

## Review

# Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects

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MS 99-256: Received 27 August 1999/Accepted 1 February 2000

## ABSTRACT

Kombucha is a slightly sweet, slightly acidic tea beverage consumed worldwide, but historically in China, Russia, and Germany. Kombucha is prepared by fermenting sweetened black tea preparations with a symbiotic culture of yeasts and bacteria. Potential health effects have created an increased interest in Kombucha. Yet, only a few research studies have shown that Kombucha has in vitro antimicrobial activity and enhances sleep and pain thresholds in rats. Furthermore, Kombucha consumption has proven to be harmful in several documented instances.

Kombucha is a traditional remedy prepared at home by fermenting sweetened black tea with a symbiotic culture of yeasts and bacteria. The product is a slightly sweet, carbonated, acidic tea beverage. It is consumed worldwide, but historically in China, Russia, and Germany. Popular media features in the United States have highlighted the beverage and its uses, suggesting that Kombucha consumption can reduce blood pressure, relieve arthritis, increase the immune response, and cure cancer. These questionable health effects have created an increased interest in Kombucha; however, these effects have not been proven scientifically.

Stadelmann reviewed the literature on Kombucha, the tea fungus, for the period from 1852 to 1961 that covered much of the European anecdotal-medical uses for the tea (32). However, greater knowledge and information is now surfacing as research is expanding parallel to the recent increase in consumption of the fermented tea.

## HISTORY OF KOMBUCHA

Kombucha has been consumed since 220 BC (32). During the Tsin Dynasty in Manchuria, the tea was sought for its suspected magical properties. As trade routes extended beyond the Far East, Kombucha traveled to Russia and Eastern Europe. The tea became very popular in Russia and was consumed as a treatment for metabolic diseases, hemorrhoids, and rheumatism. The majority of literature and information on the tea originated from Russian physicians citing medicinal uses. For example, it was noticed after World War II that the Kombucha-drinking regions of Russia had notably lower cancer rates than the nondrinking regions, despite industrial pollution and toxins present from the war (11).

During World War II, Kombucha consumption extend-

ed beyond Russia, to Western Europe and North Africa (5). European uses for the tea focused on the supposed detoxifying effects on the blood and digestive system. The fermented tea is commonly known as Kombucha in addition to many other names like, Tea Fungus, Kargasok Tea, Manchurian Mushroom, and Haipao. Current uses for the fermented tea span from consumption for better health to questionable therapeutic applications to cure chronic illnesses like cancer. However, Kombucha consumption has proven to be harmful for individuals with preexisting conditions or illness or if incorrectly prepared.

## KOMBUCHA PREPARATION

Sterile containers and utensils must be used during Kombucha preparation in order to prevent contamination from airborne molds or pathogenic organisms. The Kombucha recipe may vary; however, it is commonly made by infusing black tea leaves into freshly boiled water, sweetened with 50 to 150 g/liter (5 to 15%) sucrose, for about 10 min. After removing the tea leaves, the tea is allowed to cool to room temperature and the microbial mat/colony from a previous batch is added to the sweetened tea with about 100 ml of Kombucha from a previous fermentation. It is then covered with a clean cotton cloth and incubated at room temperature for about 7 to 10 days. If the fermentation is allowed to continue beyond 10 days, the acidity may rise to levels potentially harmful to consume. Next, the microbial colony is removed and the Kombucha beverage is ready. The recommended consumption ranges from 100 ml to 300 ml per day (6, 11). The final product is a slightly carbonated beverage comprised of organic acids, vitamins, minerals, and tea components, resembling the taste of cider.

## KOMBUCHA FERMENTATION SUBSTRATE

Sweetened, brewed tea is the substrate on which the Kombucha microorganisms thrive to produce the final prod-

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TABLE 1. *Composition of green and black tea (4)*

	Percentage of dry extract solids	
	Green tea	Black tea
Catechins	34.0	4.2
Theaflavins	—	1.8
Thearubingens	—	17.0
Flavonols	0.4	—
Flavonol glucosides	4.4	1.4
Protein	7.6	10.7
Amino acids	5.3	4.8
Caffeine	6.9	7.1
Carbohydrates	12.5	13.5
Organic acids	9.5	11.0

uct. The tea must be sweetened in order for the colony to grow and the fermentation to progress (31). The influence of different sweeteners on the metabolic activities of the tea fungus was observed by Reiss (29). Sucrose, lactose, glucose, and fructose tested and ethanol and lactic acid production by the Kombucha colony (tea fungus) were quantified for each sweetener and at varied concentrations. Sucrose proved to facilitate the fermentation the best with 50 g/liter of sucrose yielding the highest level of ethanol and lactic acid with the shortest fermentation time (29).

Reiss also grew the Kombucha colony in black tea, peppermint tea, lime blossom tea, beer, and cola and quantified ethanol, lactic acid, gluconic acid, and acetic acid (28). It was found that these metabolic products were low in the beer, cola, peppermint tea, and lime-blossom tea substrates, but present at 1.07 g/liter, 2.94 g/liter, 2.52 g/liter, and 0.08 g/liter, respectively, in black tea after a 14-day fermentation (28). As a result, the preferred substrate tested for the Kombucha colony proved to be black tea.

The caffeine content of an average cup of tea is about 40 mg (4). Caffeine and polyphenols have been shown to have antibiotic activity at high concentrations. The polyphenolic group that is most reactant during the enzymatic fermentation of fresh green leaves to black tea leaves are the catechins (4). Catechins were found to have bacteriostatic properties against *Streptococcus mutans* and other harmful organisms (26). As shown in Table 1, green tea has a much higher catechin content than black tea. As a result, green tea may have more antibiotic activity than black tea, at high concentrations (33). This is a result of processing differences between the two teas. Green tea is produced by heating the leaves right after picking to prevent any enzymatic oxidation, then dried (4). Leaves for black tea are allowed to oxidize with exposure to air and then dried to inactivate enzymatic reactions (4). The oxidation of the tea components gives black tea its deep color and unique flavor. Because of this flavor, black tea is more frequently used for Kombucha preparation (11). However, it has been shown that green tea has a more stimulating effect on the Kombucha fermentation than black tea, yielding the finished ferment in a shorter time frame (13).

## MICROBIOLOGY OF KOMBUCHA

**Microbial composition.** The fermented tea is produced by the action of a floating microbial mat/colony consisting of aerobic bacteria and yeasts. The colony's appearance often resembles a surface mold or a mushroom but is actually a floating cellulose mat produced during microbial growth. Production of the floating mat facilitates aeration for the aerobic microorganisms (3). With every batch of Kombucha a new film is formed, and this new film is the colony or mat that can be used to make subsequent batches of Kombucha.

Fontana et al. investigated cellulose biosynthesis stimulators naturally occurring in plant infusions (10). Caffeine and related compounds (theophylline and theobromine) were found to stimulate the ability of the bacteria to produce cellulose (10). Apparently, these methylxanthines inhibit the normal switch-off mechanism of cellulose synthase (10). As a result, oxygen availability to the colony is maximized in caffeinated tea (3). However, increased caffeine levels at 4 to 16 times the normal level of caffeine (40 mg) proved to inhibit the Kombucha fermentation, not stimulate it (13).

Acetic acid- and gluconic acid-producing bacteria are the prevailing prokaryotic species in the Kombucha culture (17). *Acetobacter xylinum* has been shown to be the primary bacterium in the mat (15, 17, 22, 23, 30, 31). *A. xylinum* produces acetic acid, gluconic acid, and cellulose from carbon sources like ethanol and glucose (1). *A. xylinum* is also the primary organism in Nata production and "Mother of Vinegar" (12).

The American Type Culture Collection contains the yeast isolates from the Mayser et al. study of the microbial content of a number of German household Kombucha colonies (23). This study demonstrated that the yeast composition of the colony is highly variable; but that, *Brettanomyces*, *Zygosaccharomyces*, and *Saccharomyces* occurred most frequently in the German household samples studied (23). Hesseltine reported the presence of two yeasts; *Pichia* and *Zygosaccharomyces* (NRRL Y-4810 and Y-4882) in Kombucha (15). Liu et al. isolated the yeasts *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Brettanomyces bruzellensis* from Taiwanese samples (22). Herrera and Calderon-Villagomez isolated *Brettanomyces intermedius*, *Candida famata*, *Pichia membranaefaciens*, *S. cerevisiae* subsp. *aceti*, *S. cerevisiae* subsp. *cerevisiae*, *Torulopsis delbrueckii*, *Z. bailii*, and *Zygosaccharomyces rouxii* from the Mexican tea fungus (14). *Saccharomyces*, *Torulopsis*, *Mycotorula*, *Schizosaccharomyces*, *Saccharomyces*, *Pichia*, *Torula*, *Mycoderma*, and *Candida* genera were included in Jankovic and Stojanovic's list of Kombucha yeasts (17). Roussin determined that the typical North American Kombucha yeasts were *Zygosaccharomyces*, and *S. cerevisiae* (30). From the above investigations, it is apparent that the yeast composition of the colony is variable. Table 2 summarizes the microbial composition of the colony.

In addition, Mayser et al. noted a low rate of contamination from spoilage and pathogenic microorganisms and

TABLE 2. *Kombucha* colony microbial composition

Microbe	Reference
<b>Bacteria</b>	
<i>Acetobacter xylinum</i>	19, 25, 30, 31
<i>Acetobacter aceti</i>	22
<i>Acetobacter pasteurianus</i>	22
<i>Gluconobacter</i>	22
<b>Yeasts</b>	
<i>Brettanomyces</i>	23
<i>Brettanomyces bruxellensis</i>	22
<i>Brettanomyces intermedius</i>	14
<i>Candida</i>	17
<i>Candida famata</i>	14
<i>Mycoderma</i>	17
<i>Mycotorula</i>	17
<i>Pichia</i>	15, 17
<i>Pichia membranaefaciens</i>	14
<i>Saccharomyces</i>	19, 23
<i>Saccharomyces cerevisiae</i> subsp. <i>aceti</i>	14
<i>Saccharomyces cerevisiae</i> subsp. <i>cerevisiae</i>	14, 22, 30
<i>Schizosaccharomyces</i>	17
<i>Torula</i>	17
<i>Torulaspora delbrueckii</i>	14
<i>Torulopsis</i>	17
<i>Zygosaccharomyces</i>	15, 23, 30, 31
<i>Zygosaccharomyces bailii</i>	14, 22
<i>Zygosaccharomyces rouzii</i>	14

concluded that Kombucha can safely be prepared at home without pathogenic health risk if correctly prepared (23). The product is relatively acidic (pH 2.5), which limits the ability of many other organisms, possible contaminants, to grow (22, 31). Roussin tested about 900 samples and never found the presence of the human pathogen *Candida albicans* (30). However, undesirable molds, *Aspergillus niger*, *Penicillium notatum*, and *Mucor* were visibly present in a few samples (30). The use of clean or sterile equipment, cooling the tea rapidly and quickly decreasing the pH by the addition of starter Kombucha to every batch, can reduce the risk of contamination from these and other deleterious organisms.

**The microbial symbiosis.** The distribution of the organisms in the cellulose mat is highly complex (2). Anken and Kappel used cytochrome oxidase activity to characterize the symbiotic relationship in the cellulose network (2). Their investigation demonstrated that the bacteria and yeasts of the Kombucha colony are arranged in bands and layers within the cellulose network (2). The scanning electron micrograph in Figure 1 shows the small *Acetobacter* rods next to *Saccharomyces* within the cellulose matrix (24).

Liu et al. studied the relationship of the Kombucha organisms and found that the yeasts' production of ethanol assisted the bacterial production of acetic acid (22). In addition, acetic acid production may further stimulate yeast production of ethanol (22). Finally, the simultaneous pro-

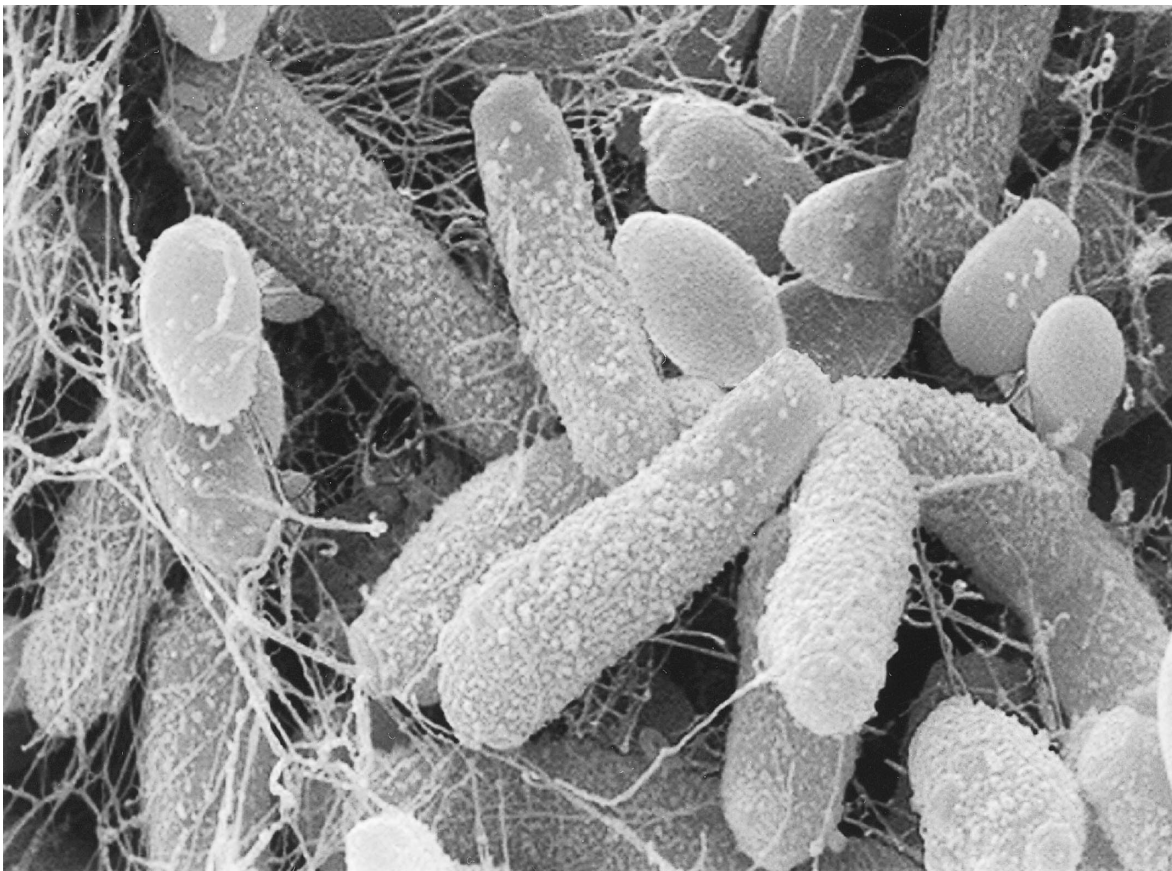
FIGURE 1. Scanning electron micrograph of a Kombucha Colony (24).  $\times 6,875$ .

TABLE 3. *Kombucha chemical composition*

Reference	Fermentation time, sweetener	Sucrose <sup>a</sup>	Glucose	Fructose	Gluconic acid	Ethanol	Acetic acid
31	10 days, 70 g/liter sucrose	18.2	28.8	16.4	2.8	3.6	2.1
	30 days, 70 g/liter sucrose	0	30.2	0.35	8.9	7	13.1
5	10 days, 70 g/liter sucrose	17	—	—	12	<1	3
	25 days, 70 g/liter sucrose	4	—	—	31	0	2
30	10 days, 8 oz sucrose (Allen)	—	—	25	3.1	—	2
	13 days, 8 oz sucrose (Laurel Farms)	—	—	15.03	6.64	—	8.61
	30 days, 8 oz sucrose (Laurel Farms)	—	—	17.04	7.21	—	3.4

<sup>a</sup> All concentrations in g/liter. The metabolites with dashes were not measured.

duction of ethanol and acetic acid prevents the competition of other microorganisms (22). This relationship illustrates the defined level of symbiosis and compatibility between the organisms in the tea colony.

**Microbial action in kombucha.** Carbon conversion of sucrose begins by the yeasts breaking down the sugar into glucose and fructose. Glucose is primarily used up by the yeasts to yield ethanol and carbon dioxide (31). The ethanol is then oxidized by the bacteria to acetaldehyde and then to acetic acid (31). As a result, the alcohol content of Kombucha is thought to never exceed 10 g/liter (35) and the acetic acid concentrations may rise to levels of high as 30 g/liter (3%) if the tea is allowed to ferment for up to 30 days (35). However, the usual concentration of acetic acid is below 10 g/liter (34). As a secondary biochemical activity of *Acetobacter*, glucose is utilized and converted to gluconic acid that is typically present in substantial quantities, about 20 g/liter (2%) (3, 31). Fructose remains part of the ferment broth and is utilized by the microorganisms to a lesser degree. As a result, the bacteria produce primarily acetic acid, gluconic acid, and cellulose (11).

#### CHEMICAL COMPOSITION OF KOMBUCHA

The most recent chemical analysis to date was Roussin's investigation. Roussin determined by high-performance liquid chromatography and mass spectrometry identification that fructose, acetic acid, and gluconic acid were the primary constituents of the tested ferments with fructose often the predominant component (30). However, Sievers et al. obtained higher levels of glucose than any other component (31).

Gluconic and acetic acid levels vary but generally are considered to be present in equal quantities in the fermented product (30). Ethyl-gluconate, oxalic acid, saccharic acid, keto-gluconic acids, succinic acid, and carbonic acids were other components typically found in the Kombucha samples tested by Roussin, usually in amounts less than 1 g/liter (30). These and all constituents in Kombucha have proven to vary (30).

Blanc characterized the metabolites of the Kombucha colony when prepared with different sucrose concentrations (0, 50 g/liter, 70 g/liter, 100 g/liter) and found that the level of metabolites present in the finished beverage was directly related to the amount of sweetener used (5). In addition,

lactic acid never reached concentrations higher than 0.6 g/liter and glucuronic acid was less than 10 mg/liter (5).

Roussin noted that the vitamin content of the fermented tea was not in sufficient concentrations to assist human health (30). In addition, the previously identified Kombucha component, glucuronic acid, was not present in any tested fermented samples (30). Roussin stated that gluconic acid derivatives were probably mistakenly identified as gluconic acid by other researchers because they have similar retention times during high-performance liquid chromatography (30). Therefore, glucuronic acid is not a typical component of Kombucha. Table 3 summarizes the primary components of Kombucha found by a number of investigators.

Additional Kombucha components may include tea components (shown in Table 1) and other minor microbial metabolites. Florenco determined that invertase, amylase, and other oxidative enzymes may be present in the tea as a result of the metabolic activities in the colony (9). Filho et al. determined the phenolic content of the tea fungus by high-performance liquid chromatographic methods (8). Orcinol, atranorin, orsellinic, slazinci, lecanoric, and fumarprotocetraric acids were found. These compounds are likely produced by the yeast in the colony.

#### EFFECTS OF KOMBUCHA

**Controversial health claims.** Insight on proven health benefits is useful because most of Kombucha's health claims are unsubstantiated scientifically. Stadelmann's compilation of Kombucha works included descriptions of medical practitioners' use of Kombucha (32). For example, Dr. Mollenda wrote that Kombucha proved itself effective at easing and promoting digestion (25). From either personal accounts or doctor-patient accounts, headaches, hemorrhoids, atherosclerosis, metabolic disorders, gout, arthritis, diabetes, sluggishness of the bowels, fatigue, stress, old age, and cancer were all documented to be cured by regular consumption of Kombucha (32). However, Professor Wiechowski, the Principal of the Pharmacological Institute of the German University in Prague, stated that Kombucha had not been pharmacologically proven effective and therefore should be considered a dietary, not therapeutic, aid (36).

**Researched effects.** Unfermented tea possesses proven beneficial health effects such as, anticarcinogenic, antitumorogenic, antimicrobial, antiatherogenic, antioxidant, and anti-inflammatory activity (33). It is important to note that these health effects are observed in populations that consume more than one cup of tea a day, usually four or more (33). Because less than one cup of Kombucha is typically consumed in a day, these types of health effects should not be associated with Kombucha consumption.

However, Steinkraus et al. showed that Kombucha has in vitro antimicrobial activity against *Helicobacter pylori* (primary cause of gastritis and peptic ulcer disease), *Escherichia coli*, *Staphylococcus aureus*, and *Agrobacterium tumefaciens* made with a low tea usage level (4.4 g/liter dry wt/vol) (34). This observed activity was attributed to the acetic acid content of Kombucha (34). Steinkraus et al. stated that they could not directly compare their results to other studies on the inhibitory activity of the tea, fermented and unfermented, because they used a substantially lower level of tea than the other researchers (34). De Silva and Saravanapavan discussed the tea cider prepared with 10 g/liter (1.0%) tea wt/vol (7). Hesseltine investigated the antibiotic activity of Kombucha prepared with 37 g/liter tea wt/vol (15). Using this undrinkable level of tea, Hesseltine reported that *Agrobacterium tumefaciens* was inhibited in the fermented tea and neutralized samples (15). Hesseltine, did not report the effect of the unfermented substrate, therefore it is unknown if the tea components contributed to the neutralized ferment's antibiotic activity.

Greenwalt et al. demonstrated that Kombucha containing 7 g/liter (0.7%) acetic acid had antimicrobial activity against *S. aureus*, *E. coli*, *Salmonella Cholerasius* serotype Typhimurium, *Bacillus cereus*, and *A. tumefaciens* in vitro (13). *C. albicans* was not inhibited by the tested Kombucha samples (13). Green and black tea preparations of various levels including the normal level of 4.4 g/liter (0.44%) dry tea and up to sixteen times that level at 70 g/liter (7.0%) dry tea were tested to compare the findings of other research studies. All organisms were inhibited similarly in the fermented tea samples. In addition, this activity was eliminated with neutralization and therefore may primarily be attributed to the acidity of the fermented tea. Very high levels (70 g/liter dry tea weight per volume) of tea did not affect the growth of the tested organisms and drinkable levels of unfermented tea (4.4 g/liter) possessed no observable antimicrobial effects (13).

As a result of this antimicrobial activity, it has been suggested that if used topically, Kombucha may aid in reducing infection in dermal injuries and burns. However, this has yet to be demonstrated in addition to potential benefits on health from this antimicrobial activity.

Kwanashie et al. (19–21) conducted a series of investigations on the health effects observed by consumption of Kombucha (also known as Kargasok tea) in rats. It was demonstrated that the tea reduced pain and assisted sleep of the rats given the beverage for two weeks (19–21). It was suggested that the observed effects may be attributed to the alcohol content of the tea, although the alcohol content was not noted or compared to a control. Furthermore,

TABLE 4. *Demonstrated effects of Kombucha*

Reference	Demonstrated effects
13, 34	Fermented tea demonstrated antimicrobial activity against <i>Escherichia coli</i> , <i>Helicobacter pylori</i> , <i>Staphylococcus aureus</i> , <i>Salmonella Cholerasius</i> serotype Typhimurium, <i>Bacillus cereus</i> , and <i>Agrobacterium tumefaciens</i> in vitro
19–21	Rat consumption of fermented tea for 2 weeks reduced pain and assisted sleep

these studies have not been validated in human tests. The discussed investigated effects of Kombucha are summarized in Table 4.

**Harmful effects of Kombucha.** Ibrahim et al. showed that there exists potential for harmful effects to occur in susceptible individuals as a result of Kombucha consumption (16). Internal lesions were observed on the organs of rats after 12 weeks of consumption (16). Mice given the same treatment had no ill effects. As a result of this study, it was concluded that the susceptibility to toxicity from Kombucha varies from species to species.

The possibility of toxic effects when Kombucha is consumed in large quantities became a concern after two incidents in the United States in 1995 (27). One individual died from perforations of the intestinal tract and severe acidosis (27). It was speculated that because they had recently increased their consumption threefold to 12 oz, that Kombucha was the cause (6). The surviving victim mentioned that they increased the length of the fermentation time from 7 days to 14 days, and they could hardly manage swallowing the very acidic tea but did anyway. It was later determined that the individuals had severe preexisting conditions that made them susceptible to acidosis (6). These two cases of illness were investigated to determine if Kombucha played a role in the development of metabolic acidosis or other toxic effects. It was concluded that Kombucha is not harmful at about 4 oz per day, however there are potential risks associated with excessive consumption or consumption by an individual with preexisting health problems (6).

The Emergency Medical Services published a warning of possible illness as a result of the high acidity or microbial contamination of Kombucha (27). It was recommended that sick or immunocompromised individuals should not drink Kombucha (27).

The final pH of fermented Kombucha is usually around 2.5. This is regarded as a high acid food by the food industry because most spoilage organisms cannot grow below a pH of 4.0 (18). However, mycotoxigenic molds may have the ability to grow in fermented tea. These molds and others are considered mycotoxigenic because of secondary metabolites produced that have toxic and carcinogenic effects (18). However, fermented Kombucha is considered safe from most pathogenic contaminants when prepared under sterile conditions.

## CONCLUSIONS

The Kombucha culture has been shown to be a symbiosis of *Acetobacter* and a variety of yeasts. The compo-

sition of the ferment has proven to vary with fructose, acetic acid, and gluconic acid, the typical primary components. The organic acids and alcohol produced by the culture have *in vitro* antimicrobial activity and improve sleep and reduce pain in rats, respectively.

When prepared under sterile conditions and allowed to ferment for 7 to 10 days, it may be safe to consume. However, there are no proven health benefits associated with its consumption, and some individuals may be sensitive to the high-acid beverage.

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