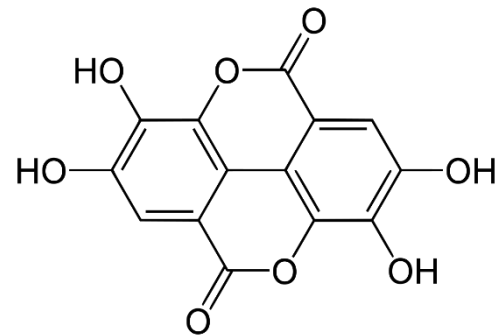
epigallocatechin gallate (EGCG)



theaflavin



gallic acid



ellagic acid

Figure 4.2: Most common phenolic compounds present in kombucha tea

VITAMINS AND MINERALS

Vitamins found in kombucha tea are several B vitamins, as well as vitamins C, A, E and K. They are mostly considered as antioxidant agents but also show additional health benefits – prevention of fatigue, loss of concentration, memory and prevention of cataracts. As mentioned earlier, vitamin C is related to glucuronic acid and the concentration of vitamin C in kombucha is dependent on concentration of sucrose added before fermentation since it is a product of glucose metabolism.

Minerals in kombucha tea are manganese, iron, nickel, copper, zinc, lead, cobalt, chromium and cadmium [8]. Jayabalan et al. (2014) found the following values: 0.004 µg/mL for cobalt, 0.462 µg/mL for manganese, 0.005 µg/mL for lead, 0.001 µg/mL for chromium. It was concluded that the value of essential minerals (Cu, Fe, Mn, Ni and Zn) increased as a result of the metabolic activity during fermentation of kombucha. Presence of fluoride, chloride, bromide, iodide, nitrate, phosphate and sulfate was also established [2].

Health benefits of kombucha consumption

Kombucha drinkers have reported several health benefits related to consumption of the beverage – antioxidant, antimicrobial, anticancer and hepatoprotective properties. However, most of those were experimental or even based on personal observations and testimonials from patients and there are still no human clinical trials that can confirm these health benefits. *In vitro* experiments have been conducted to test the possible health properties of the components present in the kombucha tea and some *in vivo* trials in rodents are being conducted. Other properties like detoxifying the body, reducing

insomnia, relieving headaches and relief against hemorrhoids, arthritis and gout have also been reported in studies. These properties are believed to result from activity of phenolic compounds in tea and phenolic compounds produced after fermentation, organic acids in kombucha tea, vitamins, microbial enzymes and proteins and lastly, probiotic activity of microorganisms [3].

ANTIOXIDANT PROPERTIES

Antioxidants are compounds that have the ability to inhibit oxidation – a chemical reaction that produces free radicals. Those are molecules that are highly reactive due to the unpaired valence electron in their structure and they can take electrons from various important macromolecules in human body such as DNA, RNA, proteins or lipids which consequently causes cell damage and aging. Oxidative stress is also often related to health disorders such as neurodegenerative diseases, cardiovascular diseases, cancer, liver diseases etc. [8]

The antioxidant activity is considered to be the result of compounds in the beverage; at first it was believed that most of that comes from tea polyphenols but it was later shown that organic acids and water – soluble vitamins (C, B1, B2, B6, B12) are also responsible. Studies have been conducted to observe the difference in antioxidant activity between unfermented tea and kombucha and it was exhibited that fermented black tea has 70% higher antioxidant activity. Explanation for that is that during fermentation bigger polyphenolic compounds are broken down by enzymes produced by microorganisms in the beverage into smaller phenolic compounds which increases the total amount of phenols [2, 8].

In vitro tests use hydroxyl and DPPH (2,2 – diphenyl – 1 - picrylhydrazyl) radicals to test the antioxidant activity. Antioxidant activity was increasing

with longer fermentation time – highest levels were on the 7th day. However, after the 14th day antioxidant activity decreases and the concentration of organic acids increases which leads to more acidic beverage and possible harmful levels for further consumption [2, 4]. Some studies also mentioned how the maximum shelf life for homemade kombucha is 4 months since the concentration of compounds – both phenolic substances and organic acids – lowers and the antioxidant activity decreases. That is due to the fact that microorganisms start using organic acids as carbon source in the absence of sugar [8].

ANTIMICROBIAL PROPERTIES

Antimicrobials are agents that kill or stop the growth of microorganisms. Depending on the microorganism they attack there are different names – antibiotics against bacteria, antifungals against fungi and antivirals against viruses. Recently microorganisms have started exhibiting resistance to available antimicrobial medication and scientists are constantly in search of new compounds that can serve this purpose.

Throughout the years *in vitro* tests of antimicrobial effects of kombucha confirmed inhibitory activity against many microorganism, including both Gram - positive and Gram - negative bacteria. Mousavi et al. (2020) mentioned some of the following microorganisms: *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens*, *Helicobacter pylori* (the causative organism of peptic ulcers and gastritis), *Enterobacter cloacae*, *Salmonella enteritidis*, *Escherichia coli* (the causative organism of common diarrhea), *Yersinia enterocolitica*, *Candida albicans*, *Shigella sonnei*, *Campylobacter jejuni* and *Staphylococcus aureus*. Additional microorganism are mentioned in Jayabalan et al. (2014) study - *Entamoeba cloacae*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Aeromonas hydrophila*, *Salmonella typhimurium*,

Staphylococcus epidermis, *Leuconostoc monocytogenes* and *Vibrio parahaemolytica*.

Compounds that show the antimicrobial activity in kombucha are organic acids and catechins. To test which compounds are responsible for antimicrobial activity of kombucha four different beverages were made - unfermented tea, kombucha fermented at room temperature for 21 days, kombucha neutralized with 1 M NaOH to reach the pH of 7.0 and tea with the same pH as kombucha adjusted with acetic acid [8]. Unfermented tea (both black and green) had no inhibitory activity against most bacterial strains but the acidic kombucha showed far better antimicrobial properties. However, antimicrobial activity has decreased significantly in neutralized kombucha which leads to the conclusion that for the most part acidity contributes to the antimicrobial health benefits. Antifungal activity was shown to increase with the fermentation time but it was completely lost in neutralized kombucha or kombucha denatured by heat. Studies are indecisive about the tea that has the highest antimicrobial activity. In some studies, black tea is preferred whereas in others green tea is preferred [2, 4]. Possible explanation is that certain compounds that are present in higher concentrations in one beverage are better at inhibiting the growth of certain microorganisms than those present when using a different tea to ferment kombucha. The kombucha that has more organic acids and is therefore more acidic attacks bacteria because acetic acid causes acidification of the cytoplasm of the bacteria which stops cell growth and kills the bacteria [8].

ANTICANCER PROPERTIES

After cardiovascular diseases, cancer is the leading cause of death worldwide. The research for anticancer drugs is still ongoing since current medication still lacks specificity and has plenty side effects [4]. Apart from personal

testimonials from patients who were drinking kombucha, "Central Oncological Research Unit" and the "Russian Academy of Sciences in Moscow" also confirmed the anticancer properties of kombucha in the 1950s.

Tea polyphenols were the first compounds that were believed to have anticancer properties due to their possibility to prevent gene mutations, inhibit cancer cell propagation, cause apoptosis of cancer cells and termination of metastasis [9]. However, additional compounds have been mentioned in newer studies: dimethyl 2 - (2 - hydroxyl - 2 - methoxypropylidene) malonate and vitexin. Anticancer and antiproliferative properties have been tested on HeLa cells (cervix epithelial carcinoma), HT-29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), A549 (human lung carcinoma), U2OS (human osteosarcoma) and 786-O (human renal carcinoma) cell lines and cytotoxic effects were noticed and documented [2, 4]. Cell invasion and cell motility of cancer cells were significantly reduced and activity of MMP - 2 and MMP - 9 (matrix metalloproteinase) was decreased. Matrix metalloproteinase are enzymes that play a crucial role in degrading the extracellular matrix proteins which allow faster invasion of cancer. Abaci et al. (2022) also wrote about the cytotoxic effect of kombucha but also its ability to induce apoptosis in the early stages. Expression of few genes (Bax, p-53, and p21) which play a crucial role in progression of apoptosis was increased.

Lastly, cancer patients were found to have lower concentrations of L - lactic acid in their tissue and since that is one of the compounds produced in kombucha it can be a suitable alternative. Higher pH (<7.56 as opposed to 7.40 in healthy individuals) is documented in cancer patients and kombucha can also be used to reequilibrate the pH value [9].

ANTIINFLAMMATORY PROPERTIES

Inflammation is an immune system response to pathogens, injury or irritation. In some disorders, this process becomes continuous and it can last for several months or years. Some of those disorders are: rheumatoid arthritis, cancer, allergies, cardiovascular diseases etc.

In vitro tests showed that activity of 5-lipoxygenase (LOX) is inhibited. LOX is an enzyme that is involved in synthesis of leukotrienes who initiate inflammation. Secretion of proinflammatory cytokines (tumor necrosis factor - α (TNF- α) and interleukins (IL) - 1β and IL - 6) has been reported to reduce after kombucha consumption. *In vivo* studies on immunosuppressive mice has also been conducted and it was concluded that kombucha had immune - stimulating effect (white blood cell count was increased, phagocytic activity of macrophages was enhanced and activity of natural killer (NK) cells was strengthened) [4, 8].

ANTIDIABETIC PROPERTIES

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [<https://www.who.int/>].

Although no purported compound or mechanism has been identified, *in vivo* studies in rats suggest that kombucha consumption improves the structure of Islets of Langerhans and regeneration of β - cells in pancreas [8]. A recent study done *in vivo*, but in humans, measured glycemic index (GI) and insulin index (II) after consumption of high - carbohydrate meal with different beverages, one of those being kombucha. Glycemic index shows how quickly carbohydrates affect glucose in human blood and insulin index shows how much certain food elevates the level of insulin in the human blood during 2

hours of consumption. Participants had to consume a meal made of rice, green peas and soy sauce with addition of 330 mL predetermined beverage – soda water, diet lemonade soft drink or kombucha. GI values were significantly lower in kombucha meals than other beverages and the reference glucose solution. Conclusion was that kombucha can produce clinically significant reductions in glycemia and insulinemia in healthy adults when consumed with a high – carbohydrate meal. However, they couldn't extrapolate the results to long – term consumption of kombucha or to people with specific diseases [10].

HEPATOPROTECTIVE PROPERTIES AND DETOXIFICATION

Kombucha has shown an ability to prevent hepatotoxicity from various environmental pollutants. Tests were conducted against paracetamol, carbontetrachloride, aflatoxin B1, cadmium chloride, TBHP (t - butyl hydroperoxide) and acetaminophen and it was shown that kombucha successfully reduces the physiological changes of these compounds on liver [2]. *In vivo* tests on mice showed decreased levels of markers (aspartate transaminase (AST), alanine aminotransferase (ALT), and triglyceride (TG)) that are released during liver damage [8].

Antioxidant properties of kombucha are also beneficial as a form of hepatoprotection since many toxic compounds can be eliminated through the body using that pathway. Detoxification helps in both the preservation of liver and prevention of cancer. It is believed that organic acids specifically glucuronic acid is responsible for removing the toxic substances from the body. Excretion of heavy metals and biotransformation of bilirubin and excess hormones can also be improved with kombucha consumption [9].

ANTIHYPERTENSIVE PROPERTIES

Hypertension is a condition that causes consistent rising of blood pressure which can be a risk for different conditions such as cardiovascular diseases [4]. It is mainly controlled by the renin - angiotensin system (RAS) through inhibition of formation of angiotensin II. Enzyme that is responsible for formation of angiotensin II is angiotensin - converting enzyme (ACE) and most of the antihypertensive drugs are based on inhibition of ACE [8].

Some test showed that green tea kombucha demonstrated higher inhibition of ACE than black tea kombucha. One human *in vivo* test is mentioned but the control group was missing and results were not explained in detail. However, it was reported that kombucha has blood - pressure lowering properties during three months of consumption [4].

OTHER BENEFITS OF KOMBUCHA CONSUMPTION

Additional health properties of kombucha have been documented in the literature throughout the years but they still haven't been explored enough to understand the exact compounds and mechanisms by which they work.

One of the risk factors for cardiovascular diseases (stroke, myocardial infarction, atherosclerosis) is high levels of cholesterol. Hypercholesterolemia is characterized by low levels of HDL- and high level of LDL-cholesterol. This condition is usually treated with drugs that inhibit the synthesis and absorption of cholesterol but *in vivo* studies on rodents were conducted to test kombucha against high LDL-cholesterol levels. Morales (2020) wrote about different experiments done on rats where diabetes was induced to animal models by using alloxan - a chemical that induces diabetes by destroying the β - cells in pancreas. Higher levels of triglycerides and LDL-cholesterol are measured after induction of diabetes on rats. After consumption of black tea kombucha,

reduction of triglycerides and LDL-cholesterol and increase of HDL-cholesterol were noticed. The same results were documented after administering kombucha tea to ducks but no human clinical trials have still been conducted. Additionally, high levels of homocysteine were lowered with kombucha consumption. Increased levels of homocysteine are documented in high – fat diets and are usually associated with vitamin B deficiency. That is also believed to be a precursor of many diseases such as cardiovascular diseases, dementia and osteoporosis [4, 8].

Flavones found in kombucha tea have exerted anti – aging activity by increasing collagen synthesis, supporting DNA repair, protecting against ultra violet (UV) radiation and increasing skin elasticity. NAD (nicotinamide adenine dinucleotide) is believed to decrease during aging which results in decreased collagen expression. Vitamin B3 found in kombucha is a precursor of NAD and can stimulate collagen biosynthesis. Vitamin B3 is also used in skin care products because it helps with hyperpigmentation. As well as vitamin B, vitamin C has shown properties in collagen biosynthesis but also antioxidant properties against free radicals caused by UV radiation. Lactic acid is a member of the group of alpha - hydroxy acids (AHA) and it has also shown anti – aging properties by collagen and hyaluronic acid biosynthesis [8].

In different literatures kombucha is documented as prebiotic, probiotic and postbiotic beverage. Microorganisms found in kombucha have probiotic properties and help promote gut health. Prebiotic and postbiotic properties are expressed by compounds that help the microorganisms in human body grow and the compounds that those microorganisms produce [3, 9].

During fermentation thick biofilm of cellulose forms in the beverage which can be further used as a wound – healing treatment or artificial skin for burns or other skin injuries. Dried biomass has also shown efficiency in removing pollutants from water as well as a supplement in diet for broiler chickens.

Increased feed consumption, body weight, performance efficiency factor (PEF) and the carcass characteristics were documented in comparison to the control group [2, 5, 9].

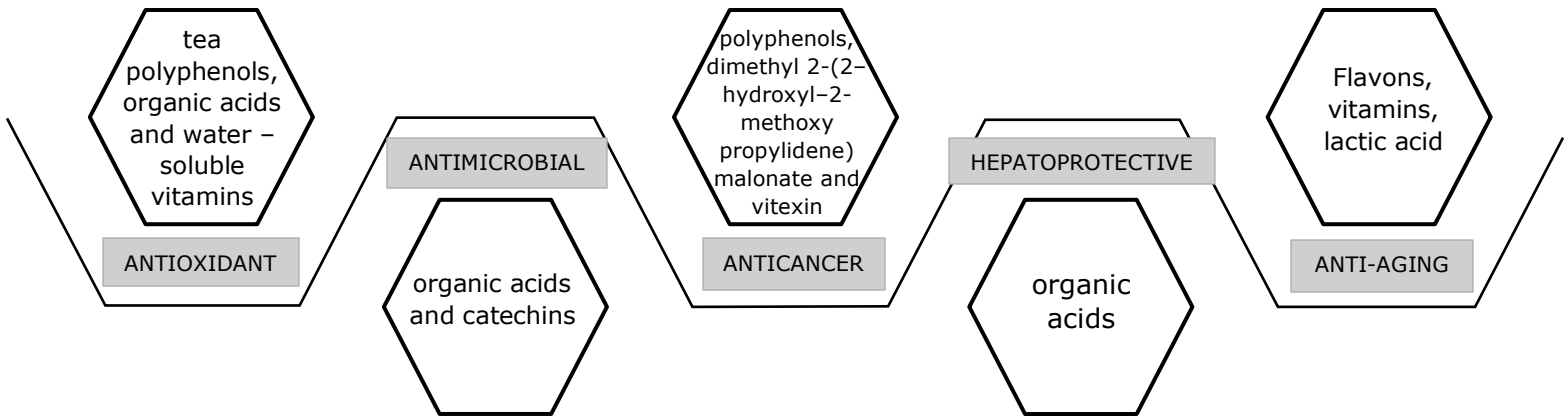


Figure 5: Components responsible for health properties of kombucha

Risks of kombucha consumption

Toxic effects of kombucha were first examined in the 90s after one individual died after kombucha consumption. Cause of death was perforations of the intestinal tract and severe acidosis. It was later discovered that the victim had preexisting conditions that could lead to acidosis. Fermentation time of the consumed kombucha was also increased from 7 to 14 days and another individual mentioned how the beverage was very acidic and hard to swallow [6].

Some individuals mentioned feeling dizziness, nausea, allergic reactions, jaundice, vomiting and head and neck pain after consuming kombucha tea. Kombucha is contraindicated in pregnant and lactating women, people with

significant renal, pulmonary or liver disease and patients with human immunodeficiency virus (HIV) [2, 11, 12].

Storage and brewing materials can also effect kombucha – lead poisoning has been documented when the beverage was stored in a ceramic pot. Glass containers are recommended for fermentation and storage to avoid the dissolving of toxic elements into the beverage. Homemade kombucha can also be dangerous if prepared incorrectly - one of the most mentioned signs being acidity/very low pH [5].

Daily dose of kombucha is also playing an important role in its health benefits. It was concluded that over 4 oz or 120 mL of kombucha tea per day can become harmful [6].

CONCLUSION

In conclusion, kombucha, a fermented beverage with origins dating back over 2000 years, has gained widespread attention due to its potential health benefits. Originating in China, it gained popularity across cultures and was later researched extensively for its healing properties. While lacking comprehensive human clinical trials, studies suggest kombucha's positive impact on human health.

The beverage is produced through the fermentation of sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY), resulting in a complex mixture of organic acids, tea polyphenols, vitamins, minerals and other bioactive compounds. Kombucha's components contribute to its antioxidant, antimicrobial, anti-inflammatory and potential anticancer properties. These compounds are formed during fermentation and are influenced by factors such as tea type, sugar content, fermentation time and microbial composition. The variability in microbial composition during fermentation, the influence of tea types and the potential interactions between compounds make kombucha a complex subject of study.

Historically, kombucha has been recognized for its healing properties, but scientific investigation into its effects on human health is still limited. While numerous *in vitro* and *in vivo* studies on rodents have illuminated many aspects, further research, particularly human trials, is imperative to unlock its full therapeutic value and establish safe consumption guidelines.

The antimicrobial potential of kombucha has been demonstrated against a range of microorganisms, suggesting its possible role in supporting immune function and inhibiting pathogens. Additionally, its potential to modulate inflammation and oxidative stress is relevant to conditions like diabetes, cancer and cardiovascular diseases. Kombucha's impact on diabetes, for

instance, has shown promise in experimental models, with potential benefits in glucose regulation and pancreatic health.

Moreover, the beverage's hepatoprotective properties, attributed to its detoxifying capacity, suggest a possible role in supporting liver function and minimizing toxic effects from various compounds. It is important to note that these findings are mostly based on *in vitro* studies and require further research to confirm their applicability to human health. Additionally, its potential as a prebiotic and probiotic source suggests gut health benefits.

Risks associated with kombucha consumption include unfavorable reactions like dizziness, nausea and allergic reactions. Improper brewing or storage may lead to harmful levels of acidity or toxic elements. Moreover, excessive consumption can be damaging rather than healthy.

In summary, kombucha holds significant promise as a functional beverage with potential health benefits, based on its complex mixture of bioactive compounds. However, its effects on human health are not fully understood, emphasizing the need for well-designed clinical trials to support the reported *in vitro* studies. As research advances, kombucha's role in promoting health and well-being may become clearer, offering an intriguing path for both scientific exploration and potential dietary inclusion.

REFERENCES

1. Wang B, Rutherford-Markwick K, Zhang XX, Mutukumira AN. Kombucha: Production and Microbiological Research. *Foods*. 2022 Oct 31;11(21):3456. doi: 10.3390/foods11213456. PMID: 36360067; PMCID: PMC9658962.
2. R. Jayabalan, R. V. Malbaša, E. S. Lončar, J. S. Vitas, and M. Sathishkumar, "A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus," *Comprehensive Reviews in Food Science and Food Safety*, vol. 13, no. 4, pp. 538–550, Jun. 2014, doi: 10.1111/1541-4337.12073.
3. Antolak H, Piechota D, Kucharska A. Kombucha Tea-A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). *Antioxidants (Basel)*. 2021 Sep 28;10(10):1541. doi: 10.3390/antiox10101541. PMID: 34679676; PMCID: PMC8532973.
4. D. Morales, "Biological activities of kombucha beverages: The need of clinical evidence," *Trends in Food Science & Technology*, vol. 105, pp. 323–333, Nov. 2020, doi: 10.1016/j.tifs.2020.09.025.
5. C. Dufresne and E. Farnworth, "Tea, Kombucha, and health: a review," *Food Research International*, vol. 33, no. 6, pp. 409–421, Jul. 2000, doi: 10.1016/s0963-9969(00)00067-3.
6. Greenwalt CJ, Steinkraus KH, Ledford RA. Kombucha, the fermented tea: microbiology, composition, and claimed health effects. *J Food Prot*. 2000 Jul;63(7):976-81. doi: 10.4315/0362-028x-63.7.976. PMID: 10914673.
7. X. Wang et al., "Chemical Profile and Antioxidant Capacity of Kombucha Tea by the Pure Cultured Kombucha," *LWT*, vol. 168, p. 113931, Oct. 2022, doi: 10.1016/j.lwt.2022.113931.

8. Abaci N, Senol Deniz FS, Orhan IE. Kombucha - An ancient fermented beverage with desired bioactivities: A narrowed review. *Food Chem X*. 2022 Apr 6;14:100302. doi: 10.1016/j.fochx.2022.100302. PMID: 35434600; PMCID: PMC9011011.
9. Mousavi SM, Hashemi SA, Zarei M, Gholami A, Lai CW, Chiang WH, Omidifar N, Bahrani S, Mazraedoost S. Recent Progress in Chemical Composition, Production, and Pharmaceutical Effects of Kombucha Beverage: A Complementary and Alternative Medicine. *Evid Based Complement Alternat Med*. 2020 Nov 18;2020:4397543. doi: 10.1155/2020/4397543. PMID: 33281911; PMCID: PMC7688354.
10. Atkinson FS, Cohen M, Lau K, Brand-Miller JC. Glycemic index and insulin index after a standard carbohydrate meal consumed with live kombucha: A randomised, placebo-controlled, crossover trial. *Front Nutr*. 2023 Feb 17;10:1036717. doi: 10.3389/fnut.2023.1036717. PMID: 36875857; PMCID: PMC9982099.
11. Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P. Understanding Kombucha Tea Fermentation: A Review. *J Food Sci*. 2018 Mar;83(3):580-588. doi: 10.1111/1750-3841.14068. PMID: 29508944
12. Kapp JM, Sumner W. Kombucha: a systematic review of the empirical evidence of human health benefit. *Ann Epidemiol*. 2019 Feb;30:66-70. doi: 10.1016/j.annepidem.2018.11.001. Epub 2018 Nov 10. PMID: 30527803.
13. Júnior JCDS, Meireles Mafaldo Í, de Lima Brito I, Tribuzy de Magalhães Cordeiro AM. Kombucha: Formulation, chemical composition, and therapeutic potentialities. *Curr Res Food Sci*. 2022 Feb 4;5:360-365. doi: 10.1016/j.crfs.2022.01.023. PMID: 35198995; PMCID: PMC8844156.

14. Batista P, Penas MR, Pintado M, Oliveira-Silva P. Kombucha: Perceptions and Future Prospects. *Foods*. 2022 Jul 4;11(13):1977. doi: 10.3390/foods11131977. PMID: 35804792; PMCID: PMC9265386.
15. Zofia NŁ, Aleksandra Z, Tomasz B, Martyna ZD, Magdalena Z, Zofia HB, Tomasz W. Effect of Fermentation Time on Antioxidant and Anti-Ageing Properties of Green Coffee Kombucha Ferments. *Molecules*. 2020 Nov 18;25(22):5394. doi: 10.3390/molecules25225394. PMID: 33218080; PMCID: PMC7698870.
16. C. D. Barbosa et al., "Microbial–physicochemical integrated analysis of kombucha fermentation," *LWT*, vol. 148, p. 111788, Aug. 2021, doi: 10.1016/j.lwt.2021.111788.

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