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Bachelor thesis

Kombucha: a systematic review of the empirical evidence of human health benefit

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Declaration

I declare that the Bachelor Thesis “Kombucha: a systematic review of the empirical evidence of human health benefit” is my own work, and all the sources cited in it are listed in the Bibliography.

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Summary

Kombucha tea is a beverage resulting from fermentation of sweetened tea with a symbiotic consortium of bacteria and yeasts (SCOBY) that has recently become popular as part of the functional food movement. This popularity is likely driven by its touted health benefits, coupled with the recent scientific movement investigating the role of the microbiome on human health. Considering this latest surge in popularity of this beverage it is necessary to compile a survey about its real effects and impacts on human health.

The purpose of this bachelor thesis is to create a literature review of the empirical health benefits of kombucha as identified from both *in vitro* and *in vivo* studies and human subject research. Various studies are compared and compiled to create the most accurate assessment of the latest and most relevant research.

A review of available literal sources showed that there are mostly *in vitro* conclusions or a limited number of *in vivo* studies conducted mostly on animals regarding the effects of kombucha beverages. Available data mostly agree on potential beneficial effects, however, no clinical studies prove these conclusions and the extent of mentioned effects on human health. Future studies on kombucha beverages should focus on clinical research to fully describe and confirm these claims.

Keywords: Kombucha, Health, Chemical composition, Beneficial property, Toxicity

Contents

1 Introduction

Both a drink and a microbial culture originating from the Orient are described as kombucha. According to one of the stories, the beneficial effects of kombucha were already known around 200 BC. Local doctor used kombucha to treat the digestive tract of the Japanese emperor. In fact, the designation of kombucha as a fungus that we know is incorrect, it is a symbiotic colony of yeasts and bacteria. Many names are used for kombucha sponge, the most commonly used being SCOBY (an acronym for symbiotic culture of bacteria and yeast). This summary will go over basic information about kombucha tea, its properties, and preparation. Various beneficial properties claimed by different sources will be compared with scientific research and will be therefore confirmed or disproved.

2 Objectives of work

Kombucha tea, a fermented beverage, has recently become popular as part of the functional food movement. This popularity is likely driven by its touted health benefits, coupled with the recent scientific movement investigating the role of the microbiome on human health. The purpose of this bachelor thesis is to create literature review of empirical health benefits of kombucha as identified from human clinical trials.

3 Literature survey

3.1 What is Kombucha

Kombucha is a traditional beverage of Asian origin obtained by fermenting sweetened black or green tea with a symbiotic culture of yeasts and bacteria. It is a moderately sweet, carbonated, acidic tea beverage. It is consumed worldwide but is most prevalent historically in China, Russia, and Germany. Popular media features have highlighted the beverage and its uses, suggesting that Kombucha consumption can have many beneficial effects on human health. Commonly listed effects are increasing of the immune response, reduction of blood pressure, antioxidant effects, and many more. These supposed health effects have created an increased interest in Kombucha tea (Greenwalt, 2000).

Many beneficial effects on the human body are attributed to kombucha drink, so it is often described as a functional food. The main beneficial effects of consumption of a functional drink include the support of immunity, supplementation of important vitamins, and protective substances. This beverage can be intended not only for drinking but can be used as a compress for burns, rashes, but also body, hair, and skincare. The production of kombucha drinks is one of the simplest fermentations that can be carried out in the home environment (Tietze, 1996).

Recently Kombucha beverage becomes increasingly popular in western cultures mainly under the functional food movement for its supposed health benefits. Kombucha is thought to decrease cholesterol levels, blood pressure, boost metabolism and weight loss, increase vitality and improve liver, gastric and immune functions. Most of these effects are based on personal testimonies and observations, but there are some indications that the consumption of kombucha beverages can be beneficial for our health thanks to detoxification, antioxidizing, energizing, and immunity-boosting effects (Laureys, 2020).

The fermented tea is a product of a microbial colony of yeasts and aerobic bacteria which is floating on the surface. In appearance, it resembles mushroom or surface mould, but it is actually cellulose material that is produced during the growth of the microbial colony. Every batch of kombucha creates a new layer of this film which facilitates conditions necessary for the aeration of aerobic microorganisms. These new colonies can be used for subsequent batches of the kombucha tea (Greenwalt, 2000).

Kombucha recipes may vary greatly, due to different traditional methods and widespread of the beverage. However, the common method of preparation is to infuse black tea leaves in freshly boiled water, then it is sweetened with 50 to 150 g/liter (5 to 15 %) sucrose. The tea needs to be sweetened in order to provide nutrients for the growth of the microbial colony. Tea leaves are then removed and the beverage is then allowed to cool to room temperature. The microbial colony is then added from the previous batch along with some of the kombucha (about 100ml). The container is then covered with a clean cotton cloth to allow access of oxygen and is incubated for a period of seven to ten days at room temperature. If the fermentation process is allowed to continue for more than ten days, the acidity levels can rise to high levels, increasing the potential to be harmful to human health. The microbial colony is then removed and the beverage is ready for consumption. The final product comprises of organic acids, minerals, tea components, and vitamins. It can be also slightly alcoholic. Final taste and appearance are comparable to various ciders (Greenwalt, 2000).

Leal et al., (2018) summarized kombucha substrate in general as a tea base with added sugar which nurtures tea fermenting bacteria and yeasts. Other authors such as Watawana et al., (2015) add that it's extremely important to use properly sanitized utensils and work in a clean environment to have control over the growth of microorganisms and to prevent unwanted contamination.

3.2 Origin and history

The exact origin is uncertain, but the first mention of the kombucha fungus or SCOBY comes from northeastern China around 220 BC. It was named after the Korean physician Komba, who was the first to bring medicinal tea to Japan, which was fermented with this fungus to heal Emperor Inkyo, who was suffering from indigestion problems. With the expansion of trade routes beyond the Far East, the kombucha gradually spread to Russia and Eastern Europe (Arcimovičová, 1998).

The first traceable record in the history of drinking kombucha drink comes from Russia and Ukraine at the end of the 19th century. Later the kombucha spreads across Europe, during World War 1, thanks to the war prisoners, it moved to new countries. Until the 1920s, the kombucha drink was very popular throughout Germany, where it was used as a traditional home remedy for stomach problems, hemorrhoids, and rheumatism. Kombucha culture itself can be often referred to by several names, of which it is most often used: SCOBY (symbiotic culture of bacteria and yeasts) or kombucha fungus. Polish doctor called Dr. Waldeck has recorded in 1927 that he was shown the recipe by a polish pharmacist for a Russian home remedy, thus recording authentic names of kombucha beverage from that period "miracle mushroom", "Volga mushroom" or "Tea-Kvass mushroom" (Tietze, 1996).

Kombucha later spreads from Asia and Europe worldwide, another notable mention is from 1980s and 90s in the United States, where it gained popularity during the epidemic of HIV. People believed that it could boost the functions of the compromised immune system. Later on, it fell out of favour due to the link of two cases of metabolic acidosis by the Center of Disease Control and Prevention.

The latest re-emergence of Kombucha from the early 21st century is prevalent to this day. Greater awareness of probiotics and functional foods elevated this beverage to the newest trends of healthy lifestyle culture and many manufacturers started to produce this product or many various home brewing kits (Petruzzello, 2017).

3.3 Tea

Tea is made mainly green or black, with black tea representing approximately 80% of all tea products. The composition and properties of tea are very good explored, making it a suitable starting point for understanding the potential of kombucha. By choosing the tea used, we can influence the taste of the resulting drink or increase its favourable effects on our body. Black tea is considered a classic kombucha drink with kombucha culture and refined sugar (Tazzini, 2014).

Tea is made from the leafy perennial crop *Camellia sinensis* and according to processing different varieties of tea are created. For the production of black tea, leaves are crushed and exposed to conditions with high humidity, which leads to enzymatic oxidation. Green tea is produced by steaming the leaves which inactivate enzymes present which prevents fermentation. Oolong tea is made from partially fermented tea leaves which are left to wilt under sunlight (Martínez Leal, 2018).

AL-Kalifawi (2014) investigated bacterial cellulose film yields in the tea broth using different concentrations of black tea and sucrose and found out that both of these factors greatly affect cellulose production. He achieved the highest yield at tea concentration of 10g/L and sucrose concentration of 100g/L.

Kallel, et. al. (2012) compared green and black tea, which both showed similar profiles for kombucha fermentation, but also described specific biochemical behaviours in different tea substrates. In this experiment, carbon sources in black tea were consumed at a faster rate, biochemical changes were faster and the contents of cellulose, acetic acid, phenolic substances, ethanol, caffeine, and protein levels were higher when compared to green tea substrate.

3.4 Microorganisms in SCOBY

SCOBY refers to a symbiotic culture of bacteria and yeasts. It is a three-dimensional zoogleal mat that consists of osmophilic yeast species in symbiotic relation with acetic acid bacteria. It resembles surface mould with a structure like jelly and it is essentially cellulose biofilm that floats on the kombucha medium is created predominantly by bacteria *Acetobacter* and *Gluconobacter* species. According to recent taxonomical studies, specie of acetic acid bacteria found in SCOBY, were reclassified into new genera *Komagateibacter* (Liu 2018). Many bacteria and yeast species are present in this mat and all are part of the microbial culture that is responsible for kombucha fermentation (Laavanya, 2021).

3.4.1 Yeasts

Yeasts present in kombucha beverage are mainly responsible for ethanol fermentation, which will be described later in text.

Watawana (2016) regards *Zygosaccharomyces* as a predominant yeast species with an 84.1% relative percentage of representation in SCOBY. Jayabalan, et. al. (2014) reported a huge variety of yeast species present in kombucha beverages that include mainly *Saccharomyces*, *Saccharomycodes, Torulospora, Schizosaccharomyces, Candida, Koleckera,* and *Zygosaccharomyces.*

Teoh (2019) conducted research to identify yeast cultures in different samples of kombucha beverage and obtained 163 yeast isolates in their samples. They tested 9 isolates from each colony and properly identified only *Schizosaccharomyces pombe* species using the Biolog identification system. Predominant culture in three of their four samples was *Zygosaccharomyces bailii,,* second most commonly found species in two of their four fermentation samples were *Torulaspora delbrueckii* and *Rhodotorula mucilaginosa.*

This study proved that there is no single species of yeasts associated with every kombucha culture universally. The most common isolates found were acid-producing, fermentative, and osmotolerant species. All of the species they found exhibited characteristics needed in kombucha fermentation. The only exception was the species *Rhodotorula mucilaginosa*, which has not been previously reported to be associated with kombucha fermentation. This species has been described as a common yeast contaminant in both beverages and food that occurs frequently and naturally in the atmosphere. The study also probed that the microbial ecology of kombucha liquor is similar to that of the pellicle present (Teoh, 2019).

3.4.2 Bacteria

Bacteria present in kombucha beverage are mainly responsible for acetic acid fermentation and lactic acid fermentation, which will be described later in text.

Torán-Pereg (2021) found out that the three main phyla of bacteria identified during his research are *Proteobacteria* and *Actinobacteria*. Proteobacteria were the most abundant species found in the pellicle made of cellulose floating in the tea. It accounted for 95% of the total population. He also identified within these phylum microorganisms that belong to the *Acetobacteraceae* family that include genera *Gluconobacter*, *Acetobacter*, and *Komagataeibacter.* and reported that the most dominant species is *Komagataeibacter xylinus* in the bacterial community, thus making it the main producer of bacterial cellulose in kombucha. The results obtained were in correspondence with the bibliography published by Marsh, et. al (2014), although he refers to the same bacteria species as the *Gluconobacter* family instead. This difference can be explained by changes in nomenclature through the years for example Liu (2018) uses both names, though favouring *Komagataeibacter* as the newer term.

The other two bacteria isolated by Torán-Pereg (2021) were *Bacillus licheniformis* and *Microbacterium sp.* Those are reported to be atypical in kombucha, but they have been described previously in the Kombucha consortium. The sample analysed was largely dominated by *Komagataeibacter* bacteria genus. Representation of other bacteria was significantly lower, but they still play role in the properties of the final product. These atypical findings of showing different representations of microorganism present, can be explained by article from Jayabalan, et. al. (2014), where it is states that the exact microbial composition of kombucha beverages is not well defined, because it varies on many aspects such as its origin, variables in substrates and production conditions.

Laureys, et. al. (2020) states that AAB are the most characteristic microorganisms found in kombucha fermentation. Genera found in kombucha are *Acetobacter*, *Gluconacetobacter*, *Komagataeibacter*, and *Gluconobacter*. The most important and characteristic microorganism in kombucha beverages *Komagataeibacter xylinus* as it is thought to be responsible for forming of the cellulose pellicle. This can be also supported by the interpretation of Gomes, et. al (2018), which states that *Komagataeibacter* accumulates up to 20% of acetic acid present in the medium. Other species for example *Acetobacter* accumulate a maximum of 8% acetic acid. Jayabalan, et. al. (2016) states that predominant AAB found in kombucha are *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, and *Gluconobacter oxydans*.

Marsh, et. al. (2014) during the analysis of multiple kombucha samples established that lactic acid bacteria (LAB) such as *Lactobacillus* and *Lactococcus* are present in SCOBY pellicle at considerable amounts. His research suggests that LAB are more represented in the kombucha colonies than previously understood. His data suggest that lactobacilli are especially prevalent at later stages of fermentation. One of the pellicles shown in his research (IRE) stood out with around 30% LAB representation in the pellicle. He also suggests that these species might be able to help with the growth of some AAB species, as *Lactobacillus* have been shown to increase the growth of *Gluconacetobacter*, and together in co-culture with they have been shown to considerably increase production of cellulose in the pellicle. **Table 1** shows results of his analysis on multiple samples of kombucha beverage.

Table 1: Bacterial genera present in different kombucha pellicles, relative abundances of the 16S bacterial genera at day 3, day 10 and in the pellicle (Marsh et al. 2014).

	Ca1	Ca2	Ire	UK	US
Day 3					
Acetobacter	0.86	0	0.43	0	0
Gluconacetobacter	86.91	97.79	93.09	98.14	95.73
Lactobacillus	3.93	1.13	3.57	1.77	1.77
Lactococcus	1.56	0	1.77	0	0
Leuconostoc	0.52	0	0	0	0
Bifidobacterium	0.30	0	0	0	0
Thermus	0.22	0	0.20	0	0
Allobaculum	0	0	0	0	0.88
Ruminococcaceae Inartae Sedis	0	0	0	0	0.19
Propionibacterium	0	0	0.20	0	0
Other	5.75	0.58	0.75	0.30	1.42
Day 10					
Acetobacter	0	0	0.19	0	0
Gluconacetobacter	92.17	93.16	87.62	94.26	95.73
Lactobacillus	5.96	6.17	9.59	1.44	3.47
Lactococcus	0	0.23	0.19	0	0.18
Leuconostoc	0	0	0	0	0
Bifidobacterium	0	0	0	0	0
Thermus	0.66	0	1.62	3.73	0
Allobaculum	0	0	0	0	0
Ruminococcaceae Inartae Sedis	0	0	0	0	0
Other	1.22	0.45	0.79	0.58	0.61
Pellicle					
Acetobacter	0	0	1.93	0.28	0
Gluconacetobacter	99.77	95.73	58.02	92.97	99.82
Lactobacillus	0	1.72	30.57	4.64	0
Lactococcus	0	1.29	7.76	1.62	0
Leuconostoc	0	0	0	0	0
Bifidobacterium	0	0	0	0	0
Thermus	0	0	0	0	0
Allobaculum	0	0	0	0	0
Ruminococcaceae Inartae Sedis	0	0	0.69	0.21	0
Propionibacterium	0	0	0.38	0	0
Other	0.23	1.26	0.66	0.28	0.18

3.5 Fermentation

Fermentation is a process of conversion of organic substances (most often carbohydrates), in which the participation of microorganisms and their enzymes produces substances that are less energy intensive. By fermentation, microorganisms gain energy similarly to respiration; fermentation is easier, but also less effective than breathing. In kombucha beverages most important fermentation processes are ethanol fermentation followed by acetic acid fermentation. Lactic acid fermentation also takes place due to presence of lactic acid bacteria, but it is not as important as the former two (Kaiser, 2021).

3.5.1 Acetic Acid Fermentation

Acetic acid fermentation is a process conducted by Acetic acid bacteria (AAB) which belong to the *Acetobacteraceae* family. They are highly capable of producing vinegar as a byproduct due to their high ability of oxidizing ethanol into acetic acid. They are also highly resistant to acid conditions caused by acetic acid in fermentation medium growing well below pH 5.0, although the pH optimum for growth is 5-6.5

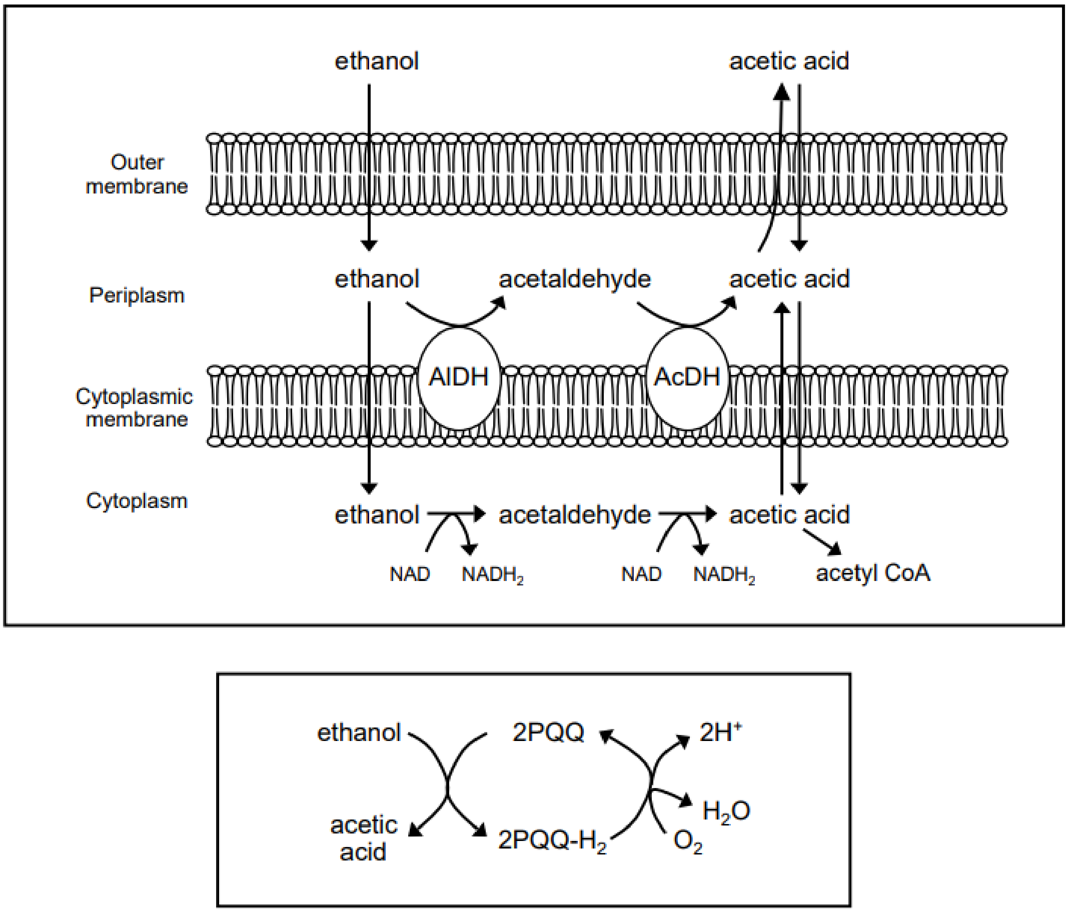

Figure 1: The formation of acetic acid from ethanol by AAB (Hutkins, 2006).

AAB are strict aerobes with a high capacity to oxidize alcohols, sugars, aldehydes in the presence of oxygen. AAB species occurrence depends entirely on the concentration of acetic acid during acetification. These species are also described as demanding, requiring high amounts of nutrition, therefore they are difficult to isolate and cultivate on artificial medium, such as fermented beverages. Products of lactic acid fermentation include vinegar, kombucha, gluconic acid, sorbose, and ascorbic acid, bacterial cellulose (Gomes, 2018).

3.5.2 Ethanol fermentation

The basic stoichiometry of alcoholic fermentation is given by the Guy - Lussac equation:

$$C_2H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Alcoholic fermentation is the most important fermentation process. It is a complex biochemical process during which yeasts, some bacteria, or microorganisms gradually decompose carbohydrates into ethanol, carbon dioxide, and other metabolic products while releasing energy in the form of heat. It is a process called glycolysis, which takes place mainly anaerobically - without air access, although slight aeration of the fermentation medium at the beginning of the fermentation is favourable for the increase in the number of cells and their fermentation activity. During glycolysis, the six-carbon glucose molecule is split into two, three-carbon molecules of pyruvate, 2NADH + 2H+, and 2 units of ATP as a result of substrate-level phosphorylation. Alcoholic fermentation is the basis for manufacturing various alcoholic beverages such as wine and beer (Kaiser, 2021).

Yeasts present in kombucha culture are the main producers of ethanol. Thanks to yeasts sucrose is hydrolyzed into glucose and fructose and through glycolysis they produce ethanol, preferring to use fructose as a substrate. AAB subsequently uses remaining glucose to form gluconic acid and ethanol to create acetic acid. The pH of the whole medium decreases with fermentation time, as more organic acids are created (Dufresne and Farnworth, 2000).

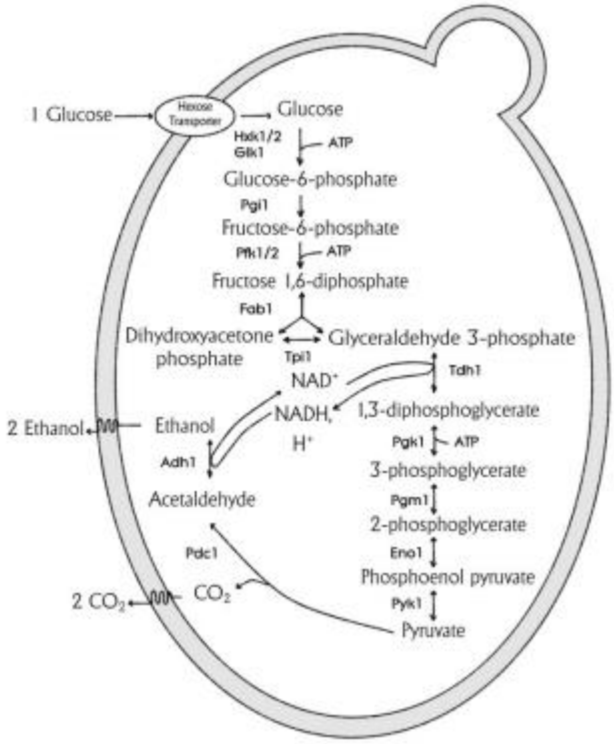

Figure 2: Ethanol fermentation – enzymatic steps on *Saccharomyces cerevisiae* (Faria-Oliveira, 2013).

3.5.3 Lactic acid fermentation

Lactic acid fermentation is a process where bacteria convert carbohydrates into lactic acid. Bacteria involved in lactic acid fermentation can be divided into two groups. Homofermentative – which create as their product almost solely lactic acid (90%) or heterofermentative, which covert fermented carbohydrate into lactic acid (50%), acetic acid, carbon dioxide, and sometimes when certain conditions are met even ethanol.

In traditional kombucha beverage the lactic acid is not one of the most characteristic compounds, but it is detected in small amounts. Lactic acid presence in kombucha is usually attributed to lactic acid bacteria present synthesizing ethanol or acetic acid (Jaylaban 2014; Dufrense 2000).

Malbaša et al., (2008) did an experiment, where they compared products of kombucha fermentation on different types of molasses and sucrose. Molasses they used were from sugar beet and were used to demonstrate the effectiveness of low-cost carbon sources on kombucha fermentation of black tea. The contents of lactic acid were related to the high quantities of invert sugars, amino nitrogen, and biotin present in the molasses. In her research, she obtained products rich in lactic acid when she used molasses as a substrate in comparison to sucrose.

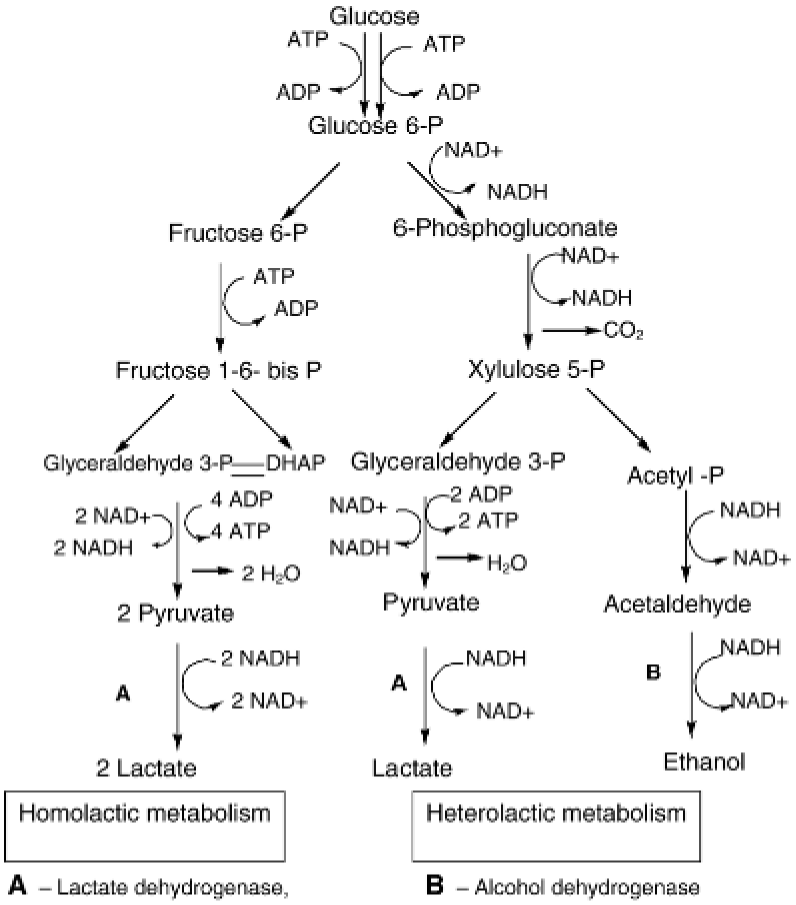

Figure 3: Metabolism of lactic acid bacteria (Reddy, 2008).

Nguyen, et. al. (2015) found out that lactic acid is more present in kombucha made from green tea medium when compared to black tea. He also conducted research, where he added supplements of LAB to kombucha beverages to enhance its biological activities. Namely glucuronic acid production, antioxidant and probiotic activity. His results showed promise that both glucuronic acid production and probiotic activity can be influenced positively, however, more evidence needs to be found.

3.6 Fermentation processes during Kombucha preparation

The fermentation process could be divided into two parts. Once the tea, preferably black (green can be also used) is prepared and sweetened by white sugar, it is then let to cool to a degree of 32 °C minimum. This is done in order not to destroy microorganisms in the colony. Then the bacterial culture is added to the surface of the solution which serves as a substrate. The solution is also acidified by the addition of vinegar or already prepared Kombucha from previous batches. The optimum temperature for the growth of acetic acid bacteria and yeasts is present in kombucha is between 25- 32 °C. A sufficient amount of oxygen must be present for the growing bacteria for primary fermentation to take place. Subsequently, the culture is allowed to incubate from 1 to 8 weeks at room temperature. The container with tea needs to be covered with a clean cloth and fastened properly to avoid contamination by other undesirable microorganisms and deny access of various organisms such as fruit flies.

The primary fermentation process begins when acetobacteria create a cellulose network floating on the surface, which enhances interactions between bacteria and yeasts. Caffeine in the tea also stimulates cellulose synthesis by bacteria. Yeast cells then convert sucrose into glucose and fructose and produce ethanol. Acetic acid bacteria then convert ethanol, fructose into acetic acid, and glucose into gluconic acid. The longer this process continues the more acidic and less sugary beverage becomes due to rising levels of acids in the medium. Low pH allows the growth of only those microorganisms that can withstand acidic conditions, thus providing some protection against invasive contaminants. The primary phase of fermentation ends when the microbial culture gets removed from the container.

The secondary part of fermentation occurs when the beverage gets bottled. The usual process consists of filtration through a cloth and subsequent bottling. This process does not remove all microorganisms from the beverage and therefore they continue the fermentation in this closed system. Microorganisms produce carbon dioxide which cannot leave the closed bottle in the same way as it does during the primary fermentation phase. A consequence of this process is the saturation of beverages and a possible increase in pH. Residual microorganisms also continue their metabolic processes consuming sugar and converting it into lactic, acetic, gluconic acids, ethanol, and carbon dioxide. The resulting beverage can therefore get even less sweet, more sparkly, and possibly more alcoholic. This process can be inhibited by placing the beverage into cold conditions, which inhibits the metabolic processes of microbial organisms (Teoh, 2004; Chi Phung, 2015; Tazzini, 2014).

3.7 Factors Influencing Kombucha Fermentation

Fermentation is a complex process influenced by many factors throughout production. Composition of the medium, the temperature throughout all the stages, pH, amounts of oxygen available, and the supply of precursors for the various reactions to happen. Composition of tea fungus itself. Any combination of these factors will affect the final product. These factors collectively coincide and together, account for composition differences. Any change would therefore affect biological activities (Villarreal-Soto, 2018).

3.7.1 Substrate

Usually, black or green tea is used, but different substrates can be used as well. For example, Ayed et al. (2016) created a kombucha beverage from grape juice, which altered both functional properties and look of the beverage, while simultaneously greatly reduced fermentation time to only 6 days. Watawana et. al. (2015) used as substrate coconut water, which affected all biological activities present.

Regardless of medium used, other properties of the substrate such as sugar concentration greatly alter fermentation processes due to them being one of the basic requirements for the fermentation to happen (Villarreal-Soto, 2018).

3.7.2 Time

The time needed for kombucha tea fermentation usually ranges from 7 to 60 days. Best results were obtained in 2 weeks on average. Antioxidant activities increase over incubation time, however organic acid concentration increases as well. Enabling them to reach levels that are potentially harmless upon direct consumption. CO2 generated over longer fermentation can be accumulated between broth and biofilm, potentially blocking the transfer of nutrients. Fermentation time is usually selected upon sensory inspection. Fermentation ranging from 6-10 days produces refreshing fruit-like beverage, while longer fermented beverage has vinegar taste due to accumulation of acids (Villarreal-Soto, 2018).

Zofia, et al. (2020) used kombucha culture in conjunction with green coffee medium to find out whether fermentation time can alter antioxidant and anti-aging properties of green coffee. Original green coffee extract and ferments after addition of SCOBY culture were evaluated for antioxidant activity, cytotoxicity, and their composition was assessed. Results of this research showed that coffee extract after longer fermentation on day 28 had the highest antioxidant activity, while shorter fermentation on day 7 had negative properties on the analyzed ferments. This research further indicates that time is an important factor in kombucha preparation.

3.7.3 Temperature

Best enzyme activity and microbial growth is acquired if the optimum temperature during fermentation is met. In general, the optimum temperature ranges between 22-30 °C for kombucha fermentation. Antioxidant activity can be also influenced by temperature changes (Villarreal-Soto, 2018).

De Filippis, et. al. (2018) conducted a duplicate experiment on fermentation under different temperatures (20 and 30 °C) in both black and green tea medium monitoring AAB specifically. Surprisingly there were no major differences observed in cultivated AAB in both media or temperatures used. AAB were more dependent on pH levels. Nevertheless, they observed that higher temperatures allowed other sub-dominant species of bacteria to develop. Mainly LAB (Lactic acid bacteria) such as *Lactobacillus* or *Streptococcus* and contaminants such as *Acinetobacter* or *Propionibacterium.* Different concentrations of polyphenols were also found at those different temperatures, reporting increased concentration in black tea medium at 20°C.

3.7.4 pH

The pH belongs to the most important parameters in the environment where the fermentation takes place because it directly affects microbial growth and antioxidant activity. It is affected by acids formed during the process, such as acetic and gluconic acids (Villarreal-Soto, 2018).

3.7.5 Oxygen

Access of oxygen is one of the imperative factors during fermentation because most of the fermentation processes are aerobic. All parts of the process need significant amounts of oxygen. For example oxidation of glucose needs 192g of oxygen to oxidize 180g of glucose. Acetic acid bacteria are strict aerobes, as they need one mole of oxygen to oxidize ethanol into acetic acid. For these reasons, oxygen supply must be maintained from the air into the broth. The supply rate of oxygen must be sufficient to satisfy the demands of all microorganisms present (Villarreal-Soto, 2018).

Lee, et.al (2014) published that maximum bacterial cellulose production occurs at 10% oxygen saturation in the medium, higher values result in gluconic acid production and lower inhibit cell growth.

3.8 Chemical composition

The chemical composition of kombucha is affected by several factors. The essential being fermentation time, type of tea and amount of sugar concentration used as a substrate medium, used temperature. All these factors can affect metabolites, their composition, and concentration. The fermentation process and its conditions should be controlled to get the best final product (Mousavi, 2020).

3.8.1 Organic Acids

The presence of organic acids in kombucha depends on the type of tea used. Organic acids change during fermentation depending on the use of black or green tea. Some of these acids in beverages have been shown to have antimicrobial activity and can even improve sleep (Jayabalan, 2007).

A study conducted by Jayabalan (2007) proved that the production of organic acids varies with time for example acetic acid increased its concentration to a maximum of 9.5g/l on the 15th day of fermentation and decreased slightly afterward. Another major metabolite was Glucuronic acid reaching a maximum of 2.3 g/l on the 12th day of fermentation. The concentration of other acids such as lactic and citric was very low and show during the early stages of fermentation. Both green and black tea was proven to be the best suitable substrate for acetic and gluconic acid production respectively by kombucha culture.

3.8.1.1 Acetic acid

It is a chemical compound responsible for the sour taste and traditional vinegar odour. Acetic acid tends to rise up to 11 g / l in a kombucha drink at 30th-day fermentation, then gradually decreases to 8 g / l at the 60th day of fermentation. Reduction of the content of acetic acid in the beverage is caused by its later usage when bacteria use it as a carbon source. The concentration of acetic acid in the drink can be influenced by the type of sugar used, for example, production, when sugar molasses is used, is much lower (Jayabalan, 2007).

3.8.1.2 Lactic acid

Lactic acid is one of the most commonly used acids in food, where it occurs either naturally or as a fermentation product (cabbage, yogurt, and many others). It is the major metabolic intermediate in most living organisms. Lactic acid, which is added to food, is obtained either by carbohydrate fermentation or by a chemical process. Both of these processes are very competitive with similar production costs (Eş, 2018).

3.8.1.3 Gluconic acid

Gluconic acid is organic acid derived by oxidation reaction from glucose. The reaction is facilitated either by glucose oxidase (fungi) or glucose hydrogenase (bacteria of *Gluconobacter* family). Gluconic acid has many applications in the food, pharmaceutical, or cosmetics industry. Gluconic acid occurs in the form of white crystals or a crystalline powder of sweet taste with a slightly acidic aftertaste. It is a sugar acid and very often it occurs in food naturally. It can be used as an antioxidant. A substance that prolongs the shelf life of food and protects it against spoilage caused by oxidation. Another use as an acidity regulator, a substance that changes or maintains the acidity or alkalinity of a food. The substance can be found in drinks, syrups, wine, mixtures for the production of sweet dough, in which it works as a baking agent (Goldberg, 2009).

3.8.1.4 Glucuronic acid

It is formed by a complex biochemical process of glucose oxidation. In a healthy person, glucuronic acid is formed in the liver. Its function is to connect with waste products of metabolism and foreign substances that enter the body through food, the respiratory tract, and through the skin. In this new alliance, it helps to eliminate harmful substances such as preservatives, nicotine, and harmful substances from the surrounding environment, such as mercury, lead, and benzene, especially in the intestines and urinary tract. A higher supply of glucuronic acid has an ideal positive effect, among other things, especially on the body's defence mechanism (Tazzini, 2014).

Glucuronic acid in kombucha is synthesized by invertase of the yeasts hydrolyses into glucose and fructose. Bacteria metabolize glucose into gluconic acid, which is later synthesized by acetic acid bacteria into glucuronic acid. This process begins as soon as the fermentation process in kombucha beverage initiates. Contents of glucuronic acid increase with fermentation time (Martínez-Leal, 2020).

3.8.2 Carbohydrates

The main function of carbohydrates in Kombucha beverages is to serve as a substrate for yeasts. Fructose is reported to be consumed at faster rates, leaving glucose as the main residual carbohydrate. Residual amounts of carbohydrates in kombucha beverages vary and decrease with longer fermentation time. However, some kombucha beverages are later sweetened to improve the sour taste (Laureys, 2020).

3.8.3 Enzymes

Enzymes are molecular active substances (proteins) that activate, accelerate and induce chemical metabolic processes, these processes could not work on their own. Each enzyme is specialized and performs a specific function. Based on their origin, enzymes are involved in the construction of complex bonds, others are involved in structural processes in the human body. Simply put, all enzymes are looking to balance all malfunctioning reactions. About 3,000 enzymes have been found in the human body. (Heßmann-Kosaris, 2002).

3.8.4 Minerals and vitamins

Minerals are inorganic substances needed in small amounts for normal bodily functions and growth. They occur in negligible concentrations in kombucha drink and are mainly copper, iron, cobalt, manganese, nickel, and zinc (Heßmann-Kosaris, 2002).

Jayabalan, et. al. (2016) states that vitamins C, B1, B2, B6, and B12 are commonly found in kombucha beverages. Kombucha beverages are rich in vitamin C content due to the production of glucuronic acids by various microorganisms, which then serves as a precursor for the biosynthesis of ascorbic acid. Vitamins of the B group are also synthesized by yeast and bacteria present in kombucha beverage Nguyen et al., (2015).

3.8.5 Ethanol

The ethanol concentration in Kombucha increases with fermentation time, followed by a decrease in concentration caused by the action of acetic bacteria using ethanol to form acetic acid. Mild alcohol consumption is reported to have a protective effect on the human body against free radicals. The amount of 0.5% alcohol in a kombucha beverage is the same as, for example, in apple cider. Kombucha is an excellent substitute for drinks with higher alcohol content, such as sparkling wine or beer. Except that those who cannot consume alcohol should not drink kombucha as well (Heßmann-Kosaris, 2002).

Talebi, et. al. (2017) did an experiment where he researched several commercially sold kombucha beverages using headspace gas chromatography technique for alcohol content. He concluded that none of the 18 samples was lower than federal limit 0.5% ABV (alcohol by volume), some even exceeded 1.5%. He also emphasizes that kombucha alcohol content changes over time, as longer storage times result in further fermentation and therefore increase in ethanol content.

3.8.6 Phenolic substances

Phenols are the most abundant antioxidants present in the diet. Phenolic substances play an important role in the prevention of several diseases related to oxidative stress, such as cancer and neurodegenerative diseases. They participate in the activity of various enzymes and cellular receptors as a means of defence against oxidative stress caused by reactive oxygen species. The polyphenols and flavonoids present in tea are responsible for its antioxidant properties. During the fermentation of kombucha, the total content of polyphenols and flavonoids increases. This increase may result in the degradation of complex tea polyphenols and flavonoids to smaller molecules by some enzymes released from the microorganisms found in the microbial culture. Such transformations are possible thanks to microorganisms that can degrade various polyphenols. The resulting fermented kombucha drink has a higher reductive power compared to a classic tea (Jayabalan, 2007; Chakravorty, 2016).

In tea leaves, they form a significant group of substances containing approximately 122 species of tannins. The tannins themselves are subject to structural changes and the practical mastery of these processes forms the basis of the technology of production and final processing of tea leaves. The content of tannins is different in different types of tea leaves. Green tea contains the most tannins (10 to 27%), while in black tea tannins are represented to a lesser extent, from 5 to 12%. Tannins give tea drinks a slightly bitter taste. Two forms of tannins were found in the leaves of the tea tree in terms of solubility. Free tannins, extracted with water and organic solvents and bound tannins, especially with proteins that are insoluble in water but can be extracted using alkaline solutions. The content of bound and free tannins depends on the degree of ripeness of the tea tree leaves. As the tea leaves age, the content of monomeric phenolic compounds decreases, and the content of tannins increases. When storing tea leaves, the tannin content is reduced by 40 to 50% (Augustín, 2001; Jayabalan, 2007).

The term tannins refer to phenolic compounds that interact with proteins. Tannins are divided into two large groups of substances, namely hydrolyzable and condensed tannins. Hydrolyzable tannins are polymers of gallic acid esters, abbreviated to catechin. Condensed tannins or also flavones are polymers of some flavonoid substances with the structure of 3-hydroxy-flavone. In practice, tannins occur in any combination of condensed and hydrolyzable tannins and are collectively called complex tannins (Cibulka, 2005).

Catechins and tannins are thought to have a direct effect on the synthesis of aromatics present in tea leaves. Tea tannins have a beneficial effect on the digestive tract because they can bind and remove harmful substances from the body. They also help with capillary bleeding, rheumatic inflammation of the heart's diaphragm, high blood pressure, and scurvy (Augustín, 2001).

3.8.7 General composition

To fully understand the potential benefits or risks of kombucha beverages, we need to know their exact composition and properties. However, both composition and metabolite concentration is heavily dependent on various factors during preparation. Such factors are for example a base medium of the fermentation, concentration of sugar and tea, length of fermentation and temperatures used, the exact microbial composition of SCOBY or tea fungus, and possible contamination. Nevertheless, Villarreal-Soto described the main metabolites and key components of kombucha beverage (Villarreal-Soto, 2018).

As stated above, the exact composition may vary across different samples of kombucha according to different methods during preparation. However, in order to generalize following composition of kombucha in **Table 2** will be used as a basic reference point unless specified otherwise.

Table 2: General chemical composition of kombucha published by Villarreal-Soto (2018).

	Compound	Average composition	Initial sucrose	Fermentation time (days)
Organic acids	Acetic acid	5.6 g/L	70 g/L	15 d
	Acetic acid	8.36 g/L	100 g/L	18 d
	Acetic acid	11 g/L	100 g/L	30 d
	Gluconic acid	39 g/L	100 g/L	60 d
	Glucuronic acid	0.016 g/L	70 g/L	21 d
	Lactic acid	0.18 g/L	100 g/L	18 d
Vitamins	Vitamin B1	0.74 mg/mL	70 g/L	15 d
	Vitamin B2	8 mg/100 mL	70 g/L	10 d
	Vitamin B6	0.52 mg/mL	70 g/L	15 d
	Vitamin B12	0.84 mg/mL	70 g/L	15 d
	Vitamin C	25 mg/mL	70 g/L	10 d
General composites	Ethanol	5.5 g/L	100 g/L	20 d
	Proteins	3 mg/mL	100 g/L	12 d
	Tea Polyphenols	7.8 Mm GAE	100 g/L	15 d
Minerals	Cu. Fe. Mn. Ni. Zn	0.1 to 0.4 µg/mL	70 g/L	15 d
Anions	F^-. Cl^-. Br^-. I^- NO_3^-. HPO_4^-. SO_4^-	0.04 to 3.20 mg/g	100 g/L	7 d

This part of the thesis will focus on various properties attributed to Kombucha beverage by various sources ranging from popular websites to promo materials, explaining what this supposed effect is and then compared with scientific research to either prove or disprove its claim.

3.9 Empirical evidence of kombucha effects on human health

3.9.1 Antioxidative effect of Kombucha beverage

Many online articles suggest that kombucha has a strong antioxidative effect, especially thanks to having high polyphenol and vitamin contents. They often attribute the ability to reduce liver toxicity to kombucha and polyphenols it contains example being Leech (2018) on web Healthline. Other authors such as Metrus (2021) on web Byrdie which focuses on health and dietary advice often extrapolate various health benefits from specific general properties of various components found in kombucha beverage.

Halliwell (2009) defined antioxidant as any substance that significantly delays or prevents the oxidizable substrate from oxidation when present at low concentrations. The term "oxidizable substrate" refers in his interpretation to almost everything found in living cells ranging from DNA, carbohydrates, lipids, and proteins.

In the human body, various chemical processes and metabolic reactions form free radicals, including reactive oxygen species (ROS). Free radicals are able to react with and modify molecules such as lipids, proteins, and DNA, therefore influencing their properties and ability to function normally. This process is called oxidative damage. The human body has a complex system of antioxidant protection, that prevents the formation of ROS and terminates reactions with biological substrates initiated by them. Antioxidants present in the diet include vitamins (such as E, C), carotenoids, some minerals which are key components of antioxidant enzymes. Studies proved that many antioxidants serve in specific conditions as "pro-oxidants", these conditions being for example their high concentrations or presence of transition metals (Stanner, 2013). Kaewkod (2019) found out in his research that displayed antioxidant activity against DPPH radicals (stable nitrogen centered free radicals).

Some plant components like polyphenols have antioxidative potential. However, there have been strong discussions whether they are *in vivo* present at sufficient amounts to influence antioxidative processes in the human body. Some studies have shown that polyphenols do not appear in high enough concentrations in the bloodstream to significantly alter the total antioxidant capacity of the human body. Stanner also estimated that 90-95% of polyphenols undergo molecular changes after ingestion, changing radically their biological activities. It is suggested that polyphenols can have an indirect antioxidative effect, however, there is a lack of concise evidence to prove otherwise (Stanner, 2013).

Cao et. al. (1996) experimented on the antioxidant capacity of vegetables and tea using the ORAC assay method. However, an experiment on green tea showed that in presence of Cu^{2+} (which can be present in kombucha beverages), negative $ORAC_{Cu}$ meaning that prooxidant activity was initiated. That did not occur during other experiments on vegetables. Further research on this matter needs to be concluded before the definitive interaction in kombucha beverages is assessed.

One of the most important aspects is the bioavailability of flavonoids from tea. Their absorption, distribution, and metabolism in the living organism are essential to assess their impact on the organism. Pure flavanols are not easily absorbed by humans, but their glycosides can be absorbed in the small intestine thanks to the active glucose transport present. Catechins and their products from black tea can be absorbed easily by humans and are also metabolized well (Hollman, 1997).

Srihari (2013) states that the presence of complex phenolic compounds in an acidic environment and various enzymes released by bacteria can lead to breaking complex compounds into smaller molecules which leads to an overall higher count of phenolic compounds in kombucha beverages. This is supported by Jayabalan (2014) who attributes the antioxidant activity of kombucha to the presence of tea polyphenols, ascorbic acid, and D-saccharic acid-1,4-lactone. He states that kombucha has *in vitro* higher antioxidant activity than regular unfermented teas because yeasts and bacteria during fermentation produce structurally modified tea polyphenols and components with lower molecular weight.

Liu (2018) states on the bioavailability of phenolic compounds in black tea that abundant compounds are theaflavin and theasinensin and their derivatives. Given the complex structures of oxidized black tea phenolics, their bioavailability is quite limited. He also states that monomeric flavan-3-ols are the main phenolic compounds in green tea whereas black tea usually oxidized dimeric and oligomeric flavan-3-ols mostly present. Their bioavailability is also low, however, they are converted by the gut microbiota into low-molecular-weight metabolites, which are much easier for the human body to absorb. The most apparent outcomes of beneficial phenolic activity are the stimulation of beneficial bacteria such as *Lactobacillus* and inhibition of pathogenic bacteria. This study also states that despite the more attention given to green tea over black tea antioxidant health benefits, their overall effects are similar with the same low bioavailability. Interaction between tea phenolics and gut microbiota needs to be studied more thoroughly to get more evidence on the beneficial effects.

Bauer-Petrovska (2000) measured vitamin and mineral contents in kombucha beverage and established that kombucha beverage has high contents of vitamins B and C. This was confirmed by later studies for example Malbaša (2011) who measured high concentrations of vitamin C with values reaching almost 30 mg/L on the tenth day of the fermentation, with the continual increase on all tested starter cultures and substrates. He also noted a correlation between the highest antioxidative properties and high content of vitamin C in series of samples. Padayatty (2003) created review of multiple studies focusing on vitamin C and its exact role as antioxidant in non-enzymatic reactions and its possible involvement in prevention of human disease. He concluded that the only clinically proven function of vitamin C against disease is prevention of scurvy. He also states that some evidence suggests that vitamin C is powerful antioxidant *in vitro*, however he found no current clinical studies that support this claim. Njus (2020) published an article on the antioxidative properties of vitamin C. He states that vitamin C acts primarily as a donor of single hydrogen atoms and that the radical anion monodehydroascorbate reacts with radicals. These properties together explain the antioxidant capacity of vitamin C, as it makes it a free radical scavenger and terminator of free radical chain reactions. He also states that vitamin C can have pro-oxidant effects when it comes in contact with ions such as Fe^{3} Cu^{2+}.

Kombucha beverages and their capacity for antioxidative effects are the most studied activities in comparison to other biological effects it possesses. Most authors usually attribute these effects to phenolic compounds, however, there are no works isolating specific phenols. Only statistical parameters can be measured (total phenolic content and antioxidant activity), but no “single-molecule” activity can be assumed based on current research. Despite this, microbial fermentation can be an efficient process to increase antioxidant activity through the conversion of phenols to free phenolics releasing in the process compounds that result in higher antioxidative capacity. Most of the research is conducted *in vitro* and more clinical evidence needs to be found (Morales, 2020).

Fermentation time directly correlates with the free radical scavenging ability of kombucha beverages, increasing with the time of fermentation. The extent of this ability is also dependant on the type of tea and microbial culture and decides which metabolites will be present. Although the time of fermentation increases the antioxidant activities of kombucha, prolonged fermentations are not advised due to the accumulation of organic acids which can be detrimental for consumption. Metabolic pathways during fermentation are instrumental for the identification of metabolites and extracellular enzymes responsible for the free radical scavenging abilities of kombucha. Manipulation of metabolic processes may be one of the ways to enhance fermentation efficiency and increase the antioxidant potential of kombucha beverages (Greenwalt 2000; Jayabalan 2014).

To summarize, the antioxidative effect of kombucha beverages is mainly due to phenolic compounds present in tea. Although the presence and effects of these compounds are proven, the limiting factor deciding the actual effect on human health is bioavailability for the human organism, which is often poor. Most important factor regarding antioxidant activity is therefore the medium (tea/drink/culture) used for preparation of kombucha.The antioxidative effect of kombucha is generally agreed upon, although, there is no evidence to what extent it can affect human health, as there was no *in vivo* experiment regarding consumption and antioxidant activity in humans.

3.9.2 Probiotic and prebiotic effect of Kombucha beverage

Popular articles suggest that kombucha is a source of probiotics due to being a fermented beverage and having several probiotic bacteria present (Lewin, 2018). Kleinfield (2020) states that yeasts and bacteria from SCOBY can improve digestion, gut health, and immune function.

Panghal (2018) defines probiotics as live microorganisms that can when introduced in adequate quantities grant benefits to health beyond inherent general nutrition. These microorganisms promote various functions in human health such as improving lactose metabolism, prevention of intestinal diseases, overall immune system boost, synthesis of vitamins, counteracting effects of pathogens present in food, and improving digestibility.

Kozyrovska et al. (2012) states that kombucha beverages are a source of probiotic bacteria, yeasts, and prebiotics (microcellulose), which can serve as fuel for the growth of helpful bacteria in our gut. She also implies that kombucha provides postbiotics such as short-chain fatty acids and other metabolites which can boost immunity. She claims that the diversity of symbiotic microorganisms naturally occurring in kombucha beverages is advantageous for human health over single strain probiotics or artificial communities of beneficial microbial strains.

Dufresne (2000) states that beneficial health effects of kombucha attributed to its probiotic content are also referred to as products that are metabolites released into the broth during fermentation by probiotic microorganisms. Mainly the acid composition, for example, the detoxification aspect is attributed to glucuronic acid.

Felix et al. (2016) states that in kombucha beverages can be over 50 different varieties of probiotics. Probiotic mixtures usually contain strains of *Lactobacillus. Saccharomyces cerevisiae* can be also present (Kozyrovska, 2012; Watawana 2015). Marsh (2014) reported *Lactobacillus* and *Lactococcus* to be dominant species in SCOBY pellicles. Predominant yeast genera were *Zygosaccharomyces.*

Bogdan et al. (2018) isolated five strains of LAB in the kombucha consortium to prove their probiotic potential. All isolates were gram-positive bacteria with a coco-bacillar shape belonging to the lactobacilli group. 99% of bacteria present were identified as strains of *Pediococcus pentosaceus*. The isolates were later tested for bile salts tolerance and three of the five strains isolated showed to be tolerant to high concentrations of bile salts. He then proposed that bacteria strains producing bacteriocin and showing the ability to tolerate high bile salts concentrations may be potential probiotics to be used in the production of functional foods. These findings are in agreement with statements of Kozyrovska (2012), who describes interactions with human health (catabolism of cholesterol, promotion of various gut biological processes, etc.) and overall promotion of gut microbiome.

Many studies proved the healing effect, prophylactic properties, and the health benefit of LAB present in kombucha beverages as probiotics. Especially people working in unhealthy environments such as miners, submarine workers, etc. can use kombucha beverages to provide necessary nutrition to promote health and wellness (Kozyrovska, 2012; Watawana 2015).

It has also been proven to a certain extent that kombucha can positively affect gastrointestinal microbiota in the human gut by affecting the balance of microbiota and therefore influencing the ability to normalize intestinal activities. LAB recognized as probiotics have proven properties on human health. Most of the studies aimed specifically on kombucha beverage consumption were done *in vitro*, however, some done *in vivo* on rats, mice, etc. by feeding them kombucha suggest several positive effects such as immunological, intestinal, urogenital, and cardiovascular (Sengun, 2020).

It is proven that LAB and yeast regarded as probiotics are present in kombucha. Although kombucha shows promise as a probiotic beverage due to the high contents of bacteria and their metabolites, the extent of these effects on humans has not yet been defined in scientific study. It should be also noted that some of the kombucha beverages available on the market are pasteurized, which would entirely negate any probiotic bacteria if no supplements are added after pasteurization. However, metabolites of these bacteria such as organic acids are still present, and their beneficial effects can be still utilized.

3.9.3 Antimicrobial effect of kombucha

It is heavily suggested that kombucha beverages can have antimicrobial properties, health advice webs imply that kombucha can kill harmful bacteria. Most of these sources attribute the antimicrobial properties to high concentrations of acetic acid (Leech 2018; Metrus 2021; Manian, 2021).

Greenwalt (1998) tested kombucha beverage with acidic contents 33 g/L (7 g/L of acetic acid) and it had ability to inhibit bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella choleraesuis* serotype *Typhimurium*, *Bacillus cereus*, *Agrobacterium tumefaciens.* He concluded that these attributes are the consequence of the acetic acid content.

Battikh et al. (2013) compared the antifungal and antibacterial activity in both green and black kombucha beverages. In his research, both versions of kombucha showed antimicrobial and antifungal properties when compared to unfermented tea, which showed almost no antimicrobial activity except for some target microorganisms. Kombucha in this study showed the strongest inhibition of *Pseudomonas aeruginosa*, *epidermidis*, *Micrococcus luteus*, and *Listeria monocytogenes*. Green tea kombucha showed to be effective against all of these bacteria, however, black tea kombucha was selective against *Listeria monocytogenes* and *Pseudomonas aeruginosa*. These properties were strengthened when the medium was further acidified with acetic acid. He also found out that the behaviours of the same strains of bacteria in black and green tea were different. This observation proved that antibacterial activity in kombucha beverages is not exclusively dependent on acetic acid and other organic acids. He implies that other biologically active parts of kombucha may show antimicrobial properties, such as bacteriocins, proteins, enzymes, and phenolic compounds from tea.

Rajaei et al. (2021) in his study used kombucha cellulose disc on pathogenic bacteria isolated from diabetic foot ulcers. Most frequent and numerous bacteria that he isolated were *Escherichia coli, Enterobacter cloacae, Citrobacter diversus.* He used the agar disk diffusion method and found out that the cellulose layer of kombucha was an excellent antibiotic against infectious bacteria in the diabetic wound, and therefore concluded that it can be utilized in both medical and therapeutic use.

Shanmugavel et al. (2017) used the kombucha consortium for the biosynthesis of silver nanoparticles (AgNPs). He prepared 5 mM of $AgNO_3$ solution and then added the SCOBY tissue. The color change from pale yellow to dark brown indicated that kombucha culture formed AgNPs, which were later tested for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) by using the agar well diffusion method and showed to be effective against both. It also showed cytotoxicity towards human breast cancer cell lines (MCF-7).

El-Wakil et al. (2019) explained the antibacterial and absorptive properties of kombucha biofilms used in wound dressings that can absorb wound exudates. He infused kombucha cellulose grown in tea extract with coffee powder to explore the capacity of cellulose to load antimicrobial properties and then using these bio-composites in treating active wounds. Sharma (2020) expanded on this using different bio-extracts of various plants to infuse kombucha SCOBY and tested their microbial properties. Both of these studies (Shanmugavel et al. 2017; El-Wakil et al. 2019) are trying to better understand the formation and usage of antibacterial nanocellulose for medical applications.

Antimicrobial activity of kombucha beverages is well proven on a wide range of microorganisms ranging from yeasts to both Gram-positive and Gram-negative bacteria. Most of the studies attribute these properties to low levels of pH and the presence of acetic acid. Proteins, enzymes, and catechins which are produced during the fermentation processes can be also regarded as key factors in the improvements of antimicrobial activity (Mousavi, 2020).

Antimicrobial effects of kombucha are almost universally accepted and no relevant research disputing these properties of kombucha beverages was found for this review. Many studies in recent years focus on kombucha and more specifically SCOBY culture, highlighting its implementations in the medical industry.

3.9.4 Anticancer activity

Many personal observations and testimonies of kombucha drinkers claim the supposed anticancer properties of kombucha beverages based on the study in Russia by the “Russian Academy of Sciences in Moscow” in 1951. Using kombucha can effectively balance blood pH for cancer patients and supplement L-lactic acid for patients with low concentrations of such acid in their connective tissues. Other components such as ascorbic acid, glucuronic, acid lactic acid, and polyphenols are capable of diminishing stomach cancer. (Dufresne 2000).

Jayabalan et al. (2011) fractionated kombucha beverage with chloroform, butanol, and ethyl acetate. They studied the anti-invasive and cytotoxic properties of kombucha. This research demonstrated the activities of tea polyphenols on the inhibitory growth activities of kombucha beverages. The chemopreventive effects of tea polyphenols have been demonstrated on various animal models on cancers afflicting lung, colon, skin, mammary glands, and others, however, the anticancer activity of kombucha beverage has not been well described. Based on the results, it can be extrapolated that the presence of tea polyphenols in kombucha beverages is the reason why it can inhibit cancer cells and cause cancer cell apoptosis. This study proved that dimethyl-2-(2-hydroxy-2-methoxypropylidene) malonate and vitexin present in ethyl acetate part of kombucha beverages shows cytotoxic activities.

Srihari et al. (2013) studied anticancer activity of kombucha beverages and found out that it could be agent in prevention of growth of metastasis in prostate cancer. Kombucha extract in this experiment was lyophilized and it significantly decreased survival rate of prostate cancer cells. This happened due to regulating of angiogenesis by affecting the expressions of its stimulators.

Kaewkod et al. (2019) studied different types of tea in kombucha preparation and their ability to inhibit colorectal cancer cells. In his study, the kombucha beverages prepared from black and green tea showed toxicity on Caco-2 colorectal cancer cells. However toxic activities against cancer were attributed to the presence of various organic acids found in the beverage, mainly acetic acid.

Jayabalan et al. (2014) states that anticancer properties of tea polyphenols accepted by most researchers the ability to inhibit gene mutation, cancer cell proliferation, apoptosis of cancer cells, and termination of metastasis. These properties are mainly associated with kombucha beverages mainly to the presence of tea polyphenols and their oxidized products which are formed during fermentation. According to Mousavi (2020) can be kombucha regarded as an anticancer agent, however, more clinical experiments and investigations should be done to prove the significance of kombucha as an anticancer agent.

The anticancer properties of kombucha are mainly attributed to organic acids and phenolic compounds present in kombucha due to the supposed inhibition or apoptosis of cancer cells. However, no study proves kombucha as a significant factor in cancer prevention in humans.

3.9.5 Hepatoprotective effects

Hepatoprotection is the ability to prevent damage caused to the liver by various toxic substances. Various studies showed promising results on animal models and cell lines proving that kombucha broth has a hepatoprotective effect against different contaminants from the environment which can cause damage to the liver and hepatotoxicity. Several *in vivo* studies listed below showed the capability of kombucha beverages (Mousavi 2020; Jayabalan 2014).

Murugesan et al. (2009) demonstrated the hepatoprotective effect of kombucha on hepatotoxicity caused by carbon tetrachloride (CCl4). He used rats in this experiment and fed three different groups with black tea, kombucha, and black tea enriched with enzymes derived from tea fungus (laccase and cellulase) respectively. The experiment was conducted over 30 days and proved the effects of kombucha by reducing the levels of hepatic enzymes in plasma. He attributed these effects to the contents of glucuronic acid and polyphenols in kombucha.

Abshenas et al. (2012) experimented on the protective effects of kombucha on mice administered with acetaminophen. Acetaminophen administration negatively affected the activities of liver enzymes in mice. Further histopathological evaluation showed that affected mice had various liver damage such as severe glycogen storage in hepatocytes, necrosis, cellular degeneration, and dilation of veins. On the other hand, the second group of mice administered with kombucha had all of these effects significantly reduced. This was attributed to the hepatoprotective capability of kombucha to reduce oxidative damage from oxidative stress thanks to glucuronic and acetic acid contents. These acids can bond with toxins and therefore inducing detoxification processes in the body. The result of this study proved the protective effect of kombucha in the treatment of acetaminophen-induced liver toxicity in mice and further establishes that the antioxidant property of kombucha makes it viable for the prevention of other organ-specific toxic properties related to oxidative stress.

Jung et al. (2019) investigated the effects of kombucha beverages on mice with nonalcoholic fatty liver disease. Mice were introduced to a special diet for seven weeks to establish a deficiency of methionine and choline. Kombucha enhanced intracellular disposal of lipids. This study showed that kombucha protects hepatocytes by influencing the metabolism of lipids and therefore reducing their toxicity. It also decreased inflammation and fibrosis, which assisted in the liver restoration of mice affected with nonalcoholic fatty liver disease.

Martínez-Leal (2020) reviewed the hepatoprotective effect of kombucha in association with glucuronic acid and stated that no further studies associated with this acid are needed as the studies concerning its beneficial effect are mostly in agreement. However, no concrete evidence suggests that glucuronic acid is the main reason for the hepatoprotective properties of kombucha. There is an especial lack of evidence regarding different tea bases of kombucha beverage and the efficiency of their respective polyphenolic compounds.

Most studies agree that glucuronic acid and polyphenols present in kombucha beverages have positive properties on reducing liver toxicity due to antioxidative potential. *In vivo* studies on rats and mice show hepatoprotective potential, however, no study testing of these effects was conducted on humans. Clinical experiments are needed to prove these effects on human subjects.

3.9.6 Management of diabetes

Some research and online articles suggest that kombucha beverages exhibit hypoglycaemic and antilipidemic properties. Kombucha being functional food is widely used as a complementary treatment of type 2 diabetes. Kombucha shows antidiabetic activity through bioactive compounds which inhibit α-amylase activity and thus suppress blood glucose levels (Dufresne 2000, Jayabalan 2014).

Aloulou et al. (2012) supplied diabetic rats with kombucha and unfermented black tea for 30 days to compare their hypoglycemic and antilipidemic effects. The blood of these rats was then collected and measured for various properties. Their pancreases were processed to establish lipase and α-amylase activities. Results of this study revealed that kombucha beverage was better at suppressing blood glucose levels and at inhibiting α-amylase and lipase activities in both plasma and the pancreas. In conclusion, kombucha can be considered as a potential supplement for the treatment and prevention of diabetes.

Zubaidah et al. (2019) compared *in vivo* antidiabetic ability between snake fruit Kombucha, black tea kombucha, and metformin. He orally administered these substances daily to three groups of rats respectively over 28 days. He then investigated fasting plasma glucose and malondialdehyde levels, lipid profiles, and superoxide dismutase activities in the blood plasma. This experiment showed interesting results regarding fasting plasma glucose levels. Metformin is an antidiabetic drug that increases liver sensitivity to insulin and increasing glucose uptake in the peripheral tissues. Black tea kombucha showed worse performance than snake fruit kombucha on decreasing levels of fasting plasma glucose with the latter having comparable effects to metformin. These effects were attributed to the high contents of antioxidant compounds such as phenolics, tannins, and some organic acids which decrease plasma glucose level through increasing cellular glucose uptake. Antioxidant compounds can also inhibit glucose absorption in the small intestine which reduces amounts of glucose that enter the bloodstream. Regarding malondialdehyde levels and superoxide dismutase activities, both kombucha beverages were either comparable or even better than metformin in body oxidation indices. This experiment proved that kombucha-derived beverages can potentially substitute metformin as diabetes control agents, although extensive human studies are needed to confirm this.

All reviewed articles showed hypoglycaemic and antilipidemic properties of kombucha beverages. It can be extrapolated that consumption of kombucha beverages has great potential as complementary substances during diabetes treatment. However, all the *in vivo* research was conducted on either rats or mice. Research on humans and the impact on human health is needed to confirm these claims. It should also be noted that kombucha beverages are by no means treatment for diabetes.

3.9.7 Other beneficial effects

The vast majority of the literature exploring kombucha is concerned with the major effects listed in the chapters above. Examined literature sources mention these following effects only briefly and they are mostly explored only theoretically, *in vitro*, or very rarely *in vivo* on rats or mice. Furthermore, there is a severe lack of research to either prove or disprove these effects, therefore there is not enough evidence to evaluate their credibility. Also, there are many more effects attributed to kombucha, which are often extrapolated from individual components of kombucha beverages. Namely tea, vitamins, organic acids, bacteria, etc. These are not included, as there is no proof of direct connection to kombucha and its effects.

Boost of organism detoxification

Dufrense (2000) states that there are many beneficial effects of kombucha beverage derived from detoxification processes enabled through bacterial acids, enzymes and other secondary metabolites produced by various kombucha fermentations. He reported other benefits such as blood detoxification, cholesterol level reduction, blood pressure reduction, regulation of appetite, and many others.

Detoxication plays an important role in human health as it cleans the organism and promotes essential fat-soluble endobiotic metabolism. This is attributed to glucuronic acid contained in kombucha beverages and it is well known detoxicant, as it conjugates metabolites toxic to human body and modifies them into better soluble compounds. Utilization of kobucha beverages can therefore help prevent tissues from preventing toxins. (Vīna et al., 2014).

It should be noted that detoxification is continuous process occurring in any organism and despite it being listed as an effect of kombucha, tea or many other functional beverages in general. However, I have found no studies proving their direct involvement in this process.

Control of obesity and hypocholesterolemic effects

Yang et al. (2009) studied hypocholesterolemic effects on mice with high cholesterol diets fed for 12 weeks. He compared traditional kombucha beverages and modified kombucha (tea broth fermented with single *Gluconacetobacter* species). Hyper cholesterol diet consisted of chow enriched by cholesterol, lard oil, and chocolate. One control group was fed only by hyper cholesterol diet, while the other two were fed traditional and modified kombucha beverages respectively. Body weights were evaluated each week and food consumption was monitored. Animals were sacrificed at the end of the experiment to evaluate the weight of organs such as the liver, spleen, and kidney. Initial body weights did not differ among the groups however, at the end of the experiment group modified kombucha tea showed reduced body growth of 16%, and the group fed with traditional kombucha beverage with a reduction of 14,7% body weight. Kombucha-fed mice showed a slight decline in food intake. This restriction in food intake correlates with blood cholesterol levels and it is possible that kombucha affected cholesterol homeostasis by suppressing food intake.

Prevention of stomach ulceration

Banerjee et al. (2010) investigated the healing activity of unfermented black and black tea fermented with kombucha culture against stomach ulceration in mice induced with single-dose indomethacin. The extent of ulcer healing was observed from histological observations. Results of this study showed that treatments with black tea affect indomethacin-induced stomach ulceration. This activity was augmented with kombucha fermentation. Higher phenolics content in kombucha beverages and constituents of organic acids accounted for a superior healing effect when compared to black tea. These results establish the potential of kombucha beverages as anti-ulcerogenic agents.

Mediation of pulmonary diseases

Pasha et al. (2005) states that chronic pulmonary degenerative diseases often cause of lung cancers and different lung infections can be caused by growing intoxication and oxidative stress. The study of fermented black tea showed several physiologically active components such as significantly increased contents of theophylline. Theophylline is a xanthine derivative that causes relaxation of airway muscles. A cup of black tea contains only 0,014 mg of theophylline while fermented black tea showed contents of 1,44 mg per cup. The usual treatment dose varies between 0,18-1,0 g per day. Therefore, he concluded that daily fermented black tea consumption can help with pulmonary diseases.

To summarize, these other effects are often mentioned in literature and attributed to kombucha beverages, however, for this review I have found only a limited number of studies listed above, which focus directly on these mentioned effects in the context of applying specifically kombucha. I have also found no clinical studies supporting these claims on humans.

3.9.8 Adverse effects and toxicity of kombucha beverages

Most of the reviewed literature in this thesis focuses on the beneficial effects of Kombucha. However, if there were any proven adverse effects, they would be detrimental to the human health benefit provided by consuming this beverage. Therefore, I have decided to investigate and incorporate evidence regarding the negative influence of kombucha in this review.

Vijayaraghavan et al. (2000) carried out a study on few groups of rats where he fed them kombucha beverages for 90 days and monitored weekly various properties such as weight, food and water intake, and general behaviour in comparison to normally fed rats. There was no significant difference in growth ratio, histological evaluations did not show any signs of toxicity and all other evaluations were within clinical limits. The study, therefore, concluded that kombucha beverage has no toxic effect. Pauline et. al, (2001) did a similar experiment where they fed three groups of rats for 15 days with different doses of kombucha (normal dose, five and ten times the dose). Rats were then examined and while estimating biochemical and histological parameters no toxicity was revealed.

There is conflicting evidence regarding the adverse effects and toxicity of kombucha beverages. Most studies explore the beneficial effects of kombucha and its components. There have not been many reported cases where kombucha beverages showed strong adverse effects, however, some individuals were reported by various sources to experience food poisoning, lead poisoning, or allergic reactions (Centers for Disease Control and Prevention 1995; Sabouraud 2009). It needs to be noted that reported cases are often associated with very specific circumstances. Such as contamination due to unhygienic conditions during preparation; lead poisoning from long-term storage in a lead container; allergic reactions. All of these cases involved only a few individuals. No scientific research to date showed substantial evidence to confirm the toxicity of kombucha beverages.

3.9.9 Fungal biomass of tea fungus and its applications

In recent years, research regarding SCOBY culture shows promise in various medical and industrial applications, which can in the future become the major factors of utilizing kombucha consortium for human benefit, therefore, it needs to be mentioned in this assessment.

. The biofilm that is formed during kombucha fermentation was traditionally considered only as inoculum for later batches of kombucha. The latest research revealed that it can be used as valuable material based on different properties of SCOBY in different fields such as food supplement, textile and electronic industry, tissue engineering, medical applications, removal of metal ions. Many small-scale experiments are being conducted to explore different usages and applications of SCOBY (Laavanya, 2021).

Zhu et al. (2014) experimented on cellulose membranes of kombucha and found out that it has interesting applications in the tissue engineering field due to its biocompatibility. These membranes are mechanically and biologically stable which is one of the reasons they are considered for the preparation of nerve conduits. These conduits were created and then tested if they are biocompatible with nerve cells. In an experiment on rats, these conduits were molded into the according proportions and implanted into rats. Morphology and function of these cells were promising and even after 6 weeks there were no signs of rejection and no hematological or histologic negative effects were observed.

4 Conclusion

Various properties, chemical composition, and effects of kombucha beverage were reviewed to assess its benefits on human health. Most of the beneficial properties of kombucha beverages are attributed to high concentrations of phenolic substances and organic acids. Acetic and glucuronic acids are almost universally recognized by most studies as powerful antioxidants and inhibitors of undesirable microbial activity. These acids and lactic acid bacteria are also the main reasons for probiotic activity attributed to kombucha.

Tea polyphenols and derived phenolic compounds are linked to the vast majority of beneficial effects of kombucha beverages, including antioxidative effects, hepatoprotective effects, and chemoprotective effects. However, there is not enough concise evidence on the bioavailability of tea polyphenols for human organisms because different approaches in kombucha preparation such as substrate types, bio composition of SCOBY, and derived fermentation processes are direct consequences of metabolite composition of the final kombucha beverage. Although, it was proven that fermented tea such as kombucha has higher bioavailability when compared to unfermented tea as the fermentation processes and microbiota in kombucha modify tea polyphenols making them more easily available for humans.

Most of the available research concerning kombucha is focused on its possible beneficial properties and not many adverse effects are proven. Only notable exceptions are related to contamination and errors during preparation and only individual cases have been reported. Vast majority of reviewed studies is done *in vitro*.

Available data mostly agree on potential beneficial effects; however, no clinical studies prove these conclusions and the extent of mentioned effects on human health. Future studies on kombucha beverages should focus on clinical research to fully describe and confirm these claims.

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