Comprehensive REVIEWS in Food Science and Food Safety

A Review on Kombucha Tea—Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus

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Abstract: Fermentation of sugared tea with a symbiotic culture of acetic acid bacteria and yeast (tea fungus) yields kombucha tea which is consumed worldwide for its refreshing and beneficial properties on human health. Important progress has been made in the past decade concerning research findings on kombucha tea and reports claiming that drinking kombucha can prevent various types of cancer and cardiovascular diseases, promote liver functions, and stimulate the immune system. Considering the widespread reports on kombucha, we recognized the need to review and update the research conducted in relation to kombucha tea, its products and tea fungus. Existing reports have suggested that the protective effects of kombucha tea are as good as those of black tea, however, more studies on kombucha tea and its composition are needed before final conclusions can be made.

Keywords: fermentation, tea, tea fungus, kombucha tea, *Medusomycess gisevii*

Introduction

Kombucha tea is a slightly sweet, slightly acidic refreshing beverage consumed worldwide. It is obtained from infusion of tea leaves by the fermentation of a symbiotic association of bacteria and yeasts forming "tea fungus" (Chen and Liu 2000). A floating cellulosic pellicle layer and the sour liquid broth are the 2 portions of kombucha tea (Figure 1). It tastes like sparkling apple cider and can be produced in the home by fermentation using mail order or locally available tea fungus. Though green tea can be used for kombucha preparation, black tea and white sugar are considered the finest substrates. Kombucha is the internationally used Germanized form of the Japanese name for this slightly fermented tea beverage. It was first used in East Asia for its healing benefits. Kombucha originated in northeast China (Manchuria) where it was prized during the Tsin Dynasty ("Ling Chi"), about 220 B.C., for its detoxifying and energizing properties. In 414 A.D., the physician Kombu brought the tea fungus to Japan and he used it to cure the digestive problems of the Emperor Inkyo. As trade routes expanded, kombucha (former trade name "Mo-Gu") found its way first into Russian (as Cainiigrib, Cainii kvass, Japonskigrib, Kambucha, Jsakvasska) and then into other eastern European areas, appearing in Germany (as Heldenpilz, Kombuchaschwamm) around the turn of the 20th century. During World War II, this beverage was again introduced into Germany, and in the 1950's it arrived in France and also in France-dominated North Africa where its consumption became quite popular. The habit of drinking fermented tea became acceptable throughout Europe until World War II which brought widespread shortages of the necessary tea leaves and sugar. In the postwar years, Italian society's passion for the beverage (called "Funkochinese") peaked in the 1950s. In the 1960s, science researchers in Switzerland reported that drinking kombucha was similarly beneficial as eating yogurt

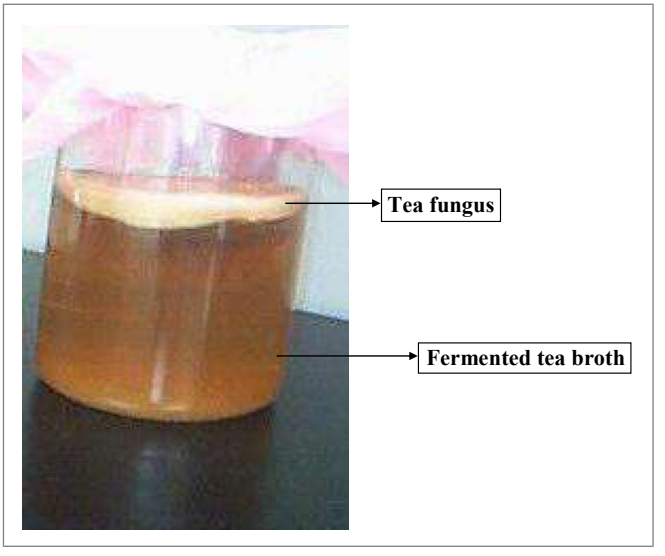

Figure 1–Kombucha black tea having fermented broth and tea fungus.

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and kombucha's popularity increased. Today, kombucha is sold worldwide in retail food stores in different flavors and kombucha culture is sold in several online shopping websites. A kombucha journal is electronically published by Gunther W. Frank and available worldwide in 30 languages (Dufresne and Farnworth 2000; Hartmann and others 2000).

Kombucha tea is prepared by placing the kombucha culture (tea fungus) into a sugared tea broth for fermentation. If the kombucha culture is cultivated according to the standard recipe with black tea, sweetened with sucrose, it turns this substrate into a refreshing beverage called tea fungus beverage with high nutritive value and medicinal properties (Lončar and others 2000). The popularity of kombucha expanded like many other traditional beverages due to its beneficial effects on human health and its ease in home preparation. The amounts of tea, sugar, and tea fungus differ in different places. The standard procedure is as follows: tap water (1 L) is boiled and during boiling 50 g sucrose is stirred in. Then 5 g tea leaves is added and removed by filtration after 5 min. After cooling to room temperature (20 ºC) the tea is inoculated with 24 g tea fungus (the culture) and poured into a beaker (1 L) previously sterilized with boiling water. The growth of undesirable microorganisms is inhibited by the addition of 0.2 L previously fermented kombucha, thus lowering the pH. The beaker is covered with a paper towel to keep insects, especially *Drosophila* fruit flies away. The incubation is carried out at 20 ºC to 22 ºC. The optimal temperature is in the wide range of 18 ºC and 26 ºC. In the next few days, the newly formed daughter culture will start to float and form a clear thin gel-like membrane across the available surface. This is the newly formed tea fungus available as a new layer above the old tea fungus which was inoculated to begin the fermentation. At this time, the tea will start to smell fermented and there will be gas bubbles appearing from the carbonic acid produced during the fermentation. The mother culture will remain at its original volume as it sinks to the bottom of the tea broth where it remains under the newly forming daughter culture. After 10 to 14 d, a new tea fungus will have developed on the surface of the tea as a disc of 2-cm thickness covering the whole diameter of the beaker. The newly formed tea fungus is removed with a spoon and kept in a small volume of fermented tea. The remaining beverage is filtered and stored in capped bottles at 4 ºC (Reiss 1994). The taste of the kombucha changes during fermentation from a pleasantly fruity sour-like sparkling flavor after a few days to a mild vinegar-like taste after a long incubation period. It is remarkable that 50 g sucrose/L provide the optimal concentrations of ethanol and lactic acid, and this sugar concentration has been used in traditional recipes for the preparation of "teakwass" (another name for kombucha) for a long time (Reiss 1994). An optimum fermentation time is required for the production of kombucha with pleasant flavor and taste. Longer fermentation produces high levels of acids (like mild vinegar) that may pose potential risks when consumed (Sreeramulu and others 2000).

Currently kombucha is alternately praised as "the ultimate health drink" or damned as "unsafe medicinal tea" (Blanc 1996; Hartmann and others 2000). There are many conception and misconception regarding the health benefits and toxicity of kombucha beverage. Though it is claimed to be beneficial for several medical ailments, very little or no clinical evidence is available for that. Studies on kombucha were reviewed earlier by Dufresne and Farnworth (2000), Yurkevich and Kutyshenko (2002), and Ernst (2003). Research on kombucha was highly boosted during the past decade, but there were no review reports published during this period. It encouraged us to collect the scientific studies reported on kombucha in the form of this review. The objective of this review was to investigate the microbiology, fermentation, composition, beneficial effects of kombucha beverage, and applications of tea fungus biomass based on the available literature.

Microorganisms of kombucha tea

Tea fungus or kombucha is the common name given to a symbiotic growth of acetic acid bacteria and osmophilic yeast species in a zoogleal mat which has to be cultured in sugared tea. According to Jarrell and others (2000), kombucha is a consortium of yeasts and bacteria. The formal botanical name *Medusomyces gisevii* was given to it by Lindau (Hesseltine 1965). Tea fungus is not a mushroom. That name is wrongly given due to the ability of bacteria to synthesize a floating cellulose network which appears like surface mold on the undisturbed, unshaken medium.

Similarly to milk-derived kefir, the exact microbial composition of kombucha cannot be given because it varies. It depends on the source of the inoculum for the tea fermentation. One of the clearer accounts of the microbes found in kombucha starter is from Hesseltine (1965). He isolated an *Acetobacter* sp. (NRRL B-2357) and 2 yeasts (NRRL YB-4810, NRRL YB-4882) from a kombucha sample received from Switzerland and used these microorganisms to produce kombucha tea.

The most abundant prokaryotes in this culture belong to the bacterial genera *Acetobacter* and *Gluconobacter*. The basic bacterium is *Acetobacter xylinum* (Danielova 1954; Konovalov and Semenova 1955; Sievers and others 1995; Roussin 1996). It produces a cellulosic floating network on the surface of the fermenting liquid. The network is the secondary metabolite of kombucha fermentation but also one of the unique features of the culture (Markov and others 2001). Sievers and others (1995) reported that the microflora embedded in the cellulose layer was a mixed culture of *A. xylinum* and a *Zygosaccharomyces* sp. The predominant acetic acid bacteria found in the tea fungus are *A. xylium*, *A. pasteurianus*, *A. aceti,* and *Gluconobacter oxydans* (Liu and others 1996). *Gluconacetobacter* sp. A4 (*G.* sp. A4), which has strong ability to produce D-saccharic acid-1,4-lactone (DSL), was the key functional bacterial species isolated from a preserved kombucha by Yang and others (2010). Strains of a new species in the genus *Acetobacter*, namely *Acetobacter. intermedius* sp. nov., were isolated from kombucha beverage and characterized by Boesch and others (1998). Dutta and Gachhui (2006, 2007) isolated the novel nitrogen-fixing *Acetobacter nitrogenifigens* sp. nov., and the nitrogen-fixing, cellulose-producing *Gluconacetobacter kombuchae* sp. nov., from kombucha tea. An investigation by Marsh and others (2014) indicated that the dominant bacteria in 5 kombucha samples (2 from Canada and one each from Ireland, the United States, and the United Kingdom) belong to *Gluconacetobacter* (over 85% in most samples) and *Lactobacillus* (up to 30%) species. *Acetobacter* was determined in very small number (lower than 2%).

In addition to acetic acid bacteria there are many yeast species in kombucha. A broad spectrum of yeasts has been reported including species of *Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Brettanomyces/Dekkera*, *Candida*, *Torulospora*, *Koleckera*, *Pichia*, *Mycotorula,* and *Mycoderma*. The yeasts of *Saccharomyces* species were identified as *Saccharomyces* sp. (Konovalov and others 1959; Kozaki and others 1972) and as *Saccharomyces cerevisiae* (Herrera and Calderon-Villagomez 1989; Liu and others 1996; Markov and others 2001; Safak and others 2002), *Saccharomyces bisporus* (Markov and others 2001), *Saccharomycoides ludwigii* (Reiss 1987; Markov and others 2001; Ramadani and Abulreesh 2010), *Schizosaccharomyces pombe* (Reiss 1987; Teoh

and others 2004), *Zygosaccharomyces* sp. (Sievers and others 1995; Markov and others 2001; Marsh and others 2014), *Zygosaccharomyces rouxii* (Herrera and Calderon-Villagomez 1989), and *Zygosaccharomyces bailii* (Herrera and Calderon-Villagomez 1989; Liu and others 1996; Jayabalan and others 2008b). The genus *Brettanomyces* was isolated by several workers. Herrera and Calderon-Villagomez (1989) isolated *Brettanomyces intermedius*, Liu and others (1996) and Teoh and others (2004) isolated *Brettanomyces bruxellensis,* and Jayabalan and others (2008b) isolated *B. claussenii*. An examination of 2 commercial kombucha and 32 cultures from private households in Germany (Mayser and others 1995) showed variable compositions of yeasts. The predominant yeasts were *Brettanomyces*, *Zygosaccharomyces*, and *Saccharomyces* spp. Roussin (1996) determined *Zygosaccharomyces* and *S. cerevisiae* as the typical yeasts in North American kombucha. Kurtzman and others (2001) isolated an ascosporogenous yeast, *Zygosaccharomyces kombuchaensis* sp. n. (type strain NRRL YB-4811, CBS 8849), from kombucha. An investigation of the physiology of *Z. kombuchaensis* sp. n., related to the spoilage yeasts *Zygosaccharomyces lentus,* clearly showed that these 2 species were not same (Steels and others 2002).

Candida sp. is included in a great number of kombucha beverages. Kozaki and others (1972) isolated *Candida famata*, *Candida guilliermondii,* and *Candida obutsa*. In kombucha samples from Mexico, Herrera and Calderon-Villagomez (1989) detected *C. famata*. Teoh and others (2004) identified *Candida stellata*. From a local kombucha in Saudi Arabia, Ramadani and Abulreesh (2010) isolated and identified 4 yeasts: *Candida guilliermondi*, *Candida colleculosa*, *Candida kefyr,* and *Candida krusei*. *C. krusei* were identified in kombucha from a district of Ankara (Turkey; Safak and others 2002).

The presence of the following was also established: *Torula* (Reiss 1987), *Torulopsis* (Konovalov and others 1959; Herrera and Calderon-Villagomez 1989; Markov and others 2001), *Torulaspora delbrueckii* (Teoh and others 2004), *Mycotorula* (Konovalov and others 1959), *Mycoderma* (Konovalov and others 1959; Reiss 1987), *Pichia* (Reiss 1987), *Pichia membranefaciens* (Kozaki and others 1972; Herrera and Calderon-Villagomez 1989), *Kloeckera apiculata* (Danielova 1954; Kozaki and others 1972; Safak and others 2002), and *Kluyveromyces africanus* (Safak and others 2002).

Chemical composition of kombucha tea

Chemical analysis of kombucha showed the presence of various organic acids, such as acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, usnic; also sugars, such as sucrose, glucose, and fructose; the vitamins B_1, B_2, B_6, B_{12}, and C; 14 amino acids, biogenic amines, purines, pigments, lipids, proteins, some hydrolytic enzymes, ethanol, antibiotically active matter, carbon dioxide, phenol, as well as some tea polyphenols, minerals, anions, DSL, as well as insufficiently known products of yeast and bacterial metabolites. The investigations of the beverage were always conducted under static conditions by the following: (Konovalov and Semenova 1955; Danielova 1957; Steiger and Steinegger 1957; Reiss 1987; Hauser 1990; Sievers and others 1995; Blanc 1996; Liu and others 1996; Roussin 1996; Petrović and others 1999; Bauer-Petrovska and Petrushevska-Tozi 2000; Chen and Liu 2000; Lončar and others 2000; Malbaša and others 2002a, 2008a, 2008b, 2011; Chu and Chen 2006; Franco and others 2006; Jayabalan and others 2007, 2008a; Kumar and others 2008; Wang and others 2010; Yang and others 2010; Yavari and others 2010, 2011; Velićanski and others 2013; Vitas and others 2013).

Yeasts and bacteria in kombucha are involved in such metabolic activities that utilize substrates by different and in complementary ways. Yeasts hydrolyze sucrose into glucose and fructose by invertase and produce ethanol via glycolysis, with a preference for fructose as a substrate. Acetic acid bacteria make use of glucose to produce gluconic acid and ethanol to produce acetic acid. The pH value of kombucha beverage decreases due to the production of organic acids during fermentation (Dufresne and Farnworth 2000).

The results presented in Table 1 indicate the predominant components of traditional kombucha beverage. These data suggest the heterogeneity of investigations performed on kombucha. The main differences in the investigated components are related to the duration of fermentation and the content of black tea. The researchers from different parts of the world (Taiwan—Chen and Liu 2000, Serbia—Lončar and others 2000, and India—Jayabalan and others 2007) used the same initial content of sucrose (10%). Researchers used different amounts of kombucha tea broth for the initial inoculation: 20% (Chen and Liu 2000), and 10% (Lončar and others 2000; Malbaša and others 2002a; Jayabalan and others 2007). The fermentation process was performed in small volume reactors (glass jar or beaker), up to 1 L. The measured values of components propose that applied parameters (fermentation temperature, fermentation time, and initial content of sucrose and black tea), as well as the composition of kombucha culture have impact on the metabolic activity of kombucha, and therefore, on the end products of the metabolism.

Acetic acid bacteria from kombucha produce acetic acid, as one of the main metabolites, when sucrose is used as a carbon source. Many authors determined the content of acetic acid in the beverage obtained after cultivation of kombucha on traditional substrate. Chen and Liu (2000) followed extended kombucha fermentation and determined the highest rate of 11 g/L after 30 d. The trend of acetic acid content was slow, increased with time, and then gradually decreased to 8 g/L, at the end of fermentation (60 d; Table 1). The same pattern was established by Jayabalan and others (2007) who monitored the fermentation until the 18th day on green tea (12 g/L) sweetened with 10% sucrose. The highest content was 9.5 g/L on the 15th day. Molasses was used in place of sucrose by Malbaša and others (2008a, 2008b). Kombucha fermentation on molasses produced only 50% of acetic acid in comparison with sucrose at the same stage of fermentation. This might be due to the poor growth of acetic acid bacteria on molasses.

Glucuronic and gluconic acids are also major organic acids that are produced as a result of the kombucha fermentation process on traditional substrate. Lončar and others (2000) determined the glucuronic acid after kombucha fermentation on sweetened black tea. The highest amount was measured after 7, and 21 d (0.0034 g/L; Table 1). Jayabalan and others (2007) established the maximum value of 2.33 g/L D-glucuronic acid after 12 d of fermentation. Chen and Liu (2000) determined that gluconic acid was not produced until the 6th day of fermentation. The ending concentration amounted the about 39 g/L after 60 d (Table 1).

Yavari and others (2010) cultivated kombucha on sour cherry juice sweetened with 0.6%, 0.8%, and 1% sucrose. Glucuronic acid was produced in very large amounts of 132.5 g/L which was determined on the 14th day of fermentation, in substrate with 0.8% sucrose. The fermentation process was conducted at 37 °C. Yavari and others (2011) used response surface methodology (RSM) to predict the value of glucuronic acid content in kombucha beverage obtained after fermentation on grape juice sweetened with

Table 1–Predominant components in kombucha tea at the end of the fermentation on sugared black tea infusion.

Component	Component content (g/L)	Initial sucrose (%)	Black tea	Fermentation temperature (°C)	Fermentation time (d)	Reference
Acetic acid	8	10	2 bags	24 ± 3	60	Chen and Liu (2000)
	4.69	10	12 g/L	24 ± 3	18	Jayabalan and others (2007)
Glucuronic acid	0.0031	5	1.5 g/L	28	21	Lončar and others (2000)
	0.0026	7	1.5 g/L	28	21	Lončar and others (2000)
	0.0034	10	1.5 g/L	28	21	Lončar and others (2000)
	1.71	10	12 g/L	24 ± 3	18	Jayabalan and others (2007)
Gluconic acid	39	10	2 bags	24 ± 3	60	Chen and Liu (2000)
Glucose	179.5	7	1.5 g/L	28	21	Malbaša and others (2002a)
	24.59	7	1.5 g/L	28	21	Lončar and others (2000)
	12	10	2 bags	24 ± 3	60	Chen and Liu (2000)
Fructose	76.9	7	1.5 g/L	28	21	Malbaša and others (2002a)
	5.40	7	1.5 g/L	28	21	Lončar and others (2000)
	55	10	2 bags	24 ± 3	60	Chen and Liu (2000)
Remained sucrose	192.8	7	1.5 g/L	28	21	Malbaša and others (2002a)
	11	10	2 bags	24 ± 3	60	Chen and Liu (2000)
	2.09	7	1.5 g/L	28	21	Lončar and others (2000)

0.7% sucrose, and the highest value was achieved after 14 d of fermentation at 37 °C. Franco and others (2006) established the presence of glucuronic (0.07 to 9.63 g/L) and gluconic (0.04 to 1.16 g/L) acids in a product obtained after kombucha cultivation on black tea sweetened with glucose (0.062% to 1.51%). Yang and others (2010) also determined the presence of gluconic acid and 2-keto gluconic acid, after cultivation of *Gluconacetobacter* sp. A4 isolated from kombucha and a strain of lactic acid bacteria, on 5 g/L black tea sweetened with 10% glucose.

L-lactic acid is not a characteristic compound for traditional kombucha beverage, but it is detected and determined. Jayabalan and others (2007) examined kombucha prepared with green tea to have a higher concentration of lactic acid than kombucha prepared from black tea and tea waste material. The maximum value of 0.54 g/L was established on the 3rd day. Malbaša and others (2008a, 2008b) measured the content of L-lactic acid after kombucha fermentation on molasses and established that it is a metabolic product present in large amounts. The presence of L-lactic acid after kombucha fermentation on molasses can be correlated to the L-lactic content of molasses itself which can be produced as a result of degradation of invert sugar in molasses. Molasses also contains amino nitrogen and biotin, which affect the intensity of kombucha fermentation.

Citric acid is also not a characteristic metabolic product of the traditional beverage. Malbaša and others (2011) determined an average value of 25 g/L citric acid in the total acids (substrate with 1.5 g/L of black tea and 7% sucrose), and Jayabalan and others (2007) measured it only on the 3rd day of fermentation, 0.03 and 0.11 g/L, in kombucha prepared with green and black tea, respectively.

Sucrose is the most common carbon source in kombucha fermentation. Its considerable amount stays largely unfermented during the process (Malbaša and others 2002a). Investigations showed that 34.06% of sucrose stays unfermented after 7 d, and after 21 d this value is 19.28% (Table 1). Chen and Liu (2000) determined that the content of sucrose linearly decreased during the first 30 d, followed by a slow-rate decline. Malbaša and others (2008b) established that utilization of 7% sucrose from molasses reached 97%, after 14 d of fermentation. The decline of sucrose concentration is more pronounced when the concentration of sucrose in molasses is optimal (7%), compared to the systems with pure sucrose. Utilization in the samples with molasses is slow when the content of sucrose is lower (Malbaša and others 2008a, 2008b). Yavari and others (2010) concluded that sucrose utilization, after the 4th day, began to speed up and this trend continued until the 14th day when the lowest sucrose content (2.1 g/L) was determined.

Malbaša and others (2002a) measured the contents of D-glucose and D-fructose in traditional kombucha and the highest values were 19.60 (on 14th day) and 10.25% (on 10th day), respectively. Lončar and others (2000) concluded that sucrose, glucose, and fructose were not utilized entirely after 21 d of fermentation and confirmed that fructose was metabolized before glucose. Chen and Liu (2000) established that glucose was not produced analogous to fructose (0.085%/d) but in lower amount (0.041%/d). The beverage, obtained on Jerusalem artichoke tuber extract, contained sugars in lower amount in comparison to sucrose substrate, except for D-fructose (10.41% on 5th day). In addition to sucrose and D-glucose, the presence of inulo-oligosaccharides were also determined (Malbaša and others 2002a).

Bauer-Petrovska and Petrushevska-Tozi (2000) quantified water-soluble vitamins in kombucha made with 0.7% sucrose and 5 g/L black tea. The values were as follows: vitamin B_1 74 mg/100 mL, vitamin B_6 52 mg/100 mL, vitamin B_{12} 84 mg/100 mL, and vitamin C 151 mg/100 mL. Malbaša and others (2011) measured the maximum content of vitamin B_2 in samples obtained with native kombucha (10th day, 7% sucrose and 1.5 g/L), on black (8.30 mg/100 mL) and green (9.60 mg/100 mL) tea. In that investigation, the content of vitamin C increased constantly in all obtained products and reached the highest value of 28.98 mg/L on 10th day in beverage produced with combination of acetic acid bacteria and *S. cerevisiae* isolated from native kombucha. This value was slightly lower (27.86 mg/L) in traditional product at the same stage of fermentation (Malbaša and others 2011). Vitamin C was also quantified in an investigation by Vitas and others (2013) by RSM methodology in the fermented milk products obtained by kombucha previously cultivated on winter savory (30 mg/L) and stinging nettle extract (45 mg/L). RSM methodology predicted values of vitamin C that are much higher in comparison to values obtained for traditional kombucha products, obtained after 7-d long fermentation period (15.19 mg/L) when the beverage is usually consumed.

The contents of manganese, iron, nickel, copper, zinc, lead, cobalt, chromium, and cadmium in the usual kombucha were determined by Bauer-Petrovska and Petrushevska-Tozi (2000). The contents of the examined minerals were in range from 0.004 μg/mL for cobalt to 0.462 μg/mL for manganese. Determination of toxic elements indicated the following values: 0.005 μg/mL for lead, 0.001 μg/mL for chromium, whereas

cadmium was not detected. It was concluded that essential minerals (Cu, Fe, Mn, Ni, and Zn) increased as a result of the metabolic activity of kombucha. The cobalt content did not increase, possibly because of its inclusion in vitamin B_{12} (Bauer-Petrovska and Petrushevska-Tozi 2000). Kumar and others (2008) established the presence of fluoride, chloride, bromide, iodide, nitrate, phosphate and sulfate in beverage with 10% sucrose and 5 g/L of black tea, after 7 d, and the highest measured value was 3.20 mg/g, for fluoride. The anionic mineral composition of kombucha and black tea was considerably different.

Chu and Chen (2006) examined a traditional beverage (4 g/L of black tea, 10% sucrose, 15 d of long fermentation period) and established that total phenol content of all kombucha samples showed a linear increase during fermentation time. Jayabalan and others (2008a) also established a highly pronounced increase of the total phenol content in all samples. Chu and Chen (2006) proved that the content was up to 7.8 mM gallic acid equivalent (GAE; 15th day of fermentation) and only around 4 mM GAE for black tea. Jayabalan and others (2007) investigated epicatechin isomers EGCG ([-]-epigallocatechin-3-gallate), EGC ([-]-epigallocatechin), ECG (-]-epicatechin-3-gallate), and EC ([-]-epicatechin) and demonstrated changeable stability during the fermentation process. Degradation of EGCG and ECG was reduced in the substrate with green tea when compared to substrates with black tea and tea waste material. Consistent degradation was observed for theaflavin and thearubigins. The highest value was measured for EC on 12th day in kombucha with green tea (around 150%), and for EGC, on the same day, in kombucha with tea waste material (around 140%) and black tea (around 115%). It is assumed that EGCG and ECG were converted to their corresponding catechin EGC and EC. The color of kombucha broth was lighter in comparison to the color of black tea and this suggested that polyphenols did undergo microbial change in the acidic environment by the enzymes liberated by bacteria and yeast (Jayabalan and others 2007).

Wang and others (2010) measured the content of DSL in kombucha, and it was in the range from 57.99 (sample from household) to 132.72 μg/mL (sample from laboratory). Yang and others (2010) established the increase of DSL content during the 8 d, when the highest value was reached followed by decrease in DSL till the end of fermentation. They concluded that lactic acid bacteria have a positive effect on DSL production, in symbiosis with *Gluconacetobacter* sp. A4. The optimum medium conditions for fermentation were glucose (10%) and black tea (5 g/L).

Chen and Liu (2000) established that the content of ethanol increased with time and reached the highest value at around 5.5 g/L, followed by a slow decline. The same pattern was observed by Reiss (1994) who concluded that ethanol production increased to a maximum on the 6th day of fermentation, with a subsequent decrease.

Jayabalan and others (2007) indicated that the protein content increased with fermentation time, in the range of 0.1 to 3.0 mg/mL, during 12 d of fermentation, in all samples. Afterwards, it continued to decrease because of yeast and bacterial extracellular protein decreases.

The composition of kombucha beverage indicates the presence of numerous compounds and it depends on cultivation substrate, time and temperature of fermentation process, as well as the microorganisms present in the culture, but also on the applied method of analysis.

Fermentation of kombucha on substrates other than tea

Traditional substrate for the kombucha fermentation is black or green tea extract sweetened with 5% to 8% sucrose. Besides traditional substrates, the possibility of use of alternative substrates has been established in various studies. Malbaša (2004) reviewed some attempts in applying nontraditional substrates for the kombucha fermentation such as Coca-Cola, red wine, white wine, vinegar, extract of Jerusalem artichoke, milk, fresh sweet whey, reconstituted sweet whey, acid whey, Echinacea, Mentha, and more.

Jayabalan and others (2007, 2008a) revealed the possibility of using tea waste material for manufacturing kombucha beverage with satisfying quality. Studies of some alternative cultivation medium have shown that green tea and lemon balm tea have more stimulating effect on the kombucha fermentation than black tea, thus providing the fermentation product in a shorter time (Greenwalt and others 1998; Velićanski and others 2007). Talawat and others (2006) prepared kombucha beverage from mulberry tea, Japanese green tea, jasmine tea, and oolong tea. Velićanski and others (2013) cultivated kombucha on sage, thyme, and peppermint teas. Some scientists attempted the kombucha fermentation on sweetened sour cherry juice (Yavari and others 2010).

A possible substrate for the kombucha fermentation is Jerusalem artichoke tuber extract which has been reported in several articles. It was found that kombucha beverage obtained on the Jerusalem artichoke tuber substrate could be appropriate as dietetic product, because of the low D-glucose and D-fructose contents, and also because of the presence of inulo-oligosaccharides which act as dietetic fibers and are expected to increase the population of resident bifidobacteria in the human intestinal flora (Malbaša and others 2002a; Lončar and others 2007).

The fact that fermentative liquids with Jerusalem artichoke tuber extracts contain almost the same metabolites as the beverage with sucrose, plus additional ingredients like fructooligosaccharides and inulin, which are prebiotics, contributes to the quality of the final product. Kombucha metabolism is more intensive on a substrate with Jerusalem artichoke tuber extract, with the same applied culture of microorganisms. Specifically, contents of L-lactic, L-ascorbic, and total organic acids are significantly higher (Malbaša and others 2002b).

Some investigations with molasses as a substrate for the kombucha fermentation have also been conducted. Molasses from sugar beet processing is attractive because of its low price and the presence of a number of components, including minerals, organic compounds, and vitamins, which are very useful for the fermentation process (Rodrigues and others 2006). The first results on the metabolic activity of kombucha on sugar beet molasses were published in 2001 (Lončar and others 2001). The next investigation (Malbaša and others 2008a) additionally confirmed that the molasses from sugar beet processing can be used as a low-cost carbon source in kombucha fermentation of black tea. The products obtained on these substrates were rich in lactic acid, which may be considered as an advantage compared to the product on sucrose. The content of lactic acid is related to the higher quantity of invert sugar, biotin, and amino nitrogen in the molasses (Malbaša and others 2008b). The chemical composition of the substrate with molasses is considerably richer, in comparison to the substrate with pure sucrose, but it was proved that 7% sucrose from molasses corresponded to an optimal concentration, which produced low levels of less desired acetic acid and high levels of physiologically important L-lactic acid.

Reiss (1994) proved the possibility of application of lactose as a source of carbon for the kombucha fermentation. There were also a few investigations related to kombucha fermentation on substrates containing lactose. Belloso Morales and Hernández-Sánchez (2003) successfully cultivated kombucha on cheese whey. Malbaša and others (2009) proved that fermented beverages can be produced by kombucha fermentation on cow milk. The metabolic activity of kombucha starters on milk was significantly different from the activity on sucrose. Even the texture and taste of the products obtained were similar to yogurt; the chemical compositions of the new beverages differed significantly from the composition of yogurt. The investigations of Vitas and others (2013) proved that the fermented milk beverages can be successfully produced by application of kombucha obtained by cultivation on sweetened stinging nettle and winter savory extracts.

Beneficial effects of kombucha tea

Kombucha tea has been claimed by kombucha drinkers all over the world to have many beneficial effects on human health. However, most of the benefits were studied in experimental models only and there is a lack of scientific evidence based on human models. Nonhuman studies regarding antimicrobial, antioxidant, hepatoprotective, and anticancer properties of kombucha tea have seen carried out and biological activities are reported in Table 2.

Reported effects of kombucha from tea drinkers' testimony and Russian researchers (Dufresne and Farnworth 2000):

Detoxify the blood
Reduce cholesterol level
Reduce atherosclerosis by regeneration of cell walls
Reduce blood pressure
Reduce inflammatory problems
Alleviate arthritis, rheumatism, and gout symptoms
Promote liver functions
Normalize intestinal activity, balance intestinal flora, cure hemorrhoids
Reduce obesity and regulate appetite
Prevent/heal bladder infection and reduce kidney calcification
Stimulate glandular systems
Protect against diabetes
Increase body resistance to cancer
Have an antibiotic effect against bacteria, viruses, and yeasts
Enhance the immune system and stimulate interferon production
Relieve bronchitis and asthma
Reduce menstrual disorders and menopausal hot flashes
Improve hair, skin, and nail health
Reduce an alcoholic's craving for alcohol
Reduce stress and nervous disturbances, and insomnia
Relieve headaches
Improve eyesight
Counteract aging
Enhance general metabolism

Kombucha tea as an antimicrobial source

Kombucha tea has been studied by many researchers for its inhibitory activity on many pathogenic microorganisms. Tea containing 4.36 g of dry tea per liter and 10% sucrose and fermented with tea fungus showed no antibiotic activity in the beverage beyond that caused by acetic acid, a primary product of the fermentation (Steinkraus and others 1996). Kombucha tea containing 33 g/L total acid (7 g/L acetic acid) had antimicrobial efficacy against *Agrobacterium tumefaciens*, *Bacillus cereus*, *Salmonella choleraesuis serotype Typhimurium*, *Staphylococcus aureus*, and *Escherichia coli,* but not for *Candida albicans* (Greenwalt and others 1998). Kombucha tea could inhibit the growth of the pathogens *Entamoeba cloacae*, *Pseudomonas aeruginosa*, *B. cereus*, *E. coli*, *Aeromonas hydrophila*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella sonnei*, *Staphylococcus epidermis*, *Leuconostoc monocytogenes*, *Yersinia enterocolitica*, *S. aureus*, *Campylobacter jejuni*, *Helicobacter pylori,* and *C. albicans* (Sreeramulu and others 2000, 2001). Kombucha tea prepared from different substrates like mulberry tea, Japanese green, jasmine tea, oolong tea, and black tea was tested on pathogenic bacteria of humans and shrimp. Results revealed that black tea kombucha possessed the greatest inhibitory activity and *Vibrio parahaemolytica* showed the highest susceptibility to the fermented tea (Talawat and others 2006). Battikh and others (2012) reported that kombucha prepared from both black tea and green tea had antimicrobial potential against the tested human pathogenic microorganisms, except *C. krusei*, and kombucha green tea exhibited the highest antimicrobial potential. Afsharmanesh and Sadaghi (2013) reported that the body weight, feed intake, and protein digestibility of broiler chickens fed with a diet having 1.2 g/kg kombucha tea (20% concentration) were significantly increased compared to the control and green tea-fed broilers. They suggested that kombucha tea can be an alternative to antibiotic growth promoters in the diets of broilers.

Research on kombucha has demonstrated its antimicrobial efficacy against pathogenic microorganisms of both Gram-positive and Gram-negative origin. Antimicrobial activity of kombucha tea is largely attributable to the presence of organic acids, particularly acetic acid, large proteins, and catechins. Acetic acid and catechins are known to inhibit a number of Gram-positive and Gram-negative microorganisms (Sreeramulu and others 2000).

Kombucha tea as an antioxidant source

There has been a global trend toward the use of phytochemicals present in natural resources as antioxidants and functional foods. Bioactive molecules of natural resources are being utilized in the food industry, and there is evidence that these molecules can act as antioxidants within the human body. Antioxidant activity of Kombucha is correlated with its many claimed beneficial effects like cancer prevention, immunity enhancement, and alleviation of inflammation and arthritis. Jayabalan and others (2008a) reported on the free radical scavenging abilities of kombucha tea prepared from green tea, black tea, and tea waste material. They have shown that total phenolic compounds, scavenging activity on DPPH radical, superoxide radical, and inhibitory activity against hydroxyl radical-mediated linoleic acid were increased with an increase in fermentation time, whereas reducing power, hydroxyl radical scavenging ability (ascorbic acid-iron EDTA), and antilipid peroxidation ability were decreased. Malbaša and others (2011) studied the influence of 3 starter cultures (mixed culture of acetic bacteria and *Zygosaccharomyces* sp., mixed culture of acetic bacteria and *S. cerevisiae*, and native local kombucha) on the antioxidant activities of green tea and black tea kombucha beverage to hydroxyl and DPPH radicals. They observed the highest antioxidant activity with native kombucha on green tea beverage and acetic acid bacteria with *Zygosaccharomyces* sp. culture on black tea beverage. The antioxidant property of kombucha tea was tested against tertiary butyl hydroperoxide (TBHP)-induced cytotoxicity using murine hepatocytes and showed that kombucha tea neutralized the TBHP-induced changes and prevented cell death. These counter effects were also shown by the unfermented black tea, but the

Table 2–Various biological activities of kombucha tea reported through studies with experimental animals and cell lines.

Biological activity	Experimental animal/cells	Treatment period/dose	Parameters studied	Reference
Hypoglycemic activity	Mice	3 d and 1.71 mL/kg body weight	Blood sugar level	Shenoy (2000)
Antioxidative stress against chromate	Rat	30 d and 0.6 mL/200 g body weight	Plasma and tissue MDA levels, delayed type hypersensitivity response, GSH, peroxidase, catalase	Sai Ram and others (2000)
Longevity	Mice	3 y and free access	Longevity, general health, and open-field exploratory behavioral outcomes	Hartmann and others (2000)
Antistress activity against cold and hypoxia	Rat	15 d and 1.6, 8.0, and 16 mL/kg body weight	Plasma/blood MDA and reduced GSH, fecal output	Pauline and others (2001)
Antioxidative stress against lead	Rat	45 d and 1 mL/kg body weight	Lipid peroxidation, creatine phosphokinase, GSH, SOD, GPx, DNA fragmentation in liver	Dipti and others (2003)
Prevention of weight loss in diabetics	Rats	15 d and different dilutions of kombucha tea (25%, 50%, 75%, and 100%) in place of water	Weight loss	Morshedi and others (2006)
Prevention of postoperative intraabdominal adhesion formation	Rats	14 d and 15 mL/kg of body weight	Adhesion intensity score, inflammatory cell reaction, number of adhesion bands	Maghsoudi and Mohammadi (2009)
Protection on chromosomal aberrations induced by γ-radiation	Human peripheral lymphocytes	250, 500, and 1000 μL doses	Chromosomal aberrations, mitotic index	Cavusoglu and Guler (2010)
Protection on nephrotoxicity induced by trichloroethylene	Rat	2 wk and 0.1 mL/100 g body weight	Lipid peroxidation, oxidative stress	Gharib (2009)
Hypocholesterolemic effect	Rat	12 wk and 66 mL/kg body weight	Total cholesterol, low-density and high-density lipoprotein cholesterol	Yang and others (2009)
Healing property on indomethancin-induced gastric ulceration	Mice	7 d and 15 mg/kg body weight	Histopathological and biochemical studies	Banerjee and others (2011)
Protection on phenol-induced cytotoxicity	Mice	20 d and 1 mL/kg body weight	Micronuclei formation	Yapar and others (2010)
Protection on mitomycin C-induced genotoxic effect	Chinese hamster cell line CHO-K1	1 h and 0.295, 1.185, 4.75 μg/mL (dry weight)	Chromosome aberrations frequency	Četojevic-Simin and others (2012)
Hypoglycemic and antilipidemic properties against alloxan	Diabetic rats	30 d and 5 mL/kg body weight	α-amylase and lipase in plasma, pancreas, and blood glucose	Aloulou and others (2012)
Cytogenic activity	Human peripheral blood lymphocytes	1 h and 40 μg/mL	Frequencies of sister chromatic exchange and micronuclei formation	Mrđanović and others (2007)
Protective effects against oxidative stress-mediated damages in alloxan-induced diabetic rats	Swiss albino male rats	14 d and 150 mg lyophilized powder of kombucha tea/kg body weight	Blood glucose, glycated hemoglobin, lipid peroxidation end products, protein carbonyl content, glutathione content, antioxidant enzyme activities	Bhattacharya and others (2013)
Amelioration of changes in trace element levels in electromagnetic field-exposed rats (950 MHz)	Male Wistar rats	9 wk and 0.1 mL/100 g body weight/d	Iron, zinc, and copper in brain, spleen, and intestine	Gharib (2013)
Antihyperglycemic effect in streptozotocin-induced diabetic rats	Male albino Wistar rats	45 d and 3, 6, 12 mg of lyophilized solvent extract of kombucha/kg body weight/day	Glycosylated hemoglobin, plasma insulin, hemoglobin, and tissue glycogen, glucose-6-phosphatase, fructose-1,6-bisphosphatase and hexokinase	Srihari and others (2013b)
Attenuation of oxidative damage in electromagnetic field-exposed rats (950 MHz)	Male Wistar rats	57 d and 0.1 mL/100 g body weight/d	Malondialdehyde, superoxide dismutase, lactate dehydrogenase, aspartate amino transferase, tissue glutathione levels in heart and lung, serum total antioxidant capacity	Gharib (2011)

kombucha tea was found to be more efficient (Bhattacharya and others 2011b).

The antioxidant activity of kombucha tea is due to the presence of tea polyphenols, ascorbic acid, and DSL. Kombucha tea was observed to have higher antioxidant activity than unfermented tea and that may be due to the production of low-molecular-weight components and structural modifications of tea polyphenols by enzymes produced by bacteria and yeast during fermentation.

Kombucha exhibited increased free radical scavenging activities during fermentation. The extent of the activity depended upon the fermentation time, type of tea material, and the normal microbiota of the kombucha culture, which in turn determined the nature of their metabolites. Although free radical scavenging properties of kombucha showed time-dependent profiles, prolonged fermentation is not recommended because of accumulation of organic acids, which might reach harmful levels for direct consumption. The identification of extracellular key enzymes responsible for the structural modification of components during kombucha fermentation and potent metabolites responsible for the free radical scavenging abilities are necessary to elucidate the metabolic pathway during kombucha fermentation. Metabolic manipulations may be one of the effective methods to enhance the antioxidant activities and fermentation efficiency of kombucha.

Kombucha tea as hepatoprotective agent

Kombucha tea has been studied for its hepatoprotective property against various environmental pollutants in animal models and cell lines and it has been shown that it can prevent hepatotoxicity induced by various pollutants. Kombucha tea (prepared from black tea) was tested against paracetamol (Pauline and others 2001), carbontetrachloride (Murugesan and others 2009), aflatoxin B_1 (Jayabalan and others 2010a), cadmium chloride (Ibrahim 2011), TBHP (Bhattacharya and others 2011b), and acetaminophen (Abshenas and others 2012; Wang and others 2014). It was demonstrated that it can effectively attenuate the physiological changes driven by these liver toxicants. The volume of kombucha tea, number of doses, treatment period, and the method of administration used in these studies were not same. In most of the studies, male albino rats (Pauline and others 2001; Murugesan and others 2009; Jayabalan and others 2010a; Ibrahim 2011; Wang and others 2014) were used and a few other studies were conducted with Balb/c mice (Abshenas and others 2012) and isolated murine hepatocytes (Bhattacharya and others 2011a). Hepatoprotective efficacy of kombucha tea was studied by measuring liver toxicity markers (serum glutamic pyruvate transaminase, serum glutamic oxaloacetic transaminase, malondialdehyde, alkaline phosphatase, gamma glutamyl transpeptidase), reduced glutathione, antioxidant enzymes (glutathione-S-transferase, glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase), various levels of creatinine and urea, nitric oxide levels in liver, and by histopathological analysis of liver tissue. More recently, apoptosis, reactive oxygen species generation, changes in mitochondrial membrane potential, cytochrome c release, activation of caspases (3 and 9) and Apaf-1 were studied to show the hepatoprotective property of Kombucha tea against TBHP (Bhattacharya and others 2011b).

Antioxidant activity and its ability to facilitate both antioxidant and detoxification processes in the liver were ascribed to the hepatoprotection offered by kombucha tea. Wang and others (2014) reported that hepatoprotective effects of kombucha tea against acetaminophen is largely attributed to the presence of DSL, and *Gluconacetobacter* sp. A4 was the primary producer of it. Most of the studies concluded that kombucha tea could be beneficial against liver diseases, for which oxidative stress is a well-known causative factor.

Kombucha tea as an anticancer source

Chemoprevention using a combination of dietary phytochemicals with diverse mechanisms has been proposed as a successful approach to control different types of cancer with fewer side effects. Kombucha tea has been seriously claimed to have anticancer property by kombucha drinkers for many years. Based on personal observations and testimonials, it has been claimed to have anticancer properties and has also been claimed by a population study conducted in Russia by the "Central Oncological Research Unit" and the "Russian Academy of Sciences in Moscow" in 1951 (Dufresne and Farnworth 2000). Cetojevic-Simin and others (2008) investigated the antiproliferative activity of kombucha beverages from black tea and winter savory tea (*Satureja montana* L.) on HeLa cells (cervix epithelial carcinoma), HT-29 (colon adenocarcinoma), and MCF-7 (breast adenocarcinoma) using the sulforhodamine B colorimetric assay. They reported that the antiproliferative effect of kombucha winter savory tea was comparable to that of traditional kombucha black tea; and concluded that kombucha prepared from winter savory tea might have more active antiproliferative components than simple water extracts of winter savory tea. An ethyl acetate fraction of kombucha black tea which contained dimethyl 2-(2-hydroxy-2-methoxypropylidene) malonate and vitexin at a concentration of 100 μg/mL caused cytotoxic effects on 786-O (human renal carcinoma) and U2OS (human osteosarcoma) cells, significantly reduced the cell invasion and cell motility in A549 (human lung carcinoma), U2OS and 786-O cells, and reduced the activities of matrix metalloproteinase-2 (MMP-2) and MMP-9 in 786-O cells and MMP-2 activity in A549 cells (Jayabalan and others 2011). Lyophilized kombucha tea extract significantly decreased the survival of prostate cancer cells by downregulating the expression of angiogenesis stimulators like matrix metalloproteinase, cyclooxygenase-2, interleukin-8, endothelial growth factor, and human inducible factor-1α (Srihari and others 2013a). This study showed the remarkable potential of kombucha in inhibiting angiogenesis through alterations in the expression of angiogenic stimulators.

The possible anticancer mechanisms of tea polyphenols accepted by most researchers now are as follows: (1) inhibition of gene mutation; (2) inhibition of cancer-cell proliferation; (3) induction of cancer-cell apoptosis; and (4) termination of metastasis (Conney and others 2002; Ioannides and Yoxall 2003; Park and Dong 2003). Anticancer properties of kombucha tea might be due to the presence of tea polyphenols and their degradation products formed during fermentation.

Reported toxicity of kombucha tea

Although kombucha tea has been reported to have curative effects, there is some evidence of toxicity associated with it. Some individuals have reported dizziness and nausea after consuming certain kombucha products. Two cases of unexplained severe illness have also been reported following kombucha consumption (Centers for Disease Control and Prevention 1995). Kombucha tea is contraindicated in pregnant and lactating women. It has been found to cause lead poisoning and gastrointestinal toxicity in 2 individuals. The presence of anthrax *Bacillus* in kombucha tea fermented in unhygienic condition was reported by Sadjadi (1998). Further, Gamundi and Valdivia (1995) stated the risks of

consuming kombucha beverage by HIV-positive patients. Side effects like allergic reactions, jaundice, nausea, vomiting, and head and neck pain related to consumption of kombucha were reported in 4 patients (Srinivasan and others 1997). A married couple who had been drinking kombucha tea for 6 mo, which was brewed in a ceramic pot, was reported to have symptomatic lead poisoning requiring chelation therapy (Phan and others 1998). It was postulated that acids in the drink eluted lead from the glaze pigment used in the ceramic pot. Sabouraud and others (2009) reported cases of lead poisoning in adults identified as anemia due to the lead-glazed earthenware jug which was used to store kombucha. A case of acute renal failure with lactic acidosis and hyperthermia within 15 h of kombucha tea ingestion by a 22-y-old HIV-positive male with a blood lactate level of 12.9 mmol/L and serum creatinine of 2.1 mg/dL was recorded (Kole and others 2009). However, all of these cases were very isolated and involved only a small number of individuals. Moreover, there is no substantial evidence to confirm the toxicity of any kombucha tea or the occurrence of illness by earlier studies (Vijayaraghavan and others 2000).

Nontoxic nature of kombucha tea

The U.S. Food and Drug Administration and Kappa Laboratories, Miami, Florida, U.S.A. (1995), have carried out microbiological and biochemical tests and reported that kombucha tea is safe for human consumption. Vijayaraghavan and others (2000) studied the subacute (90 d) oral toxicity potency of kombucha tea using rats by recording body weight, feed intake, water intake, general behavior, and histological examinations. They concluded that kombucha feeding for 90 d to rats did not show any toxic signs. Hematological and biochemical variables of rats studied were within clinical limits. Their study indicated that rats fed kombucha tea for 90 d did not show any toxic effects. Pauline and others (2001) studied the toxicity of kombucha tea by feeding the rats orally for 15 d using 3 different doses of kombucha tea (normal dose and 5 and 10 times that dose) and by measuring various biochemical and histopathological parameters. They observed that kombucha tea displayed no significant toxicity.

Tea fungus (fungal biomass) and its applications

Cellulose produced during the fermentation by *A. xylinum* appears as a thin membrane on the surface of tea broth where the cell mass of bacteria and yeast is attached (Figure 2A and 2B). This mixture of microorganisms and cellulose is likely why kombucha is also called "tea fungus" (Sreeramulu and others 2000). Cellulose prepared from pellicles of *A. xylinum* has a unique characteristic in terms of its chemical stability, molecular structure, and mechanical strength (Czaja and others 2006). A similar cellulose network floating on the surface of various fruit juices fermented by a symbiotic culture composed of *A. xylinum* and yeasts, and called "note," is consumed in the Philippines as a delicacy. The cellulose network produced by a pure culture of *A. xylinum* is used for the treatment of skin burns and other dermal injuries in Brazil (Blanc 1996). Caffeine and related compounds (theophylline and theobromine) are identified as activators for cellulose production in *A. xylinum* (Lončar and others 2001). In ancient days, this cellulose biofilm was used for the treatment of wounds. Microbial cellulose synthesized in abundance by *A. xylinum* shows vast potential as a novel wound healing system (Czaja and others 2006).

Dried tea fungal biomass has been efficiently utilized as a biosorbent to remove metal pollutants from waste water by several researchers worldwide (Murugesan and others 2005; Mamisahebei and others 2007; Razmovski and Šćiban 2008). The charges possessed by the bacteria and yeasts present in the cellulose biomass were correlated with absorbent ability. Mamisahebei and others (2007) investigated the efficiency of tea fungal biomass pretreated with $FeCl_3$ to remove arsenic from aqueous solution and found that maximum capacities of tea fungal biomass for arsenic (V) were obtained at 3.98×10^{-3} mmol/g at pH of 6 to 8. Razmovski and Šćiban (2008) studied the efficiency of waste tea fungal biomass to remove Cr(VI) and Cu(II) ions from aqueous solutions in a batch biosorption system and reported that the optimum pH values for biosorption of Cr(VI) and Cu(II) by waste tea fungal biomass were 2.0 and 4.0, respectively. Murugesan and others (2005) studied the proximate composition of tea fungal biomass and reported that it contains 179.38 g crude protein, 120 g crude fiber, 4.82 g phosphorus, 6.56 g calcium, and 8.92 MJ metabolizable energy per kilogram of biomass. They also reported that the supplementation of tea fungal biomass at 150 g/kg poultry feed increased feed consumption, body weight, performance efficiency factor (PEF), and the carcass characteristics (dressed weight, eviscerated weight, liver, heart and gizzard) of test broilers significantly over the control.

Tea fungus was found to be rich in crude fiber, crude protein, and the amino acid lysine, and an increase in fermentation time increased the biochemical components of tea fungus (Jayabalan and others 2010b). Coculturing *Gluconacetobacter hansenii* CGMCC 1671 and *S. cerevisiae* CGMCC 1670 in traditional kombucha with 10.37% inoculum, initial pH 4.96, and medium volume of 77.13 mL in a 250 mL flask resulted in 300.093 mg/g of bacterial

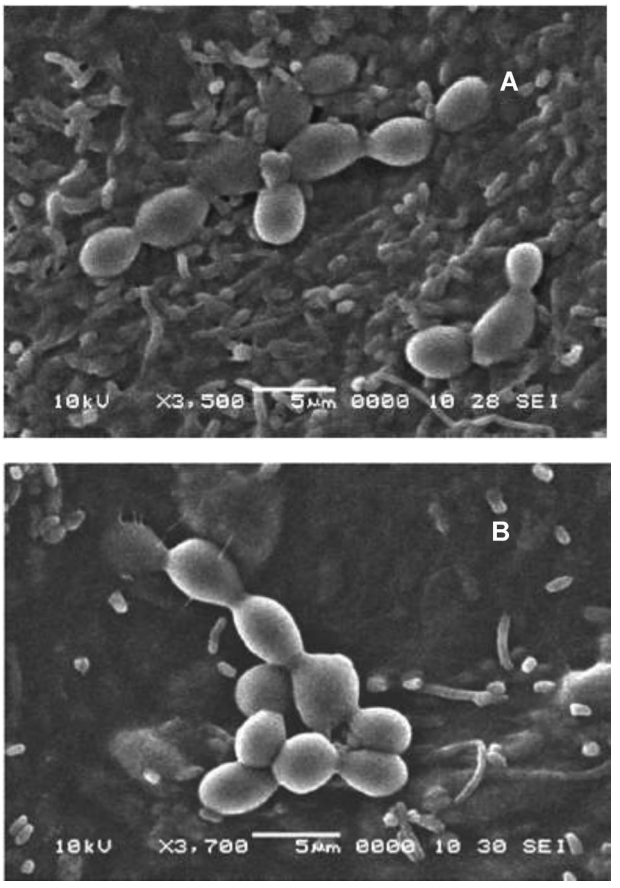

Figure 2–(A, B)–Scanning electron microscope image of the consortia of yeasts and bacteria in a portion of tea fungus (magnification 2a = 3500× and 2b = 2700× (reproduced with prior permission; El-Taher 2011).

cellulose (Tan and others 2012). The researchers concluded that coculturing pure strains of traditional kombucha can be used to provide bacterial cellulose of high grade in addition to produce the high-quality kombucha beverage. Tea broth with a sucrose concentration of 9% produced the highest yield of bacterial cellulose (66.9%), and the thickness and yield of this bacterial cellulose increased with fermentation time and surface area:depth ratio (Goh and others 2012a). Characterization of microbial cellulose produced from kombucha after 8 d of fermentation, by employing SEM, FTIR, X-ray diffractometry, adsorption isotherm, and by measuring the swelling properties, was done by Goh and others (2012b). Their results on SEM showed that an ultrafine network makes up the cellulose layer. FTIR confirmed the presence of a characteristic region of anomeric carbons and β-1,4-linkages. Cellulose was confirmed to be free from contaminants such as lignin or hemicellulose. X-ray diffraction studies showed that the overall degree of crystallinity index of dried tea fungal biomass was slightly lower than that of microbial cellulose. Hence, it can also be used for the preparation of cellulose-based chemicals like carboxymethylcellulose and can be fermented to bioethanol. Zhu and others (2013) demonstrated that kombucha cellulose had good biocompatibility with primary cultured Schwann cells (neurilemma cells), and the kombucha cellulose did not show histological and hematological toxic effects on nerve tissues *in vivo*.

Conclusions and future prospects

Kombucha drink is consumed worldwide as a homemade refreshing beverage and it is also commercially sold by some companies. Different tea leaf varieties, amounts of sugar, fermentation time, and composition of tea fungus may account for differences in composition and therefore also the biological activities of kombucha tea. There is still a dispute over the beneficial effects of kombucha drink. There has been no evidence published to date on the biological activities of kombucha in human trials. All the biological activities have been investigated using animal experimental models. Toxicity reports on kombucha drink are very rare and scattered. Toxicity must be evaluated thoroughly using modern procedures. Tea fungus is an excellent example of biofilm and studies on its cellulose chemistry must be encouraged. Cellulose in tea fungus can be used as a successful alternative to traditional cellulose in various applications. Although kombucha tea cannot be granted official health claims at this time, it can be recognized as an important part of a sound diet. Not exactly a traditional beverage, kombucha tea is now regarded as a "health" drink, a source of pharmacologically active molecules, an important member of the antioxidant food group, and a functional food with potential beneficial health properties. Research on kombucha demonstrating its beneficial effects and their mechanisms will most likely continue to increase substantially in the next few years. It is apparent that kombucha tea is a source of a wide range of bioactive components that are digested, absorbed, and metabolized by the body, and exert their effects at the cellular level. Kombucha tea's current status as a functional food as summarized in this review, lends credibility to what has been believed by kombucha tea drinkers for a long time.

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Authors' Contribution

Rasu Jayabalan did the writing of abstract, introduction, beneficial effects of kombucha tea, antimicrobial activity, antioxidant activity, hepatoprotective property, anticancer property, Table 2, kombucha toxicity, and kombucha-nontoxic drink.

Radomir Malbasa did the writing of fermentation of kombucha on substrates other than tea.

Eva Loncar did the writing of microbiology of kombucha.

Jasmina Vitas did the writing of chemical composition of kombucha tea.

Muthuswamy Sathishkumar did the writing of tea fungal biomass and its applications and also conclusions and future prospects.

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