Intl. J. Food. Ferment. Technol. 6(1): 13-24, June, 2016

DOI: 10.5958/2277-9396.2016.00022.2

REVIEW PAPER

Kombucha: Technology, Microbiology, Production, Composition and Therapeutic Value

Vikas Kumar*[1] and V.K. Joshi[2]

[1]Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Jalandhar, Punjab, India
[2]Former, Department of Food Science and Technology, Dr.Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP)-173230, India

Corresponding author: vkchoprafst@rediffmail.com

Paper No. 108 Received: 17-10-2015 Accepted: 5-4-2016

Abstract

Kombucha, Tea Kvass, Japanese or Indonesian tea fungus and Manchurian are the most common names for the symbiotic association of bacteria and osmophilic yeast in a form of thick jelly membrane which is cultured in sugared tea. It is slightly sweet, acetic acid-flavoured beverage also called tea eider the traditional substrate for *kombucha* preparation is black tea sweetened with 5 to 15% of sucrose and produced during 6 to 10 days of fermentation under aerobic conditions, at a temperature range of 20 to 30°C. The fermentation is two steps fermentation in which, the yeasts ferment the sugar to ethanol, which is further oxidised by the acetic acid bacteria to produce acetic acid which reduces the pH of medium. Except black tea, different types of other tea's such as orthodox tea and herbal tea have been used for the production of apple tea wine, using natural and inoculated fermentation. Besides acetic acid, the fermented liquid contains gluconic, glucuronic and lactic acid, among them all glucuronic acid is the main therapeutic agent in *Kombucha*. *Kombucha* metabolism produces glucose, fructose, small amounts of ethanol, carbon-dioxide, vitamins C, B_1 B_2, B_3, B_6, B_{12}, folic acid, different organic acids, mainly acetic, gluconic, L-lactic, glucuronic, enzymes and some antibiotically active compounds, and many others. Beverage also contains most of tea ingredients like tea catechines and caffeine. The beverage has been claimed is considered a prophylactic agent and is considered beneficial to human health.

Keywords: *Kumbucha*, tea, fermentation, Manchurian, tea kvass, glucuronic acid, therapeutic value

Tea (*Camellia sinensis* L.) is the most important non-alcoholic beverage having worldwide popularity. It is consumed as a morning drink by 2/3rd of the world population. It is mainly consumed in the form of 'fermented tea' or 'black tea', 'non-fermented' or 'green tea' and 'semi-fermented' or 'oolong tea' which is also popular in Japan and China. Tea leaves have more than 700 chemical constituent, among which flavonoids, amino acids, vitamins (C,E,K), caffeine and polysaccharides are important to human health. However, the stimulative effect of tea is due to caffeine (1.25-45.5%) (Kurian and Peter, 2007). Tea, mostly black tea, is the second most consumed drink in the world after water and well ahead of coffee, beer, wine and carbonated soft drinks (Hui, 1992). The black tea infusion contains proteins, amino acids, volatile compounds, lipids, enzymes and polyphenols, which make it as a good fermentation medium (Hui, 1978). Microbial fermentation of black tea leads to production of *Kombucha* or tea cider, known to have therapeutic values. (Rosma *et al.,* 2016)

Black tea is produced by a process that oxidizes the polyphenols in the leaves to such substances as are characteristics of black tea and responsible for the

colour, the caffeine content and taste of infusion. Black tea has a strong body due to tannins, which are a group of astringent polyphenolic compounds such as flavonoids (theaflavin and thearubigin) and others derivatives of polyphenols. The natural tannins are powerful reducing agents and exhibit a marked tendency to absorb oxygen, thereby, making tea infusions a possible health drink due to its antioxidant property. Not only in tea, tannins impart body to various fermentation products especially in wines and fermented fruit juices, besides enhancing their flavour profile (Fleet, 2001). Black tea is a good fermentation medium because its infusion contains proteins, aminoacids, volatile compounds, lipids, enzymes and more importantly polyphenols (Martin and Arnold, 1978). Black tea fermented with yeast accumulates the vitamins A, C and B complex, making it a nutritious and a therapeutic agent (Chand and Gopal, 2005), besides increasing shelf-life (Liyanage *et al.* 1988).

Tea cider also known as *Kombucha* (traditional fermented product), is a fermented tea that is often drunk for medicinal purposes. *Kombucha* tea has been used in Russia for several centuries. The modern form of Russian *Kombucha* tea is widely popular and is known as "tea kvass" or simply "kvass" (Murugesan *et al.* 2009). This refreshing beverage tasting like sparkling apple cider is often produced at home by fermentation using a tea fungus passed from house-to-house (Dufresne and Farnworth, 2000). Fermented tea decoctions such as "*Kombucha*" have been prepared by co-fermentation with yeast and acetic acid bacteria, and are known to have health benefits (Gutapadu *et al.* 2000).

The purpose of this review is to bring out the technology, microbiology, production, composition, therapeutic value and try to establish a better understanding of *Kombucha* and its possible health benefits. A thorough knowledge of tea, its composition and effects on metabolism and health provides a starting point in understanding the potential of *Kombucha*.

HISTORY

Most of the world population, especially people in highly developed countries, has demonstrated increased awareness and interest in functional food, i.e., food that positively effects upon bio-regulatory functions and human health. Such an interest lasted for a few decades, having great impact in the development of food industry. The consumption of *Kombucha* was first practiced in 220 B.C. in Manchuria (Jayabalan *et al.*, 2014), the tea was sought for its magical properties. As trade routes extended beyond the Far-East, It, then, spread to Russia where *Kombucha* is called *teakwas*. This beverage was introduced into Germany during World War II, in the 50's arrived into France and France-dominated North Africa (Blanc, 1996). Presently, *Kombucha* is popular in the United States, due to its refreshing power and curative effects.

MICROBIOLOGY

Acetic acid bacteria (*Acetobacter xylinum, Acetobacter aceti, Acetobacter pasteurianus, Gluconobacter oxydans*) (Greenwalt *et al.* 2000) and yeast (*Saccharomyces sp., Zygosaccharomyces sp., Torulopsis sp., Pichia sp., Bret anomyces sp.*) are the main microbes having the symbiosis in tea fungus responsible for *kombucha* fermentation. The variation of its composition could be due to geographic, climatic and cultural conditions as well as depends on the types of wild yeast and bacteria that exist locally (Petrovska and Tozi, 2000 and Teoh *et al.* 2004). The fermentation is two steps fermentation in which, yeasts ferment the sugar to ethanol, which is further oxidised by the acetic acid bacteria to produce acetic acid in the second fermentation. The result is reduced pH of medium. Both ethanol and acetic acid have antimicrobial activity against pathogenic bacteria, thereby, providing protection against contamination of the tea fungus (Liu *et al.* 1996). Besides acetic acid, the fermented liquid contains gluconic, glucuronic and lactic acid. Glucuronic acid is the main therapeutic agent in *Kombucha*, as a detoxification agent (Loncar *et al.* 2000). Many flavour compounds, including alcohols, aldehydes, ketones, esters and amino

acids have been identified from fermented broth (Teoh *et al.* 2004). A culture of *Kombucha* is a living organism exposed to many influences, which gives the final beverage a different chemical composition and taste (Petrovska and Tozi, 2000). Besides these, recently Kumar (2014) reported that the bacteria responsible for the natural fermentation of apple tea wine belong to *Bacillus* whereas the yeast responsible for the natural fermentation of apple tea wine belong to genus *Saccharomyces*. List of bacteria and yeasts associated with fermentation of *Kombucha* is presented in Table 1.

Table 1: Microbial Population of *Komucha*

Microorganism	References
Bacteria	
Acetobacter xylinum	Kozaki, 1972, Sievers *et al.* 1995, Blanc, 1996 Greenwalt *et al.* 2000, Mrdanovic *et al.* 2007
Acetobacter aceti	Liu *et al.* 1996, Greenwalt *et al.* 2000, Mrdanovic *et al.* 2007
Acetobacter ketogenum	Morales and Sanchez, 2003
Acetobacter pasteurianus	Liu *et al.* 1996, Greenwalt *et al.* 2000,
Bacterium gluconicum	Morales and Sanchez, 2003, Mrdanovic *et al.* 2007
Bacterium katogenum	Mrdanovic *et al.* 2007
Bacterium xylinum	Morales and Sanchez, 2003, Mrdanovic *et al.* 2007
Bacterium xylinoides	Morales and Sanchez, 2003, Mrdanovic *et al.* 2007
Gluconobacter oxydans	Liu *et al.* 1996, Greenwalt *et al.* 2000, Mrdanovic *et al.* 2007
Yeast	
Bret anomyces sp.	Kozaki, 1972, Mayser *et al.* 1995
Bret anomyces bruxellensis	Liu *et al.* 1996, Mrdanovic *et al.* 2007
Bret anomyces intermedius	Greenwalt *et al.* 2000,
Candida	Jankovic and Stojanovic, 1994
Candida guilliermondii	Kozaki, 1972, Greenwalt *et al.* 2000,
Candida famata	Greenwalt *et al.* 2000,
Candida stellata	
Mycoderma	Jankovic and Stojanovic, 1994
Mycotorula	Jankovic and Stojanovic, 1994
Pichia	Jankovic and Stojanovic, 1994
Pichia fermentans	
Pichia membranaefaciens	Kozaki, 1972, Greenwalt *et al.* 2000
Saccharomyces sp.	Kozaki, 1972,
Saccharomyces cerevisiae subsp. *aceti*	Greenwalt *et al.* 2000
Saccharomyces cerevisiae subsp. *cerevisiae*	Liu *et al.* 1996, Greenwalt *et al.* 2000
Saccharomycodes ludwigii	
Schizosaccharomyces	Jankovic and Stojanovic, 1994
Schizosaccharomyces pombe	
Torula	Jankovic and Stojanovic, 1994
Torulaspora delbrueckii	Greenwalt *et al.* 2000
Torulopsis famata	Kozaki, 1972,
Zygosaccharomyces sp.	Sievers *et al.* 1995
Zygosaccharomyces bailii	Liu *et al.* 1996, Greenwalt *et al.* 2000
Zygosaccharomyces rouzii	Greenwalt *et al.* 2000
Zygosaccharomyces rouxii	Blanc, 1996

PREPARATION OF *KOMBUCHA*

The process of preparation of *Kombucha* is shown in Fig. 1 and described here.

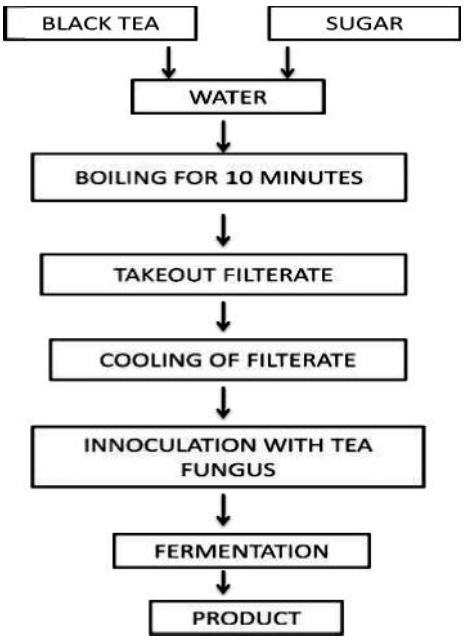


Fig. 1: Schematic description of *Kombucha* manufacture

Raw material

Brewed tea is the substrate on which the microorganisms grow to produce the final product that is *Kombucha*. Black tea is usually used for *Kombucha* preparation, green tea and herbs are also used (Kumar, *et al.* 2016). It has been shown that green tea has a more stimulating effect on the *Kombucha* fermentation than black tea, yielding the fermentation in a short time frame (Greenwalt, *et al.* 1998). Black tea and white sugar are the best substrates for the preparation of *Kombucha*, although green tea can also be used (Reiss, 1994).

Radomir *et al.* (2006) conducted research on influence of black tea concentrate on *Kombucha* fermentation and reported that it is possible to perform *Kombucha* fermentation in substrates with higher black tea concentration than is the traditional one, but metabolites content in fermentative liquids is not proportional to the amount of used tea and sucrose. When solution with tea concentration of 15 g/L was diluted with cold tap water in such ratio that final solution is corresponding to standard substrate for fermentation with 1.5 g/L of black tea and 70 g/L of sucrose, the compared metabolites contents in fermentative samples of standard and diluted concentrate were very similar.

Recently, Kumar *et al.* (2015) and Kumar *et al.* (2016) conducted studies to check the suitability of different types of tea (black tea, orthodox tea and herbal tea) at different concentrations (2 to 5%) for the preparation of apple tea wine and reported that apple tea wine prepared using 4% CTC tea was the best on the basis of physico-chemical and the sensory quality characteristics.

Must Preparation

Tea leaves are added to boiling water and allowed to infuse for about 10 min af er which the leaves are removed and sweetened with sucrose 50 to 150g/ml (5 to 15%). Sucrose (Greenwalt *et al.* 2000) is dissolved in the hot tea. This must is allowed to cool at room temperature (Jayabalan *et al.* 2014).

Recently, Joshi and Kumar (2016) conducted studies to check the effect of different sugar sources (apple juice concentrate, sucrose and honey), nitrogen sources (DAHP, ammonium sulphate and peptone) and inocula (*Saccharomyces cerevisiae* var. *ellipsoideus*, consortia 1 and 2) on the quality characteristics of tea wine and reported that the wine prepared by ameliorating the apple tea must with apple juice concentrate, DAHP and inoculated with *Saccharomyces* was the best.

Inoculation and fermentation

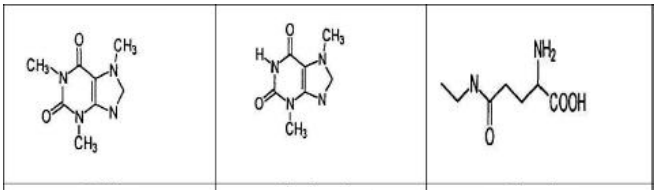
Tea is poured into a wide-mouthed clean vessel and the microbial mat or colony from previous batch of *Kombucha* is added to the sweetened tea with about 100 ml of *Kombucha* from previous fermentation. Markov *et al.* (2006) reported that isolated strains of yeasts and acetic acid bacteria from tea fungus may be used as started cultures for obtaining of *Kombucha*. Further, it was revealed that, the fermentation was faster in medium inoculated with fermentation broth compared to the fermentation with the starter cultures. The fermentation time is dependent on initial count of yeasts cells.

The tea fungus is laid on the tea surface, and the jar is carefully covered with a clean cloth and fastened properly. The preparation incubated at room temperature (between 20° and 30°C) for 7-10 days. If the fermentation is allowed to continue beyond the 10 days, the acidity may rise to levels potentially harmful to consume. During fermentation, a daughter tea fungus is formed at the tea surface.

The tea fungus is removed from the surface and kept in a small volume of fermented tea. The beverage is passed through cheese-cloth and stored in capped bot les at 4°C (Jayabalan *et al.* 2014). It has been reported the taste of the *Kombucha* changes during fermentation from a pleasant fruit sour-like lightly sparkling flavour af er a few days, to a mild vinegar-like taste with prolonged incubation (Blanc, 1996; Reiss, 1994; Sievers *et al.* 1995). The schematic description of *Kombucha* manufacture is presented in Fig. 2.

Kombucha is traditionally prepared by fermentation of sweetened (sucrose) black tea. This medium (freshly prepared medium) is usually inoculated

with cellulose pellicle formed during the previous cultivation and incubated statically under aerobic conditions for 7-10 days.



Caffeine	Theobromine	Theanine

Fig. 2: Important ingredients present in *Kombucha*

Source: Harbowy and Balentine, 1997

The media for tea fungus cultivation could also be successfully inoculated with fermented liquid from previous fermentation, where the concentration of cells is generally higher than those in cellulosic pellicles (Liu *et al.* 1996). Production of vitamin C and other valuable compounds, in *Kombucha* during fermentation process, is strongly related to the process duration, fermentation temperature, *Kombucha* symbiosis and inoculum concentration, source of carbon atoms and other working conditions by Petrovic *et al.* (1995–1996). It has also been reported (Petrovic *et al.* 1995–1996) that the product of 7th day of fermentation possesses optimal sensory characteristics; increase of acidity during fermentation longer than 7 days makes liquor of the *Kombucha* too sour and unpleasant. However, some authors (Chen and Liu, 2000) have investigated changes in major compounds of tea fungus metabolites during prolonged fermentation of up to 60 days. Temperature of fermentation significantly influences kinetics of substrate intake and product formation. It was proved that the composition of fermented tea greatly depend on the individual *Kombucha* association (Reiss, 1994; Blanc, 1996) as well as on the concentration of inoculum solution. As far as sucrose substrate is concerned, it seems that concentration of 50 g $^{-1}$ gives optimal concentration of both ethanol and lactic acid (Reiss, 1994).

The composition and properties of tea are well documented, but scarce scientific information is available, concerning the composition and the effects of *Kombucha* on health. Benefits have been reported by testimony of users in different conditions and with variable consumption.

BIOCHEMICAL CHANGES IN *KOMBUCHA*

The tea fungus produces a cellulosic pellicle or mat, oxidized ethanol, producesd acetic, L(+)lactic acid, gluconic acid and glucuronic acid (Frank, 1991). The fermentation and oxidation processes starts, when the tea fungus is placed in a freshly prepared infusion of tea and sugar. When grown in sucrose medium, the yeast break down sucrose in glucose and fructose, then produce carbon dioxide and ethanol, which are oxidized to acetaldehyde by acetic acid bacteria. The tea fungus produces many other substances, like gluconic acid and vitamins, which with the supply of tea nutrients, give the drink its unusual flavor and healing properties. During the process, the glucose is polymerized and produces cellulose and hemi-celluloses (Greenwalt *et al.* 1998; Bauer and Petrushevska, 2000).

To produce *Kombucha*, black tea ingredients and sucrose undergo progressive modification by the action of the tea fungus. The main metabolites identified in the fermented beverage are: acetic, lactic, gluconic and glucuronic acids, ethanol and glycerol (Blanc, 1996; Liu *et al.* 1996). Some chemical structures of important ingredients reported in *Kombucha* are given in Fig. 2. The presence of usnic acid in *Kombucha* reported once has not been confirmed in recent studies (Blanc, 1996). Usnic acid had been previously identified in lichens and can deactivate some groups of viruses. The metabolite composition and concentration depends on the tea fungus source, sugar concentration, and the time course of fermentation. With 50 g/l sucrose, concentrations of ethanol and of lactic acid are optimal (Reiss, 1994). Yeast and bacteria in the tea fungus make use of substrates by different and complementary ways. Yeast cells hydrolyse sucrose into glucose and fructose, and produce ethanol, with a preference for fructose as a substrate (Sievers *et al.* 1995). Acetic bacteria utilize glucose to produce gluconic acid (Sievers *et al.* 1995),

and ethanol to produce acetic acid (Yurkevich and Kuty Shenko, 1998). The presence of lactic acid was not observed in these studies but has been reported in another. In this study, the lactic acid synthesis is attributed to the action of lactic bacteria on ethanol and acetic acid (Reiss, 1994). It is also reported that the fermentation process induces the synthesis of the B complex of vitamins and folic acid. The pH value of *Kombucha* decreases during the fermentation process following the increase in the organic acid content (Blanc, 1996; Riess, 1994; Sievers *et al.* 1995). Similar finding have been observed by Kumar *et al.* (2015), Kumar *et al.* (2016) and Joshi and Kumar (2016) during the fermentation of apple tea wine.

Components of Kombucha

Kombucha contains the organic acids, active enzymes, amino acids, and polyphenols produced by these microbes. The precise quantities of these component a sample can only be determined by laboratory analysis. Finished *Kombucha* may contain any of the following components. These components are acetic acid (mildly antibacterial), butyric acid, B-vitamins (Aleksandra *et al.* 2007), alcohol, gluconic acid, lactic acid, malic acid, oxalic acid and usnic acid.

Normal *Kombucha* contains less than 0.5% alcohol, which classifies *kombucha* as a non-alcoholic beverage. Older, more acidic, *kombucha* might contain 1.0% or 1.5% alcohol, depending on more anaerobic brewing time and higher proportions of sugar and yeast.

Nutritive and Therapeutic Value

Tea cider (*Kombucha*) is associated with many health benefits. It possesses characteristics of functional food and known for a few thousand years. It originated in China, 220 BC, Korea and Japan. There, it was popular due to detoxifying and energizing properties as well as curing digestive problems (Jayabalan *et al.* 2014). It contains liver detoxifiers, antioxidants, polyphenols, probiotics and free-form amino acids. *Kombucha* has been studied intensively since 1852; few of the health properties have been demonstrated by scientific and experimental studies (Dufresne and Farnworth, 2000). As a traditional medicine, the *Kombucha* drink was used as healing liquor in the treatment of many diseases, and at present it is considered to be a folk-remedy (Petrovska and Tozi, 2000). Hence, it is used as an alternative therapy (Blanc, 1996). Beneficial effects attributed to consumption of *Kombucha* (mushroom) tea have included prevention of a few cancer, relief of arthritis, treatment of insomnia, hemorrhoids, digestive disorders, heart disease, allergies, asthma, decrease of blood pressure, increase of vitality, increase of T cell count and stimulation of regrowth of hair. Because, the tea is believed to stimulate the immune system, it has become popular among the elderly persons (Sreeramulu *et al.* 2000, Cavusoglu and Guler, 2010).

The recommended consumption of *Kombucha* ranges from 100 to 300 ml per day (Frank, 1991). The beverage has been claimed to be a prophylactic agent and to be beneficial to human health as a diuretic in edemas, in atherosclerosis, in case of gout, sluggish bowels, for stones, etc. (Kaufmann, 1966). Fermentation also induces biosynthesis of ascorbic acid. Vitamin C is an important natural antioxidant, which serves as human health protector and a drug. One who has started with application of vitamin C as a drug was a leading vitamin C clinician (Klenner, 1971). Petrovic and Loncar (1996) and Malbasa *et al.* (2002), have determined content of vitamin C in fermentative liquids of tea fungus. Activity of vitamin C as well as activities of other compounds present in *Kombucha* fermentation system is modified in a positive way by the chemical environment in the fermented beverage. For example, it has been reported that tocopherol and ascorbic acid exert strong synergetic effects on the antioxidant of tea catechins (Hara *et al.* 1995). Therefore, vitamin C and other constituents of *Kombucha* beverage protect human health more efficient than the same isolated compounds. Vitamin C is also an essential component of the human diet. It enhances iron adsorption (Cook and Redd, 2001; Halberg and Hulthen, 2000), prevents megaloblastic anemia (Jacob, 1994) and reduces stomach cancer (Hemila and Herman, 1995). Ascorbic acid inhibits iron absorption by tannins. Decreased tannins and increased ascorbic acid concentration, in fermented tea, are very useful for iron absorption and improved

digestion (Pasha and Reddy, 2005). Due to the rich biomass in tea fungus (*Medusomyces gisevii*), it can be utilized as protein supplement in animal feed (Jayabalan *et al.* 2010). They further revealed that tea fungus is rich in crude protein, crude fibre, and amino acid lysine. The biochemical characteristics of tea fungus studied were increased throughout the fermentation time during the study of biochemical characteristics of tea fungus produced during *Kombucha* fermentation.

Petrovska and Tozi, (2000) quantified contents of minor and trace elements viz., manganese, iron, nickel, copper, zinc, lead, cobalt, chromium and cadmium in the Kombucha drink and sweet tea infusion which are presented in Table 2.

Table 2: Minor and trace elements content (μg mL^{-1}) in the *Kombucha* drink and sweet tea infusion

	***Kombucha* drink**	**Tea decocts**	**Increasing or decreasing factor**
Manganese	0.462 ± 0.024	0.362 ± 0.015	1.28
Iron	0.353 ± 0.018	0.257 ± 0.013	1.37
Nickel	0.346 ± 0.016	0.282 ± 0.008	1.23
Copper	0.237 ± 0.004	0.160 ± 0.007	1.48
Zinc	0.154 ± 0.008	0.132 ± 0.004	1.17
Lead	0.005 ± 0.001	0.012 ± 0.001	0.42
Cobalt	0.004 ± 0.0008	0.004 ± 0.0009	0
Chromium	0.001 ± 0.0001	0.002 ± 0.0001	0.5
Cadmium	0.001 ± 0.000 001	0.± 0.000	0

(n = 5)

Source: Petrovska and Tozi, 2000.

The average mineral composition of the essential elements was in concentrations from 0.004 μg mL^{-1} for cobalt to 0.462 μg mL^{-1} for manganese. Investigations of some toxic elements gave the following concentration values: 0.005 μg mL^{-1} for lead, 0.001 μg mL^{-1} for chromium. Cadmium was not detected.

Mineral content of the essential elements (Cu, Fe, Mn, Ni and Zn) for normal physiological processes in the organism increased as a result of the Kombucha cultivation in the sweet black tea infusion. The cobalt content did not increase in Kombucha beverage probably because of its incorporation in vitamin B_{12}. Cadmium was not found in the investigated samples. Decreased lead concentration in the drink proved the *Kombucha* was able to detoxify beverages, possibly by its high glucuronic acid content. Kombucha also decreased the very low chromium concentration present in the investigated tea decoct sample. They further revealed that, an average consumption of approximately 0.3μ litre per day, could satisfy all Ni and Co daily needs, partial Fe and Cu needs, but not the Zn and Mn daily needs.

The product has the bacterial β-glucuronidase enzyme that can interfere with proper disposal of a chemotherapeutic agent, and that antibiotics against gut bacteria can prevent toxicity of some chemotherapy drugs (Srinivasan *et al.* 1997), supporting the idea that glucaric acid is an active component of *Kombucha*. Low alcoholic beverages have gained importance in preventing cardiovascular diseases, by preventing the formation of LDL and increases the HDL levels (having protective effect against heart disease (Joshi *et al.* 1999).

Glucuronic acid, a constituent of *Kombucha*, forms compound with metabolic wastes as well as drugs and poisons and aids in detoxication. The tea fungus has been recommended as a therapy for gout, rheumatism, furunculosis, early artherosclerosis, high blood pressure, nervousness, and the symptoms of old age. *Kombucha* made the blood more acidic, it rejuvenates the elderly, causes grey hair to become dark again, tightens the skin, and enhances feelings of vitality and health. Among the Vitamins, Vitamin B_1, B_2, B_3, B_6, B_{12} and folic acids is also produced in *Kombucha* (Frank, 1991).

The beneficial effects of *Kombucha* tea are attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, aminoacids, antibiotics and a variety of micronutrients produced during the fermentation (Jayabalan *et al.* 2008). Many of these compounds are found in the tea composition (Table 3).

Table 3: The Composition of a Typical Tea Beverage, per cent (wt/wt) Solids

	Green Tea	Black Tea
Catechins	30%	9%
Theaflavins	—	4%
Simple polyphenols	2%	3%
Flavonols	2%	1%
Other polyphenols	6%	23%
Theanine	3%	3%
Aminoacids	3%	3%
Peptided Protein	6%	6%
Organic acids	2%	2%
Sugars	7%	7%
Other carbohydrates	4%	4%
Lipids	3%	3%
Caffeine	3%	3%
Other methylxanthines	<1%	<1%
Potassium	5%	5%
Other mineraldash	5%	5%
Aroma	Trace	Trace

Source: Harbowy and Balentine, 1997

Jayabalan *et al.* (2008) demonstrated that *Kombucha* tea prepared from green tea, black tea and tea waste material have excellent antioxidant activities. *Kombucha* exhibited increased free-radical scavenging activities during fermentation. The extent of the activity however, depends upon the fermentation time, type of tea material and the normal microbiota of *Kombucha* culture, which in turn determined the forms of their metabolites. Although free-radical scavenging properties of *Kombucha* showed the time-dependent profiles, prolonged fermentation was 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 elevate he antioxidant activities and fermentation efficiency of *Kombucha.*

Pasha and Reddy (2005) reported the nutritional and medicinal improvement of black tea by yeast fermentation. During experiment, black tea was fermented with *Dabaryomyces hansenii* for 10 days which resulted in accumulation of major vitamins, such as A, B1, B2, B12 and C in sufficient quantities to fulfil the recommended dietary allowances (RDA). Fermentation of tea by yeast resulted also in reduction of caffeine and excess tannins in significant amounts. Af er fermentation, the amount of theophylline was increased to make fermented tea a potent bronchodilator.

However, *Kombucha* consumption has proven to be harmful for individuals with preexisting conditions or illness or if incorrectly prepared (Greenwalt *et al.* 2000). Some results by researchers revealed that *Kombucha* can become contaminated with potentially harmful microorganisms, such as mould which may make it harmful for human consumption (Mayser *et al.* 1995, Cavusoglu and Guler, 2010). Contamination may potentially produce serious adverse effects, and consumption of this may cause several problems such as nausea, jaundice, shortness of breath, throat tightness vomiting, akathasia, headache, xerostomia, dizziness, liver inflammation, chronic liver disease and neck pain (Peron *et al.* 1995, Jayabalan *et al.* 2007, Cavusoglu and Guler, 2010).

Antimicrobial Activity of *Kombucha*

Application of natural antibacterial agents has been increasingly noticed as a novel trend in biological preservation of foods in recent years (Schillinger *et al.* 1996). Natural antibacterial agents have been increasingly applied for the biological preservation of food in recent years (Kumar *et al.,* 2016). Antimicrobial activities of microbial fermented tea are much less known than its health beneficial properties. These antimicrobial activities are generated in natural microbial fermentation process with tea leaves as substrates. The antimicrobial components produced during the fermentation process have shown inhibitory effects against several food-borne and pathogenic bacteria (Sreeramulu *et al.* 2001). With the trend of increasing use of natural and biological

preservatives in food products, natural antimicrobial agents from microbial fermented tea may offer an innovative and interesting measure for such applications (Kumar *et al.,* 2016).

The acidity and mild alcoholic element of *kombucha* resists contamination by most airborne molds or bacterial spores. As a result, *kombucha* is relatively easy to maintain as a culture outside of sterile conditions. The bacteria and yeasts in *kombucha* may also produce antimicrobial defense molecules. *Kombucha* is a home-brewing product which means the preparation conditions are not sterile. Majority of the tests for *Kombucha* indicated that a low rate of contamination from spoilage and pathogenic microorganisms, suggesting that *Kombucha* has antimicrobial properties to pathogenic and other "bad" microorganisms (Mayser *et al.,* 1995).

Some studies reported that antimicrobial activity of *Kombucha* against a range of bacteria, made with a low tea usage level (4.4 g/L), was attributable to the acetic-acid content (Steinkraus *et al.* 1996, Greenwalt *et al.,* 1998). While Sreeramulu *et al.* (2001) revealed that the antimicrobial components of *Kombucha* are compounds other than organic acids, ethanol, proteins or tannins in tea or their derivates after systematic investigation.

Rodrigo *et al.* (2009) conducted research on antimicrobial activity of broth fermented with *Kombucha* colonies. They reported that The fermented growth was efficient against *Microsporum canis* (LM-828), *Escherichia coli* (CCT-0355) and *Salmonella typhi* (CCT-1511). The best conditions of inhibition against *M. canis* (> 32mm) and *E. coli* (16 mm) was observed at pH 4.0, 55% of commercial sugar and 0.10 g/l of $MgSO_4$, and for *S. typhi* (32 mm) without $MgSO_4$. Gutapadu *et al.* (2000) investigated antimicrobial activity of *Kombucha* against a number of pathogenic microorganisms.

Staphylococcus aureus, *Shigella sonnei*, *Escherichia coli*, *Aeromonas hydrophila*, *Yersinia enterolitica*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus epidermis*, *Campylobacter jejuni*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Bacillus cereus*, *Helicobacter pylori*, and *Listeria monocytogenes* were found to be sensitive to *Kombucha*. Acetic acid is considered to be responsible for the inhibitory effect toward a number of microbes tested. However, *Kombucha* proved to exert antimicrobial activities against *E. coli*, *Sh. sonnei*, *Sal. typhimurium*, *Sal. enteritidis*, and *Cm. jejuni*, even at neutral pH and after thermal denaturation. They also suggests the presence of antimicrobial compounds other than acetic acid and large proteins in *Kombucha*.

Greenwalt *et al.* (1998) conducted research to determine and characterization of antimicrobial activity of the fermented tea *Kombucha*. They reported that the fermented samples containing 33g/l total acid (7g/l acetic acid) was significant against the tested gram positive and gram negative pathogenic organisms (*Agrobacterium tumefaciens, Bacillus cereus, Salmonella cholerasuis* serotype *typhimurium, Staphylococcus aureus and Escherichia coli*). *Candida albicans* was not inhibited by *Kombucha*. Tea, at drinkable levels, demonstrated no antimicrobial properties, even at the highest levels tested; 70 g/l (7%) dry tea. The antimicrobial activity of *Kombucha* was attributed to its acetic acid content. Kumar *et al.* (2016) studied the antimicrobial effect of different types of apple tea wine prepared using different types of tea at different concentrations against *Escherichia coli* (IGMC), *Enterococcus faecalis* (MTCC 2729), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MRSA 252) and *Bacillus cereus* (CRI) and reported that all apple tea wines showed antimicrobial activity (inhibition zone >7 mm) against all these microbes.

Conclusion and future prospects

Tea cider known as *"Kombucha"* or "tea kvass", tasting like sparkling apple cider is commonly produced at home by fermentation using a tea fungus (acetic acid bacteria and yeast). No doubt, black tea and white sugar are the common substrates for the preparation of the *Kombucha* but recently researchers took shift and used different types of the tea and sugar sources as well as nitrogen sources for *Kombucha* preparation with improved quality characteristics. A glimpse of literature revealed that *Kombucha* is a good source of the bioactive compounds which make

it as a functional beverage. But, still a few aspects are untouched in the *Kombucha* industry such as utilization of fruit juices as a substrate along with the tea for the preparation of Kombucha, maturation of *Kombucha* and effect of *Kombucha* on human being in *in-vitro* trials which need to be explored for further improvement in *Kombucha.*

References

Aleksandra Velicanski, Dragoljub Cvetkovic, Sinisa Markov, Vesna Tumbas and Sladjana, Savatovic. 2007. Antimicrobial and Antioxidant Activity Of Lemon Balm Kombucha. *Acta periodica technologica*. **38**: 165.

Bauer-Petrovska B, and Petrushevska-Tozi L. 2000. Mineral and water soluble vitamins content in *Kombucha* drink. *Inter J Food Sci Tech*. **35**: 201-205.

Blanc, P.J. 1996. Characterization of tea fungus metabolites. *Biotechnology Let ers*. **18**(2): 139–142.

Cavusoglu Kultigin and Guler Perihan. 2010. Protective effect of *kombucha mushroom* (KM) tea on chromosomal aberrations induced by gamma radiation in human peripheral lymphocytes *in-vitro*. *Journal of Environmental Biology*. **31**(5): 851-856.

Chand P and Gopal R. 2005. Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chem*. **89**(3): 449-453.

Chen, C. and Liu, B.Y. 2000. Changes in major components of tea fungus metabolites during prolonged fermentation, *Journal of Applied Microbiology*. **89**: 834–839.

Cook, J.D. and Redd, M.B. 2001. Effect of ascorbic acid intake on newborn iron absorption from a complete diet. *American Journal of Clinical Nutrition*. **73**: 93–98.

Dufresne, C. and Farnworth E. 2000. Tea, *Kombucha*, and health: A review. *Food Research International*, **33**(6): 409–421.

Dufresne, C. and Farnworth, E. 2000. Tea, *Kombucha* and health: a review. *Food Res. Int*. **33**: 409– 421.

Fleet, G.H. 2001. Wine. In: Food Microbiology: Fundamentals and Frontiers. Michel, P.D., Larry, R.B. and Thomas, J.M. (eds.). ASM press, Washington DC. pp. 722-772.

Frank, G.W. 1991. *Kombucha*. In: *Healthy Beverage and Natural Remedy from the Far East*. Wilhelm Ennsthaller, Austria.

Greenwalt, C.J., Ledford, R.A. and Steinkraus, K.H. 1998. Determination and characterization of antimicrobial activity of the fermented tea *Kombucha*. *Lebensm. Wiss. Technol*. **31**: 291-266.

Greenwalt, C.J., Ledford, R.A. and Steinkraus, K.H. 1998. Determination and characterisation of the antimicrobial activity of the fermented tea *Kombucha*. *Lebensm. Wiss. -Technol.* **31**: 291-296.

Greenwalt, C.J., Steinkraus, K.H. and Ledford, R.A. 2000. *Kombucha*, the fermented tea: microbiology, composition, and claimed health effects. *Journal of Food Protection*. **63**: 976-981.

Gut apadu, S., Yang, Z. and Knol, W. 2000. *Kombucha* fermentation and its antimicrobial activity. *J. Agric. Food Chem.* **48**(6): 2589-2594.

Halberg, L. and Hulthen, L. 2000. Prediction of dietary iron absorption: An algorithm for calculating absorption and bio-availability of dietary iron, *American Journal of Clinical Nutrition.* **71**: 1147–1160.

Hara, Y., Luo, S.J., Wickremashinghe, R.L. and Yamanishi, T. 1995. Uses and benefits of tea. *Food Reviews International*. **11**: 527–542.

Harbowy, E. Mat hew, and Balentine, A Douglas. 1997. Tea Chemistry. *Critical Reviews in Plant Sciences*. **16**(5): 415-480.

Hemila, H. and Herman, Z. 1995. Vitamin C and the common cold: A retrospective analysis of chalmers review. *Journal of the American College of Nutrition*. **14**: 116–123.

Hui, Y.H. 1978. Tannins. In: Encyclopedia of Food Science. Vol. 3. John Wiley and Sons, USA. pp. 732-734.

Hui, Y.H. 1992. Tea. In: Encyclopedia of Food Science and Technology. Vol 4. John Wiley and Sons, USA. pp. 2525-2537.

Jacob, R.A. 1994. Vitamin C, *In* Shils, M E, Olson J A and Shike M. (eds). Modern Nutrition in Health and Disease, 8th editions, 432–448 (Lea an Febiger, Philadelphia, PA, USA).

Jankovic, I. and Stojanovic, M. 1994. Microbial and chemical composition, growth, therapeutical, and antimicrobial characteristics of Tea fungus. *Microbiologija*. **31**(1): 35-43.

Jayabalan Rasu, Malini Kesavan, Sathishkumar Muthuswamy, Swaminathan Krishnaswami and Yun Sei-Eok. 2010. Biochemical characteristics of tea fungus produced during *Kombucha* fermentation. *Food Sci. Biotechnol*. **19**(3): 843-847.

Jayabalan, R., Marimuthu, S. and Swaminathan, K. 2007. Changes in content of organic acids and tea polyphenols during *Kombucha* tea fermentation. *Food. Chem*., **102**: 392-398.

Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M. and Swaminathan, K. 2008. Changes in free-radical scavenging ability of *Kombucha* tea during fermentation. *Food Chemistry.* **109**: 227–234.

Jayabalan Rasu, Malbasa Radomir, V., Loncar Eva, S., Vitas Jasmina S. and Satish Kumar Muthuswamy. 2014. A review on *Kombucha* tea - microbiology, composition, fermentation,

beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety.* 13: 538-55.

Joshi, V.K., Sandhu, D.K. and Thakur, N.S. 1999. Fruit based alcoholic beverages. In: Biotechnology: Food Fermentation (Microbiology, Biochemistry and Technology). Joshi V K and Pandey A (eds.). Vol. II. Educational Publisher & Distributors, Ernakulum, New Delhi. p. 647.

Joshi, V.K. and Kumar Vikas. 2016. Influence of different sugar sources, nitrogen sources and *inocula* on the quality characteristics of apple tea wine. *Journal of Institute of Brewing.* (In press)

Klenner, F. 1971. Observation of the dose and administration of ascorbic acid when employed beyond the range of A vitamin in human pathology. *Journal of Applied Nutrition*, **23**: 1–26.

Kozaki, M., Koizuri, A. and Kitahara, K. 1972. Microorganisms of zoological mats formed in tea decoction. *J. Food Hyg. Society* (Japan). **13**: 89-96.

Kumar Vikas, Joshi, V.K., Vyas Gitanjali, Thakur, N.S. and Sharma Nivedita. 2016. Process optimization for preparation of apple tea wine with analysis of its physico-chemical, sensory and antimicrobial activities against food borne pathogens. *Nutrafoods.* **15**: 111-121.

Kumar Vikas, Joshi Vinod K., Vyas Gitanjali, Thakur, N.S. and Sharma Nivedita. 2016. Process optimization for preparation of apple tea wine with analysis of its physico-chemical, sensory and antimicrobial activities against food borne pathogens. *Nutrafoods.* **15**: 111-121.

Kumar Vikas, Joshi, V.K., Vyas Gitanjali and Tanwar Beenu. 2015. Effect of different types of fermentation (inoculated and natural fermentation) on the functional properties of apple tea wine. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **6**(3): 847-854.

Kumar Vikas. 2014. Preparation and evaluation of Tea cider. PhD Thesis. Dr. Y S Parmar UHF Nauni solan, Himachal Pradesh, India.

Kurian Alice and Peter, K.V. 2007. Tea. In: Commercial crops technology, Horticulture science series-8. Alice Kurian and K.V. Peter (eds.). New India Publishing Agency, New Delhi, p. 480.

Liu, C.H., Hsu, W.H., Lee, F.L. and Liao, C.C. 1996. The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiology*, **13**: 407-415.

Liyanage, A.C., Silva, M.J. and Ekanayaka, A. 1988. Analysis of major fat y acids in tea. *Sri Lanka J. Tea Sci.* **3**: 46-49.

Loncar, S.E., Petrovic, E.S., Malbasa, V.R. and Verac, M.R. 2000. Biosynthesis of glucuronic acid by means of tea fungys, *Nahrung*.

Malbasa, R., Loncar, E. and Kolarov, L.J. 2002. L-lactic, L-ascorbic, total and volatile acids contents in dietetic *Kombucha* beverage, *Romanian Biotechnological Let ers,* **7**(5): *ht p://www.unibuc.ro/eBooks/ biologie/RBL/vol7nr5/art2.htm*

Markov Sinisa, Cvetkovic Dragoljub and Bukvic Branka. 2006. Use of tea fungus isolate as starter culture for obtaining *Kombucha*. *Annals of the Faculty of Engineering Hunedoara*. Tome IV, Fascicole 3, (ISSN 1584-2673), pp. 73-78.

Martin, S.P. and Arnold, H.J. 1978. Tannins. In: Encyclopedia of Food Science. Vol 3. John Wiley and Sons, USA. pp. 732-734.

Mayser, P., Fromme, S., Leitzmann, C. and Grunder, K. 1995. The yeast spectrum of the '*tea fungus Kombucha'*. *Mycoses*. **38**: 289-295.

Morales, G.B. and Sanchez, H.H. 2003. Manufacture of a beverage from cheese whey using "tea fungus" fermentation. *Rev Latinoam Microbiol*. **45**: 5.

Mrdanovic, J., Bogdanovic, G., Cvetkovic, D., Velicanski, A. and Cetojevic-Simin, D. 2007. The frequency of sister chromatid exchange and micronuclei in evaluation of cytogenetic activity of Kombucha on human peripheral blood lymphocytes. *Arch. Oncol*. 15: 85-88.

Murugesan, G.S., Sathishkumar, M., Jayabalan, R., Binupriya, A.R., Swaminathan, K. and Yun, S.E. 2009. Hepatoprotective and curative properties of *Kombucha* tea against carbon tetrachloride-induced toxicity. *Journal of microbiology and biotechnology* **19**(4): 397–402.

Pasha Chand, Reddy Gopal. 2005. Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chemistry*. **89**: 449–453.

Pasha, C.H. and Reddy, G. 2005. Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chem*, **89**: 449–453.

Peron, A.D., Pat erson, J.A. and Yanofsky, N.N. 1995. *Kombucha* "mushroom" hepatotoxicity. *Ann. Emerg. Med*., **26**: 660-661.

Petrovic S. and Loncar, E. 1996. Content of water-soluble vitamins in fermentative liquids of tea fungus. *Mikrobiologija*. **33**: 101–106.

Petrovic, S., Loncar, E, Ruzic, N. and Kolarov, L.J. 1995–1996. Nutritive characteristics of tea fungus metabolites. *Faculty of Technology, Novi Sad, Proceedings*. **26–27**: 257–269.

Petrovska Biljana Bauer and Tozi Lidija Petrushevska. 2000. Mineral and water soluble vitamin content in the Kombucha drink. *International Journal of Food Science and Technology*. 35: 201–205.

Radomir, V. Malbasa Eva S Loncar and Ljiljana, A. Kolarov. 2006. Influence of black tea concentrate on *Kombucha* fermentation. *APTEFF*. **37**: 137-143.

Reiss, J. 1994. Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift fur Lebensmitel-Untersuchung und-For-schung*, **198**: 258-261.

Reiss, J. 1994. Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift fur Lebensmitel-Untersuchung und-For- schung*, **198**: 258-261.

Rodrigo Jose Santos Júnior, Rejane Andrade Batista, Sheyla Alves Rodrigues, Lauro Xavier Filho and Alvaro Silva Lima. 2009. Antimicrobial activity of broth fermented with *Kombucha* colonies. *J. Microbial Biochem. Technol.* **1**(1): 72-78.

Rosma, A. Amarjit Singh, Anton, Ann at al. 2016. Indigenous fermented foods: Fermented Meat Products, fish and fish products, Alkaline fermented foods, tea and related products. In V.K. Joshi (ed.). Indigenous fermented foods of South Asia, CRC Press, Taylor and Francis group, Florida, pp. 645-713.

Schillinger, U., Geisen, R. and Holzapfel, W.H. 1996. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends in Food Science and Technology*. **7**(5): 158-164.

Sievers, M, Lanini, C., Weber, A., Schuler Schmid, U. and Teuber, M. 1995. Microbiology and fermentation balance in *Kombucha* beverage obtained from a tea fungus fermentation. *Systematic and Applied Microbiology*, **18**: 590-594.

Sreeramulu, G., Zhu, Y. and Knol, W. 2000. Kombucha fermentation and its antimicrobial activity. *J. Agric. Food. Chem.*, **48**: 2589-2594.

Sreeramulu, G., Zhu, Y. and Knol, W. 2001. Characterization of antimicrobial activity in *Kombucha* fermentation. *Acta Biotechnol.* **21**: 49-56.

Srinivasan Radhika, Smolinske Susan, Pharmd and Greenbaum David. 1997. Probable Gastrointestinal Toxicity of *Kombucha* Tea, Is This Beverage Healthy or Harmful? *Journal of General Internal Medicine* **12**: 643–645.

Steinkraus, K.H., Shapiro, K.B., Hotchfiss, J.H. and Mortlock, R.P. 1996. Investigation into the antibiotic activity of tea fungus/*Kombucha* beverage. *Acta Biotechnologica*, **16**: 199–205.

Teoh, A.L., Heard, G. and Cox, J. 2004. Yeast ecology of *Kombucha* fermentation. *Inter J Food Microb*. **95**: 119-126.

Yurkevich, D.I. and Kutyshenko, V.P. 1998. Study of glucose utilisation during the growth of tea fungus by 1H NMR spectroscopy. *Biofizika*, **43**: 319-322.