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# *KOMBUCHA* DRINK: PRODUCTION, QUALITY, AND SAFETY ASPECTS

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## 8.1 Introduction

Evolution of ethnic foods and drinks is related to the developing civilizations across the globe. Besides combating general hunger, satiety and taste, various foods, and drinks are often consumed for their assumed and proven health benefits. Hot water extract of the dried leaves of tea plant (*Camellia sinensis*), with the popular global name "tea" is the oldest herbal medicine known for its stimulating and detoxifying properties. Tea has two main varieties, namely *sinensis* and *assamica*. Tea cultivation and drinking is believed to be originated in China about 5000 years back. Owing to its health-promoting and disease-resistant attributes, tea was considered as a "sacred drink" by the Japanese and Korean people, who perform special tea drinking sessions as a part of gathering rituals (Nishimura et al., 2010). According to a popular Chinese belief, tea is one of the seven basic necessities for life. British colonization caused maximum spread of tea processing and preparation practices across the globe. Today, tea is the most popular processed beverage. A range of tea classes, namely green tea, oolong tea, and their value-added variants are commercially available. In India, milk is added to the boiling black tea with certain spices make it tastier. Milk tea in Hong-Kong is prepared by using evaporated milk instead of liquid milk. Herbal "karkade" tea of Egypt is ascribed to have medicinal qualities. Thai cuisine "iced tea" variants are now popular across the global cold chain beverage market. In Myanmar, a pickle called "lahpet" is prepared by solid-state fermentation of tea leaves. Libyan tea variants have a strong green or black tea liquor base added

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with different ingredients and typically completed in three rounds of drinking. A similar three step drinking ritual is also followed for "green gunpowder tea" within a span of several hours in the Sahelian region of Sahara. People in East Frisian region of Germany traditionally prepare a three layered drink with layers of "wolkje" (a heavy cream cloud), black of green tea extract and a "kluntje"(a white sugar candy) at the bottom. Russian "chiffir" is a very concentrated tea which is often drunk after dilution. A popular tea culture is also prevalent in Canada. Tribes in Ontario use cedar tea during "sweat lodge sessions" and believe in its body purifying properties. Tea availability, its varied forms and their contribution to physical and mental wellness remains their key to popularity as well as global acceptability.

*Kombucha* is a beverage produced by a traditionally practiced fermentation technique involving fermentation of sugar-dissolved black tea by a symbiotic culture of bacteria and yeast (SCOBY). This bell-shaped cellulosic spongy SCOBY culture is also known as "tea fungus." This fermented beverage, when freshly prepared, tastes like sweet sparkling apple cider. A prolonged fermentation results in development of vinegar-like acidic flavor to the sparkling beverage. The main bacterial strains identified the traditional SCOBY are *Acetobacter xylinoides*, *Komagataeibacter xylinus*, *Gluconacetobacter xylinus*, *Acetobacter aceti*, and *Acetobacter pasteurianus*. Yeasts, namely *Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Torulaspora*, *Zygosaccharomyces bailii*, *Brettanomyces bruxellensis*, *Brettanomyces lambicus*, *Brettanomyces custersii*, *Candida*, and *Pichia* species have been reported (Zhang et al., 2011; Yamada et al., 2012). *Acetobacter* species reportedly forms the cellulosic network which forms as the physical base for the symbiosis development. A cumulative fermentation of sugar by yeasts and bacterial species results in the formation of ethanol and acids which are natural preservatives responsible for long shelf-life of the fermented beverage. Besides these, a number of other health-supporting bioactive components are known exist and be formed in the liquor during the course of fermentation. They add to the nutraceutical level of the tea-based drink. *Kombucha* has been traditionally claimed to have antibiotic properties, improve gastrointestinal and glandular functions, relieves joint rheumatism, positive cholesterolemic effects, detoxification of blood, ageing problems, etc. Research on tea composition and its health-promoting factors has been carried out in plenty during the late 20th and 21st centuries. Although effects of *kombucha* drinking on animal models have been confirmed, comparable mechanisms in humans remain uncertain till date. Serious health hazards have also been reported and attributed to drinking *kombucha*. Despite of being a popular folk remedy to various illnesses, the product needs authentic validation. Composition of *kombucha* and its functionality has very recently gained its due scientific and commercial popularity.

### 8.1.1 History of Kombucha

The earliest available report on *kombucha* consumption dates back to 220 BC in China, during the Chin dynasty. They termed the tea fungus as the divine "Ling-tche" or "Che". In AD 414, a Korean doctor named Kombu was summoned to cure the digestive troubles of Japanese king Inkyo. Kombu brought the divine Che with him, which can be believed to be later popularized in Japan as *Kombucha*. A wide acceptability of the drink in those periods is generally harangued. The Japanese warriors used to take the refreshing and strengthening drink even to the battle fields (Roche, 1998). Some other names for the beverage and its variants are the *Eastern tea*, *Fungus japonicus*, *olinca*, *Pichia fermentans*, *Cembuya orientalist*, *Combuchu*, *Tschambucco*, *Volga spring*, *Mo-Gu*, *Champignon de longue vie*, *Teekwass*, *Kwassan*, and *Kargasok tea*. Other than China, Korea, and Japan, a popular practice of yeast-cultured tea consumption was prevalent in the Kargasok region of Russia. The folks of the region were known to live a healthy lifestyle, showed retarded ageing with an average lifespan exceeding hundred years (Williams, 2001). With extension of trade routes, tea fungus, and the *kombucha* beverage found their way to migrate throughout the world. *Kombucha* drinking and retailing became a popular practice in Europe until the World War II, when an unavoidable scarcity of tea and sugar (the basic ingredient materials for the fermentation) occurred. Another historical reason which can be also considered for lesser information on *kombucha* is the ethnic religious belief and taboo associated with the drink and the tea fungus. It is especially applicable for the Asian countries where it is believed to have originated. The beverage was often prepared in households, suitably in prayer areas. There is strong evidence that the Moravian monasteries kept their fermented tea beverage "Olinka" as a closely guarded secret. Praying areas were necessarily kept clean and had lesser chances of contamination. This practice, gradually made the *kombucha* practice "divine" and a myth on it was developed. It was considered unholy to sell the tea fungus and it passed from household to household as a gift. The authors could observe similar believes persisting even today in *kombucha* culturing households of Assam, India (not reported). *Kombucha* tea was high in demand in Italy and other parts of the world until the 1950s, when a rumor on toxic effect of the drink was spread. Probably as a result of this, research was initiated in the 1960s by Swiss scientists who reported that drinking *kombucha* was similarly beneficial as eating yogurt. After the Chernobyl disaster of Russia in 1986, damage analysis indicated that people consuming *kombucha* on a regular basis were resistant to the nuclear radiation effects. *Kombucha* has regained its popularity in the past two decades and its commercial brewing has seen a notable escalation especially in the United States in addition to other parts of the world.

## 8.1.2 Brewing Technique

Black tea sweetened with sucrose is considered to be the ideal substrate for *kombucha* beverage fermentation. Green tea is the popular alternative to black tea. The amount and size of added SCOBY culture may also vary. A standard method as described by Jayabalan et al. (2014) is given in Fig. 8.1. This method is based on several other previously published reports on *kombucha*. Briefly, 50 g of sucrose is

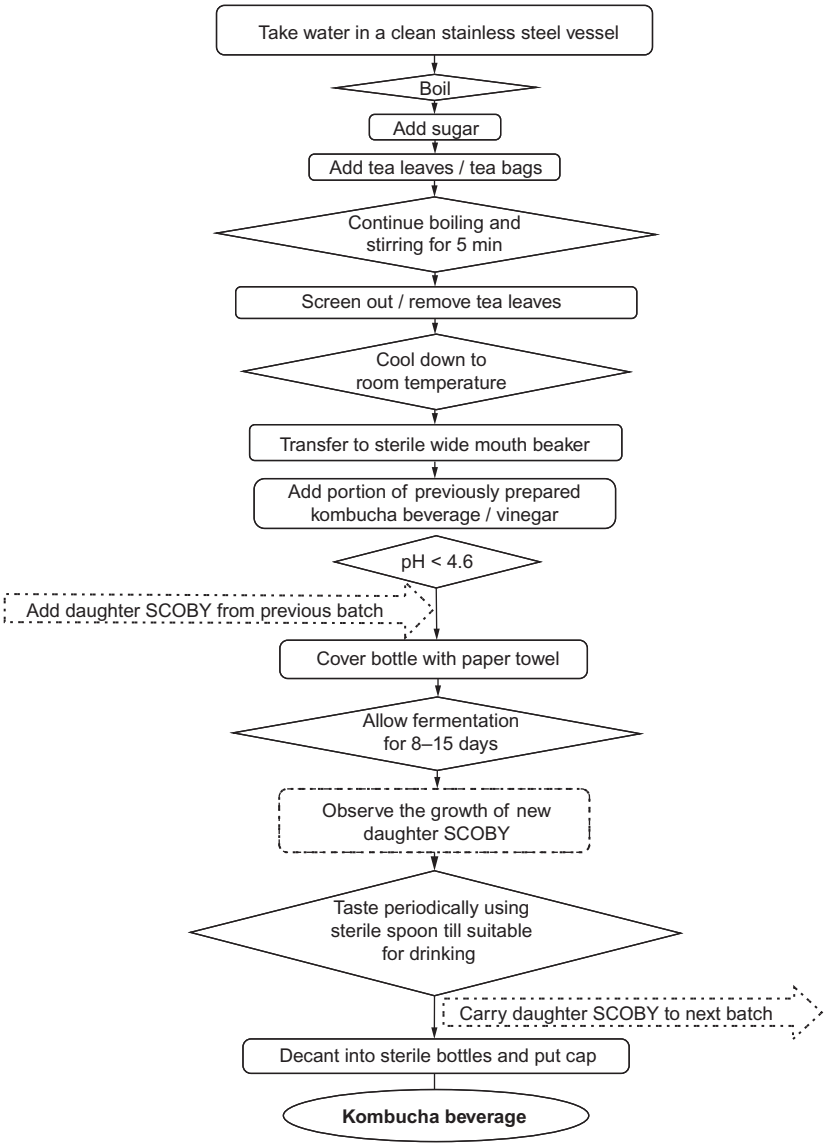


**Fig. 8.1** Process flow diagram for the production of kombucha beverage.

dissolved in 1 L of boiling tap water. However, a 70 g/L concentration is popularly practiced by most *kombucha* researchers (Malbaša et al., 2008a, b). To the sugar solution, 5 g tea leaves are added, stirred, and removed by screening after 5 min. Alternatively, tea bags can also be used to ease the operation (Morshedi and Dashti-Rahmatabadi, 2010). The mixture is cooled down to 20°C before inoculating with 24 g of the SCOBY. The solution with the SCOBY is then transferred to a heat sterilized beaker. Previously fermented *kombucha* beverage (200 mL) is added to lower the pH. A low pH (<4.6) controls the growth of undesirable contaminating microbes. Drosophila fruit flies are attracted to acidic sweetened solutions and often contaminate them. To avoid such contamination, the mouth of the beaker is covered with a paper towel. The mixture is then incubated within a temperature range of 18°C and 28°C. In a few days a "daughter" SCOBY emerges from the inoculated mother SCOBY and microbes suspended in the broth. Step wise demonstration of formation of daughter SCOBY from mother SCOBY during *kombucha* fermentation is presented in Fig. 8.2. It is a clear, thin gel-like membrane floating at the top of the mother SCOBY and may be attached to the mother SCOBY at certain points. Distinct gas bubbles and fermented smell is sensed at this stage. With progress in fermentation, the mother SCOBY tends to sink to the bottom of the fermenting beaker. The daughter SCOBY increase in size and covers the surface of the liquid by the 10th to 14th day of incubation with pH level as low as 2.0. The duration may vary depending on the SCOBY composition and rate of fermentation. It is then carefully removed with a spoon and kept in small volume of fermented tea to avoid drying and for future use. The remaining tea is filtered, filled in capped bottles, and stored at 4°C for consumption. It is necessary to sterilize the bottles to avoid cross-contamination. The authors states that a 50 g sucrose/L of solution is optimum for the necessary production of ethanol and lactic acid in the *kombucha*. However, necessity of optimizing the fermentation time for best quality and safe *kombucha* was also suggested. Malbaša et al. (2006) scaled-up the black tea fermentation in differently sized fermenting vessels and related the process variables and changed pH with the geometrical shape of the vessels. Large-scale vessels with similar geometry showed correlation between vessel size and process duration as a function of pH change. High volume *kombucha* fermentation can also significantly lower the input costs of fungus, electricity, machinery, and rent land expenses (Mohammadshirazi and Kalhor, 2016). Many modern *kombucha* breweries have installed large-scale batch fermentation set-ups for its industrial-scale production. Although the domestic-scale method and conditions of fermentation have remained almost the same for large-scale processes, critical care should be taken for the product safety. Ideally, clean water, efficient

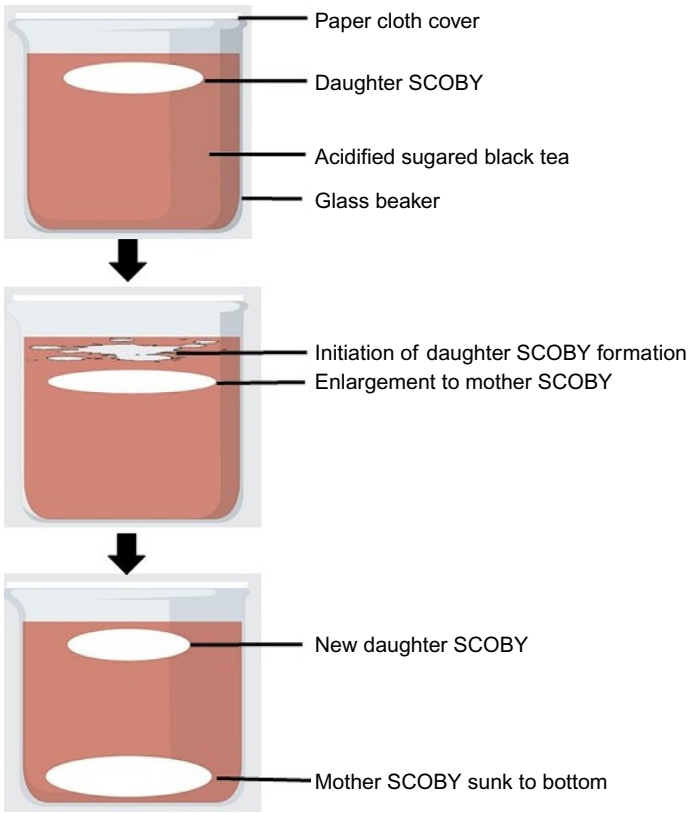


**Fig. 8.2** Step wise demonstration of formation of daughter SCOBY from mother SCOBY during *kombucha* fermentation.

sterilization of fermenter, and bottles should be ensured. Hard water with dissolved minerals, salt, and alkali may hamper the growth of the SCOBY. Use of sterilized deionized water is a safer option. If an automated fermenter is not used and fermentation is done in a wide mouthed vessel, the mouth of the vessel is kept covered with a paper towel during fermentation. The towel should be dry-sterile. The closure should be suitable and sufficient to avoid insect and airborne microbes, but should allow gas passage. *Kombucha* fermentation required partial aerobic conditions and sufficient release of $CO_2$ formed during fermentation in order to produce the characteristic acids and metabolites. A two-stage method has been described by several *kombucha* makers in their blogs and reported to produce better quality beverage. In this, the daughter SCOBY is used for a second stage of the *kombucha* fermentation. Alternatively, some follow a secondary fermentation technique in which the daughter SCOBY is also removed and yeasts that remain suspended in the liquor is allowed to further ferment the material under anaerobic environment. This reportedly increases the dissolved $CO_2$ content in the beverage.

Several home-scale *kombucha* makers have recently come up with larger-scale enterprises. *GT's* Living Foods started selling bottled *kombucha* drink in 1995 in Los Angeles Health Food Store, Erewhon. It is known to be the first commercial retailing of *kombucha* in the modern world. Recently, *kombucha* microbreweries have taken up production of the beverage and also its different flavored variants. The Happy Leaf Kombucha Microbrewery of Denver, Colorado started in 2013. They produce a range of differently flavored *kombucha* drinks. The fermentation is carried out in fermentation tanks of more than 3500 L capacity. The SCOBY weight in such tanks increases even up to 90 kg. When the pH level drops to about 2.7, the liquor is transferred to a stainless steel, glycol-lined brite tank. The *kombucha* here is kept at subzero temperature to deactivate the fermenting bacteria and yeast in the liquor. Different flavors are added to it in this stage. The flavors take 2–5 days for full integration in the liquor depending on the specific potency of the flavor compound. Transferring of *kombucha* from fermenter tank to brite tank may cause loss of dissolved $CO_2$ due to agitation. Besides, consumers often demand for higher and bubbly fizz in their *kombucha* drink. Happy leaf kombucha, after flavor addition, is strained and pumped into kegs along with pressurized $CO_2$ dissolution. The force carbonated *kombucha* is then manually bottled, capped, labeled, and boxed. A temperature of 2–4°C is maintained in the bottles and kegs to ensure freshness of the drinks. A nonexhaustive list of different manufacturers of *kombucha* and *kombucha* products, their brand name, product type, etc. are provided in Table 8.1.

## 8.2 Tea and Non-Tea *Kombucha*

Black tea is the most common form of commercial powdered tea and is used as the predominant raw material for *kombucha* fermentation (Malbaša et al., 2006; Jayabalan et al., 2014; Wilburn and Ryan, 2016). Yeast cells in the SCOBY hydrolyze sucrose into glucose and fructose by the enzyme invertase and also produce ethanol. Acetic acid bacteria utilize glucose and ethanol to produce gluconic acid and acetic acid, respectively. Glucose also transforms to glucuronic acid by oxidation at the C-6 position of its molecule. Lactic acid is formed from glucose and sucrose by the lactic acid bacteria. Fermentation time, type of raw material, and the native microbiota of the SCOBY are the dependent factors responsible for formation of functional metabolites during fermentation. Lončar et al. (2006) suggested that the process duration is a more influencing factor than temperature and inoculum concentration on the composition of *kombucha* beverage. The gradually lowering pH during the course of fermentation provides various suitable condition sets for formation of metabolites and their conformational changes. However, considering the complex

# Table 8.1 *Kombucha* and *Kombucha* Product Manufacturers (Nonexhaustive List) and Their Product Types

| Company | Establishment Year | Brand | Company Websites/ Weblinks | Products |
|---|---|---|---|---|
| Townshend's Tea Company | 2008 | Brew Dr. Kombucha | http://brewdrkombucha.com/ | Organic herbal blended *kombucha* |
| The Kombucha Shop | 2013 | The Kombucha Shop | https://www.thekombu-chashop.com | *Kombucha* brewing kit, jars, bottles and accessories, organic blended *kombucha* premix, *kombucha* culture and starter |
| Humm Kombucha | 2008 | Humm | https://hummkombucha.com | Basic and flavored *kombucha* |
| Ithaca Kombucha Company | 2015 | IKC Lucky Brew | http://www.ithacakombucha-company.com | *Kombucha* sauce, ingredients, flavored *kombucha* drinks |
| Kombucha Dog | 2008 | KOMBUCHADOG | http://www.kombuchadog.com | Blended *kombucha* beverage |
| Puget Sound Kombucha Company | 2012 | PUGET SOUND KOMBUCHA | https://soundkombucha.com | Blended *kombucha* beverages |
| The Kombucha Tea Company | Not known | THE KOMBUCHA TEA CO. | https://thekombuchateaco.com | Blended *kombucha* beverages |
| Revive Kombucha | 2010 | Revive | https://www.revivekombucha.com | Blended *kombucha* beverages |
| Health-Ade kombuca | 2012 | HEALTH-ADE KOMBUCHA | https://health-ade.com | Blended *kombucha* beverages |
| Fine Feathers Kombucha Company | 2014 | Fine feathers KOMBUCHA CO. | finefeatherskombucha.com | Flavored and blended *kombucha* |
| Kombucha Brooklyn | 2009 | KBBK KOMBUCHA BROOKLYN | https://kombuchabrooklyn.com | *Kombucha* cultures, home brewing kits and equipments, ingredients |
| Marin Kombucha | 2015 | MARIN KOMBUCHA | marinkombucha.com | Oak-aged flavored *kombucha* |
| Buchi Kombucha | 2008 | Buchi KOMBUCHA | http://www.drinkbuchi.com | Blended *kombucha* |
| Salt Spring Island Kombucha | Not known | SALT SPRING ISLAND KOMBUCHA | https://www.ssikombucha.com | Organic herbal blended *kombucha* |

Data collected from the official website of the company.

microbiota in the SCOBY and their versatility in SCOBY collected from different regions, several other factors should also be considered while designing a *kombucha* process (Dufresne and Farnworth, 2000).

Jayabalan et al. (2007) prepared *kombucha* using black tea, green tea, and tea manufacture waste. The authors observed marked variation in changes of organic acids (lactic, acetic, gluconic, and glucuronic acid) and tea polyphenols (catechins, namely epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate) during fermentation of the beverage. Increase in acetic acid concentration continued up to 15th day of fermentation and was highest in green tea *kombucha* (9.5 g/L). It showed a gradual decrease after that. Glucuronic acid, the key organic acid in *kombucha* responsible for its detoxifying effect was highest on the 12th day for black tea (2.33 g/L) followed by green tea (1.73 g/L). Depletion in lactic acid content was observed for all tea variants after the 3rd day of fermentation. Trace levels of citric acid (0.03–0.17 g/L) could be detected on 3rd day incubated samples. A general observation was that all the four catechins exhibited degradation up to 9th day of *kombucha* fermentation. This decrease in epicatechin and epigallocatechin was lowest for green tea *kombucha* followed by black tea *kombucha* and tea industry waste *kombucha*. A notable increase in all the catechin concentration in all three *kombucha* variants was observed on the 12th day. This was attributed to rupture of acid-sensitive microbial cells producing these compounds on lowering of pH to near 2.0. However, concentration of epicatechin and epigallocatechin could only rise to levels higher than that of the initial concentration (0th day). A possible biotransformation of epigallocatechin-3-gallate and epicatechin-3-gallate to their catechin forms due to pH changes was attributed to this difference. Theaflavin and thearubigins are polyphenols present only in black tea. These two polyphenols showed uniform degradation in the black tea *kombucha* fermentation medium till 18th day of fermentation. Unidentified microbial enzymes causing conversion of the bioactive compounds in *kombucha* drink were another possibility for their structural instability, as suggested by the authors. Antioxidant activity of tea polyphenols is also dependent on their conformational structures, the substituent groups attached to their molecular ring structures and their degree of polymerization (Botten et al., 2015). Three structural criteria for a compound with free-radical scavenging activity are that it should possess at least (i) a 3-hydroxy group on a unsaturated C ring or (ii) a 2,3-double bond with the 3-OH group and 4-one in the C ring or (iii) an ortho-OH substitution pattern in the B ring where the OH groups are not glycated. Catechin and epicatechin molecules fulfill the first and third structural criteria. In another study, increased free-radical scavenging activity of the three aforementioned *kombucha* variants was observed during fermentation (Jayabalan et al., 2008).

Structural instability of epigallocatechin-3-gallate and epicatechin-3-gallate due to some identified enzymatic actions could be related to better scavenging performance on nitrogen and superoxide radicals (1,1-diphenyl-2-picrylhydrazyl DPPH, xanthine-xanthine oxidase system, and ammonium thiocyanate assay) and poorer scavenging activity on hydroxyl radicals (ascorbic acid-iron EDTA and thiobarbituric assay). These studies indicated that black tea is the superior raw material for *kombucha* fermentation.

Tea waste was found to be a potentially lower grade material, although its use in *kombucha* preparation may serve as a way of waste utilization in the tea industry. Stabilizing catechins in green tea by metabolic manipulation of SCOBY culture could make it suitable for commercial *kombucha* preparation. Under the given conditions, a 12 day fermentation time could be considered optimum for all the *kombucha* variants as a prolonged fermentation time could yield more of the organic acids, reaching harmful levels for direct consumption. Ion chromatographic study by Kumar et al. (2008) revealed that black and *kombucha* tea differ significantly in their anionic mineral composition, namely fluoride, chloride, bromide, iodide, nitrate, phosphate, and sulfate. The acetate and formate ions formed during *kombucha* fermentation and their co-elution with fluoride was a major observation. Peak for chloride showed a drop while intensity of peak for sulfate and iodide increased drastically. Although black and green tea remains to be the traditional and ideal substrates for *kombucha* preparation, newer plant sources have also been proposed for their applicability for *kombucha* preparation.

If the SCOBY growth is not inhibited by the constituents and metabolites formed are in parity with the health stimulating effects of tea *kombucha*, any edible plant extract mixed with the right amount of sugar can be used for preparing *kombucha* drink. Such trials have been taken up by a number of authors and such products have been termed as "*kombucha* adjuncts" (Battikh et al., 2012).

Molasses from sugarcane and sugar beet are popular raw materials in the alcohol and vinegar industries. It is the semisolid by-product left after sugar crystals are formed and removed during sugar crystallization process. Sugar beet molasses can be a cost-effective alternative raw material to tea syrup as was thoroughly investigated by several researchers (Lončar et al., 2000; Malbaša et al., 2008a, b). In their study, the authors observed that the concentration of molasses in the media is inversely proportional to drop in pH values and directly to the rise in acetic acid concentration. The pH drop was attributed to formation of lactic acid. The authors opined that a higher rate of acetic acid fermentation can be achieved by using low concentration (<50 g/L) of molasses sucrose solution than pure sucrose (70 g/L). In these studies, molasses proved to be a better option than pure sucrose if predominant formation of

lactic acid in the *kombucha* is targeted. Interestingly, a high pH (>6.0) in the most concentrated molasses sugar sample (70 g/L) also showed a high content of total acids (approximately 2.4 g/L). This indicated that molasses adjunct of *kombucha* would have a reduced sour taste in spite of the high content of organic acids, which can be a suitable quality attribute for commercial *kombucha* beverage.

Battikh et al. (2012) cultured *kombucha* SCOBY in sugared herbal extracts of thyme (*Thymus vulgaris* L.), lemon verbena (*Lippia citriodora*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*), and pepper mint (*Mentha piperita*). These adjuncts along with typical black tea cultured *kombucha* were tested for antibacterial and antifungal activity against seven each of pathogenic bacterial and candida yeasts strains using agar diffusion method. Fermented extracts of lemon verbena and fennel showed the highest activity, especially against the *Candida* species. Thyme *kombucha* did not show any microbial growth inhibition. Lactic and acetic acid produced during fermentation contributes an add-on effect to the antifungal activities of the native herbal extracts. Thermal sensitivity of other antimicrobial agents in the native and fermented adjuncts was another observation from the study. It is very likely that some functional compounds present in the raw materials, such as tea catechins are modified to dysfunctional epimeric forms during the thermal treatment exerted for preparing the extracts (Mika et al., 2015).

*Kombucha* liquor broth blended with wheat grass (*Triticum aestivum* L.) juice was found to successfully fermented by a typical *kombucha* SCOBY with major bacterial components identified as *Gluconacetobacter rhaeticus* and *Gluconobacter roseus* and the yeast component as *Dekkera bruxellensis*. The resulting liquor blend exhibited markedly high oxygen scavenging as well as oxygen radical absorption capacities. These effects were highest for a 1:1 (v/v) blend of black tea with wheatgrass juice fermented for 3 days (Sun et al., 2015). Commercial fermented products are often sold in pasteurized forms so as to destroy the potential pathogens as well as to avoid excessive fermentation. Pulsed electric field (PEF) is an efficient modern technique used to destroy microorganisms in beverages (Barba et al., 2015). Rapid electric pulses are generated to produce alternating magnetic fields around the microbes, which destroys their cell membranes leading to their lethality. Bark and leaves of Oak (*Quercus* sp.) are rich sources of polyphenols and antioxidants. Their extracts are used to prepare refreshing drinks in Mexico (Rosales-Castro et al., 2012). Oak leaf *kombucha* adjunct produced by Vázquez-Cabral et al., 2017was found to be effective against oxidative damage and inflammation in human macrophage cells. Freshly prepared oak leaf *kombucha* analogue, when treated with PEF showed no change in pH and soluble solid contents. Low-energy PEF treatments at higher feed flow could sufficiently

destroy the yeasts and bacteria, with minimum color change and render products with higher polyphenolic content and antioxidant capacity (Vazquez-Cabral et al., 2016). Release of bioactive compound from the PEF degenerated cells can be attributed to such positive changes. King coconut (*Cocos nucifera* var. aurantiaca) water could be successfully used as an alternative media for *kombucha* fermentation (Watawana et al., 2016). The fermented coconut water was more acidic, yellowish, thicker, and higher in antioxidant and starch hydrolase inhibitory activities than a non-fermented control sample. Addition of banana (*Musa sapientum*) peels and nettles (*Urticadioica*) leaf infusions, both having reportedly high antioxidant and antimicrobial properties resulted in increased polyphenol content in the fermented tea broth. Fermentation reportedly increased antioxidant capacities of both banana peel as well as nettles (Pure and Pure, 2016a, b). Lemon balm (*Melissa officinalis* L.) is a well aromatic herb which is also used as a medicinal plant. Its aqueous extract, popularly called "lemon balm tea" is used for gastrointestinal treatments and as antibacterial and antifungal agents (Dastmalchi et al., 2008). Such similar health-promoting nature makes it another suitable candidate as *kombucha* adjunct. Četojević-Simin et al. (2012) prepared *kombucha* beverage from lemon balm tea which showed significant antimicrobial activity against bacteria but not molds. Antigenotoxic effects of lemon balm tea and its *kombucha* were confirmed in Chinese hamster cell line CHO-K1 by decreased chromosome aberrations frequency. Lemon balm, instead of tea has also been successfully used for *kombucha* fermentation by Velićanski et al. (2014) and reportedly showed higher antioxidant activity against DPPH radicals than traditional black tea *kombucha*. Garlic fermented in *kombucha* vinegar (beverage, pH 2) showed higher retention of organosulfurs, presence of linoleic and α-linoleic acids indicating that *kombucha* can be a suitable medium for high-quality fermented garlic (Pure and Pure, 2016a, b).

Fermented milk products have been important non-tea models for researchers in *kombucha* fermentation. *Kombucha* SCOBY contains a large proportion of lactic acid bacteria, which primarily makes it a suitable inoculum for milk fermentation. Local *kombucha* cultures grown on sweetened black tea, green tea, and topinambur juice were concentrated by vacuum evaporation and applied in concentrations of 10% and 15% (v/v) to milk for fermentation (Malbaša et al., 2009). Fermentations were stopped at pH 4.4. A commercial yogurt culture gave a linear fermentation rate curve while *kombucha* cultures gave sigmoidal patterns. Viscosity was higher for *kombucha* fermented samples. The samples showed high values of sensorial acceptance after 5 days of storage. *Kombucha* fermented milk exhibits an earlier protein gelation (at higher pH) than commercial yogurt

and probiotic cultures, giving a better textured product than that of probiotic fermented milk (Hrnjez et al., 2014a). Mathematical models for predicting texture of milk fermented by *kombucha* cultures of peppermint (*Mentha piperita*, Lamiaceae), wild thyme (*Thymus serpyllum*, *Lamiaceae*), winter savory (*Satureja montana*, *Lamiaceae*), and stinging nettle (*Urtica dioica*, *Lamiaceae*) was developed by Malbaša et al. (2015). Hrnjez et al. (2014b) also suggested that *kombucha* SCOBY culture can be effectively used for milk fermentation to produce milk products suitable for human health. The authors used *kombucha* starter culture for fermenting milk without any sugar added to the milk. They compared its effect with that of commercial probiotic and yogurt starter cultures. Similar trend of change in pH, degree of proteolysis of milk proteins, vitamin C content, and sensory properties were obtained during 14 days of fermentation. Oxygen scavenging activity was found to lower down with time of fermentation. Angiotensin I hormone in the blood stream is converted to angiotensin II by the angiotensin converting enzyme (ACE) present in lung capillaries and kidney endothelium. Angiotensin II is an active vasoconstrictor responsible for high blood pressure. Besides, ACE also causes degradation of the vasodilator bradykinin which acts against blood vessel constriction. Milk fermented with *kombucha* culture showed notably high ACE inhibitory effect at the end of 14 days storage than samples fermented with yogurt and probiotic cultures. Although no direct correlation between degree of proteolysis and ACE inhibition was found, it was opined that proteinase and peptidase activities in the culture lead to formation of functional peptides with ACE inhibiting properties. Some polyphenol compounds and $Ca^{2+}$ ion present in the *kombucha* culture could also show such inhibitory activity (Gonzalez-Gonzalez et al., 2011). Recently, Elkhtab et al. (2017) isolated peptide fractions of *kombucha* fermented milk using HPLC equipped with reverse-phase column and further purified and identified them with liquid chromatography-mass spectrometry/mass spectrometry. Several identified new potential peptides were chemically synthesized. Three of such peptides, namely VAPFPEVFGK, LVYPFPGPLH, and FVAPEPFVFGKE showed high potency against ACE *in vitro*.

## 8.3 *Kombucha* Metabolites

A substantiate load of functional molecules in the *kombucha* drink makes it a product with multifaceted biochemistry. Such molecules are proved as well as believed to have accelerating capacities in human metabolism. Most of the *kombucha* metabolites are known to originate from the raw material tea. Tea contains a number of polyphenols, flavonols (theaflavins and thearubigins), catechins, caffeine, catechin

gallates, adenine, theobromine, theophylline, gallic acids, tannins, and gallotannin, which makes it a complex system with potentially high antioxidant property. The antioxidant activity of a molecule is the measure of its free-radical scavenging property. It is further dependent on the isomeric structure, substitute groups attached to the flavonoid rings and their degree of polymerization (Loganayaki et al., 2013). Structures of many of these metabolites are transformed and new structures are formed during the acid-alcoholic fermentation of *kombucha* tea. Among the new functional compounds formed in the fermented brew, acetic acid, lactic acid, gluconic acid, and glucuronic acid are the characteristic ones. Invertase enzymes secreted by yeasts in the SCOBY hydrolyze the sugar substrate to its monomers, namely glucose and fructose and further convert them to ethanol through glycolysis. The partially aerobic condition results in activity of the acetic bacterial strains to act upon glucose and ethanol to produce gluconic acid and acetic acid, respectively. Lactic acid is produced by action of lactic acid bacteria on sugars. A detail on their metabolic formation has been recently reported by Jayabalan et al. (2014). Other products, namely, citric acid, tartaric acid, malonic acid, oxalic acid, succinic acid, pyruvic acid, B complex vitamins, essential minerals, ascorbic acid, amino acids, biogenic amines, purines, antibiotics are also reportedly formed. The exact mechanism of their formation is not yet defined. Variable formation and proportion of these characteristics as well as noncharacteristic metabolites have been mostly attributed to difference in microbial distribution in the SCOBY, composition of the tea leaves, and concentration of sugar and fermentation rate. Many health promoting activities of metabolites present in *kombucha* tea have been identified. Many such metabolites, although supposed to be present and exerting nutraceutical support to the consumers, have not been identified yet. The exact metabolism of all the *kombucha* metabolites in tea consumers' physiological pathways is yet to be studied in detail. However, a number of general nutritional properties and benefits related to the *kombucha* drink's bioactive potential have been studied. Physiological and health effects of various constituents (metabolites) of *kombucha* drink obtained from different readily available sources are also listed in Table 8.2.

## 8.4 Nutritional and Bioactive Potential

*Kombucha* drinking has been believed to be linked with a number of physiological improvements of human. General observation and survey-based reports indicate such improvement in general health of population masses who consume *kombucha* on a regular basis. Such reports are also associated with tea consumption practices that are

# Table 8.2 Physiological and Health Effects of Various Constituents (Metabolites) of *Kombucha* Drink Obtained from Different Readily Available Sources

| Metabolite Name | Sources | Physiological and Health Effects | References |
|---|---|---|---|
| Acetic acid | Different vinegars | Hypertension, hyperlipidemia, obesity, hypolipidemia | Samad et al. (2016) and Chen et al. (2016) |
| Lactic acid | Probiotic culture of lactic acid bacteria | Colon health, vaginal and uterine health, reduce mercury toxicity from foods, brain health, delivery of bioactive macromolecules, immune modulation | Tachedjian et al. (2017), Genís et al. (2017), and Jadán-Piedra et al. (2017) |
| Gluconic acid | Commercial gluconates | Colon health and tissue repair | Asono et al. (1994) |
| Glucuronic acid | Commercial gluconolactone and glucouronolactone | Precursor of ascorbic acid in mammals, inflammatory regulation | Lewis et al. (2013) |
| Invertase | Yeasts (*Saccharomyces cerevisiae*, *Candida utilis*, *Aspergillus niger*) and commercial β-fructofuranosidase | Manufacture of artificial honey, antimicrobial and antioxidant activity | Kulshrestha et al. (2013) |
| Citric acid | Commercial, citrus fruits, *Penicillium* and *Aspergillus* fermentation | Regulates the enzyme acetyl-CoA carboxylase, improves bone density | Kamzolova and Morgunov (2017) |
| Tartaric acid | Wine | Expectorant, antioxidant, improve intestine function, glucose tolerance | Shahidi et al. (2008a) |
| Malonic and malic acid | Commercial and apples | Acidity regulator, reduce muscle pain in fibromylagia | Shahidi et al. (2008b) |
| Succinic acid | *Microorganisms* (*Escherichia coli*, *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*) | Acidity regulator, flavoring agent | McKinlay et al. (2007) and Liu et al. (2008) |

## Table 8.2 Physiological and Health Effects of Various Constituents (Metabolites) of *Kombucha* Drink Obtained from Different Readily Available Sources

| Metabolite Name | Sources | Physiological and Health Effects | References |
|---|---|---|---|
| Pyruvic acid | Pyruvate salt of calcium added in health supplements | Weight loss, improves cardiac function | Onakpoya et al. (2014) and Jaimes et al. (2016) |
| Usnic acid | Lichen | Antimicrobial | Guo et al. (2008) |
| Ascorbic acid | Wine, commercial | Antioxidant, preservative, co-factor in a number of metabolic pathways | Quadros et al. (2016) |
| Biogenic amines | Wine, seafoods, meat, cheese, fermented sausage and vegetables | Neurotransmitter | Chase and Koelle (2007) |
| Purines | Meat products especially organs | Neuro-regeneration and neuro-protection | Lahiri et al. (2007) |
| Theaflavins | Black tea | Antioxidant, anti-HIV-1 activity, reduce blood cholesterol, anticancer | Liu et al. (2005) and Senanayake (2013) |
| Catechins | Black tea, green tea, wine, cocoa, pome fruit | Blood flow regulation, intestinal absorption, antidiabetic effect | Matsui (2015a, b) |
| Gallic acid | *Punicagranatum* L., wine | Antifibrotic effect, prevent Parkinson's disease, Antipathogenic effect | Vaquero et al. (2007) |

associated with metabolites present in tea (Suzuki et al., 2012). Reports on added benefits of *kombucha* beverage has been often doubted as an exaggerated hype (Dufresne and Farnworth, 2000; Greenwalt et al., 2000). Considering its long history of global consumption as a health supporting drink, *kombucha* is being continuously explored for its inherent properties and validated several facts of general believes associated with it. A few such reports available till date are discussed here, while numerous other credible beneficial molecular mechanisms are yet to be explored and revealed.

### 8.4.1 Antiinflammatory and Anticancer

Cellular inflammation has been a prime risk factor for cancer development. Molecular supplementations in the form of drugs or nutraceuticals have been successful in regulating inflammation and inflammatory ailments in humans (Nasri et al., 2014; Larussa et al., 2017). Srihari et al. (2013) examined the antiangiogenic effect of lyophilized and reconstituted *kombucha* in human androgen-independent prostate cancer cells. Effect on the angiogenesis stimulators or regulators, namely human inducible factor-1α, vascular endothelial growth factor, interleukin-8, cyclooxygenase-2, and matrix metallophosphatase were studied. Remarkable inhibition by downregulation of the stimulators was reported, suggesting remedial use of *kombucha* for prostate cancer patients. *Kombucha* may thereby also assist in developed susceptibility by decreasing resistance of tumor cells, causing decreased cell proliferation, progression, metastasis, and angiogenesis. Tissue damage in alloxan-induced diabetic rats could also be cured by dose-dependent feeding of *kombucha* beverage (Bhattacharya et al., 2013). A dose of 150 mg of *kombucha* extract per kilogram bodyweight fed for 14 days could also ameliorate DNA fragmentation and caspase-3 activation in the pancreatic tissue of diabetic rats. Chromate compounds are well-known carcinogenic agents. Oral feeding of chromate in rats caused significant elevation in plasma and tissue lipid peroxidation (an oxidative reaction) and reduction in delayed hypertensive response, indicative of oxidative stress development (Ram et al., 2000). *Kombucha* tea feeding could completely reverse such changes in the subject rats. Studies have revealed that ultrahigh-frequency microwave radiation from mobile phones can cause increased trace element levels in human brain, kidney, and heart tissues resulting in oxidative stress development (Ragy, 2015; El-Bediwi et al., 2011). A study was conducted by Gharib (2014) where rats were exposed to such high-frequency electromagnetic field (950 MHz) for 8 weeks. Rats fed with *kombucha* tea while being exposed to radiation exhibited reduced response to these adverse effects, suggesting radical-scavenging and ameliorative effect of *kombucha* on electromagnetic damage in mammalian tissues. *Kombucha* adjunct from three oak species were found to possess antiinflammatory properties when tested in lipopolysaccharide-stimulated human macrophage cells (Vázquez-Cabral et al., 2017). Production of pro-inflammatory compounds namely nitric oxide, macrophage derived TNF-alpha, and IL-6 was significantly reduced on *kombucha* addition. Sun et al. (2015) opined that *kombucha* fermentation of black tea blended with wheat grass juice can result in markedly better concentration of antioxidant compounds than traditional black tea fermented *kombucha* drink. Enzyme β-glucuronidase is responsible for hydrolyzing glucuronides in the lumen of the gut and generating carcinogenic substances.

Brewed *kombucha* tea produces D-saccharic acid-1,4-lactone, a metabolite which inhibits the activity of β-glucuronidase and could prevent colorectal cancer in humans (Wang et al., 2010).

## 8.4.2 Antihypertensive

Blood pressure is primarily regulated by the pressure exerted by the pumping of blood by the heart and the resistance of the blood vessels to it. Both of these are regulated by hormonal activities triggered and inhibited through a number of physical and biochemical factors. Induced myocardial membrane infarction in rats caused by isoproterenol could be stabilized by regular feeding of *kombucha* tea (Lobo et al., 2016). Isoproterenol resulted in a drastic lowering of tissue antioxidants and increased levels of ester, free cholesterol, triglycerides, free fatty acids, plasma phospholipid, and glycoprotein components in plasma and heart. Decrease in the phospholipid content of heart tissues with decreased $Na^+/K^+$ ATPase activity and increased $Ca^{2+}$ and $Mg^{2+}$ ATPase activities indicated destabilization of the myocardial membranes. Pretreatment with *kombucha* tea was able to bring these components to near normalcy. *Kombucha* SCOBY fermented milk has been opined to be a successful food commodity with acceptable texture and organoleptic properties similar to commercial yogurt (Elkhtab et al., 2017). Hydrolysis of milk proteins by the SCOBY was found to produce peptides with reported antihypertensive action which support human circulatory system. The identified peptides inhibit the ACE enzyme which converts hormone angiotensin I to angiotensin II, the main participant in increasing blood pressure. Remarkably higher ACE-inhibition by *kombucha* fermented milk than commercial probiotic and yogurt forming strains was also reported by Hrnjez et al. (2014b). The highest inhibitory rate was obtained after the 14th day of fermentation, while it was minimum on the 7th day. Time of fermentation, therefore plays an important part in the formation of such functional molecules in a *kombucha* drink.

## 8.4.3 Antidiabetic

Abnormality in sugar metabolism is often termed as diabetes. It is generally a genetic as well as an age or lifestyle related disorder. A reduced insulin hormone level in blood plasma and a number of other factors associated with it are the primary causes of diabetes development. Rats with diabetes mellitus induced by injecting streptozotocin could be cured by *kombucha* drinking (Srihari et al., 2013). Diabetic rats daily fed with *kombucha* (6 mg/kg body weight) for 45 days showed significantly decreased glycosylated hemoglobin ($HbA_{1c}$) level and increased level of plasma insulin, hemoglobin, and tissue glycogen

with normalized plasma glucose levels. Activities of gluconeogenic enzymes (glucose-6-phosphatase, fructose-1,6-bisphosphatase) and glycolytic enzymes (hexokinase) were reversed in the rat tissues indicating hypoglycemic effects of *kombucha* at multiple sites of glucose regulatory pathways. Complex tea polyphenols in *kombucha* such as theaflavins and thearubigins coupled with organic acids and B-complex vitamins might prevent the damage and death of pancreatic β-cells, and/or stimulate their regeneration in diabetic rats (Fu et al., 2017). In another study, alloxan-induced diabetic rats were fed with black tea and *kombucha* prepared from black tea (Bhattacharya et al., 2013). A dose of 150-mg lyophilized extract/kg body weight fed for 14 days was found to effectively restore the induced diabetic changes. *Kombucha* beverage was more efficient than unfermented black tea in reversing the induced diabetic physiology in the rats. Formation of some novel unidentified bioactive molecules during fermentation was considered to be a probable reason for this therapeutic efficacy. Reduction of blood glucose in diabetes mellitus induced rats by a 5.5 mL dose of a 12 day fermented *kombucha* feeding for 30 days was also reported by Aloulou et al. (2012). Kallel et al. (2012) studied on the potential impact of *kombucha* drinking on starch digestion. Phenolics formed during *kombucha* fermentation could strongly inhibit the activity of procrine pancreatic α-amylase. The active compounds were suspected to be monomeric and oligomeric phenolic compounds. The inhibition efficiency of the *kombucha* increased with fermentation progress till the 15th day. Doses of 1000 times diluted *kombucha* tea sample could also effectively cause the inhibition (55%). The exact mechanism of the inhibition is not yet reported. *Kombucha* tea doses can be potentially tested in humans for lowering the blood glucose level and thereby can contribute in developing an effective and bio-based (natural) antiglycemic therapy.

### 8.4.4 Hepatoprotective

Use of *kombucha* for treating complicated liver disorders is one of the earliest known information on the effectiveness of this functional drink (Yarbrough, 2017). Its hepatoprotective functionality in rats and mice models has been recently confirmed. Bhattacharya et al. (2011) induced cytotoxicity in murine hepatocyte cells in vitro using tertiary butyl hydroperoxide, a well-known reactive oxygen species. This caused reduction in cell viability, enhanced membrane damage, and disturbed intracellular antioxidant machineries. Flow cytometry indicated apoptosis in the infected tissue through degeneration of the mitochondrial pathway. A simultaneous treatment with *kombucha* beverage could maintain membrane integrity and prevented the change in antioxidant status brought about by mitochondrial abnormality. Multiple other reports on hepatoprotective action of fermented

*kombucha* and its efficiency over unfermented black tea are also available. Hepatic cytotoxicity in those reports was induced by other agents, namely paracetamol (Pauline et al., 2001), carbon tetrachloride (Murugesan et al., 2009), aflatoxin B1 (Jayabalan et al., 2010), and thioacetamide (Kabiri et al., 2014). In most reports, some unidentified fermentation hydrolysate compounds in *kombucha* tea with greater antioxidant properties were opined to be functional for the anticytotoxicity properties.

### 8.4.5 Antimicrobial

Pathogen inhibiting property of herbal and probiotic drinks is an important feature considering colon health regulation by their usage (Sugiharto, 2016). A pool of research on antimicrobial effects of tea and *kombucha* drink as well as *kombucha* adjuncts from other plant sources is available (Reygaert, 2014; Siddiqui et al., 2016). From an early study, Greenwalt et al. (2000) opined that the additional antimicrobial effect exerted upon both pathogenic Gram positive and Gram negative bacteria by *kombucha* after tea fermentation is due to the acetic acid produced during the process. The raw material tea itself has the rest of the property which is carried over to the *kombucha* drink. In contrary, Pure and Pure (2016a, b) did not find any antibacterial activity in infusions from black tea, banana peel, nettles, and *kombucha* made from them. A low concentration of extracts used was opined to be the cause of the negative results, which indicated a dose-dependency of the material to perform bacterial growth inhibition. However, lemon balm infusion fermented with *kombucha* SCOBY resulted in antibacterial activity against 11 wild bacterial species, which was attributed to acetic acid concentration and other tea components and SCOBY metabolites having antibacterial properties.

## 8.5 Antinutritional and Safety Issues

With recent increase in popularity of herbal medicines and supplements like *kombucha* tea, concern for the risk and safety issues related to those is also gaining potential. Apprehensions on safety of *kombucha* drinking are not new. After a popular culture of *kombucha* drinking developed during the 1950s, unsubstantiated rumor on possible cancer inducing effect of Russian tea Kwass (a *kombucha*-like drink) was spread. The tea has been popularly considered as “naturally safe” with minimal chances of postproduction contamination. The United States Food and Drug Administration (FDA), surveying commercial producers, have found no pathogenic bacteria or hygiene violations in *kombucha* tea (Food and Drug Administration, FDA, Cautions Consumers on *Kombucha*: Mushroom Tea. News Release, US Department of

Health and Human Services, Public Health Service, Food and Drug Administration, Washington, DC, March 1995). A low chance of microbial contamination is attributed to development of high acidity and low pH during fermentation (Mayser et al., 1995). High acid formation on prolonged fermentation is also a risk factor associated with the drink. One case of hyperthermia, lactic acidosis, and acute renal failure within 15 h of *Kombucha* tea ingestion was confirmed in a 22-year-old HIV-positive male (SungHeeKole et al., 2009). Though a number of acids, principally acetic acid, lactic acid, and citric acid are formed during *kombucha* fermentation but possible formation of pathogenic mold species at such low pH cannot be nullified. Any harmful effect on colon microflora due to the acidity of the fermented liquor needs investigation. While *kombucha* tea has been effective against various pathogenic bacteria and molds, unacceptable mold growth over *kombucha* drink is a notable hazard (Nummer, 2013). Furthermore, inorganic contaminants getting added during processing is also not ignorable. Safe and hygienic handling of the SCOBY as well as the fermenting broth has always been suggested. It is always suggested to brew the drink in food-grade glass, plastic, or stainless steel vessels with minimum chances of element leaching or rusting. Symptomatic lead poisoning in a married couple regularly drinking *kombucha* tea fermented in a ceramic pot was reported (Phan et al., 1998). The acid formed in the tea leached lead from the ceramic glazing material into the tea as postulated by the authors. Srinivasan et al. (1997) reported onset of hepatic and allergic complications in four patients consuming *kombucha* tea. The symptoms ceased when consumption was stopped, indicating etiological association. The mechanism of these side effects was reportedly uncertain and might have been caused by contaminants. In a recent report, a 58-year-old woman consuming *kombucha* tea for a month developed epi-gastric pain, clay-colored bowel movement, dark urine, pruritus, and jaundice. She showed signs of cholestatic liver injury due to a high alkaline phosphatase to aminotransferase ratio coupled with bilirubinemia. A biopsy indicated drug-induced hepatotoxicity (Gedela et al., 2016). However, in all the cases, direct relation of the disorders with *kombucha* consumption could not be ascertained. Kallel et al. (2012) reported on inhibitory action of some *kombucha* tea polyphenols on starch digestion and glucose absorption. While such inhibition may be beneficial for glycemic control, the same may be harmful to persons with normal sugar metabolism and persons requiring high-energy sugar-based supplements. A significant content of tea tannin may also serve as an antinutritional factor in *kombucha* drinkers by causing reduced intake, lower nutrient digestibility, decreased absorption, and protein availability (Sabahelkhier et al., 2014).

The Bureau of Food Safety and Laboratory Services under the Pennsylvania Department of Agriculture have given few guidelines for *kombucha* fermentation and bottling. Under this, *kombucha* production in a licensed retail food facility requires an approved Hazard Analysis and Critical Control Point (HACCP) plan. A food safety monitoring of pH change using calibrated digital pH meter during fermentation has been reported necessarily. The non-TCS (time temperature control for safety) food necessarily should aim for a pH level below 4.6 for pasteurized and 4.2 or below for unpasteurized bottled *kombucha* within 7 days of initiating the fermentation. A pH below 2.5 may be considered as unsafe for consumption and a product tasting highly acidic should not be served to consumers. A dose of 118 m *kombucha* per day is recommended. Immunocompromised individuals should avoid its consumption. No health claim of the beverage should be made on the label. The alcohol content must remain below 0.5% and be sold as nonalcoholic beverage even after residual fermentation after packaging and bottling. Such post bottling fermentation can be prevented by pasteurizing the prepared *kombucha* tea at 82°C for 15 s to destroy fermentative yeasts. 0.1% concentrations of sodium benzoate and potassium sorbate can also be used to prevent mold growth. A commercial SCOBY culture should be purchased to initiate a *kombucha* process line. Daughter SCOBY with no signs of mold or unusual contamination should only be used in the subsequent batch. A detail of these guidelines have been also published by Nummer (2013).

## 8.6 Research Gaps and Potential Scope

The *kombucha* beverage has found its way from the historical monasteries, through rural households to the modern industrial processing for consumer market. Research on the potential health promoting drink has seen a drastic elevation in the past two decades and its rising popularity can be largely attributed to those findings. While extensively going through the researches and reports published till date, a number of gaps in the existing knowledge could be clearly identified.

The SCOBY is a least understood entity. Species identification in *kombucha* SCOBY has been carried out by different workers. A number of bacteria and yeasts have been reported to be present in the fermenting cultures, producing different metabolites. The microbial population and their ratio in the SCOBY vary from culture to culture. Existence of specific microbes depends largely on physical conditions like temperature, altitude, salinity, etc., which may cause such alteration in the symbiotic cultures. Chu and Chen (2006) reported distinct difference in rise of antioxidant properties in *kombucha* drinks

fermented using eight different SCOBY cultures collected from different parts of Taiwan. In a recent study by Marsh et al. (2014), five SCOBY pellicles collected from commercial suppliers of Canada, United Kingdom, United States, and Ireland were tested for their bacterial and fungal compositions using nucleic acid sequencing-based technique. *Gluconacetobacter* was found to be the major (>85%) species followed by *Lactobacillus* species. *Zygosaccharomyces* species dominated the yeast population (>95%) in the tea as well as the SCOBY. Schematic representation of the distribution of microbial population in SCOBY is shown in Fig. 8.3. Similar studies have also been carried out by using rRNA amplification technique. A detailed study with broader range of SCOBY cultures is necessary. Although most of the metabolism-related studies have termed the fermenting SCOBY as *kombucha*, tea fungus, etc., their targeted functionality would vary considerably with differences in the microbial population. In many studies, the SCOBY cultures are reported to be collected from commercial reliable sources. The microflora needs precise identification with detailing of each species' proportion before using it for *kombucha* production. This will help in clearly understanding the probable metabolites being produced by them. In almost all the available studies, the health promoting effects and bioactivity of the drinks have been conclusively attributed to the actions of unspecified enzymes on unidentified secondary substrates to form some unknown functional metabolites. Profiling of all the *kombucha* metabolites, their formation and bioactive pathways should be thoroughly investigated. Besides, human trials should be conducted and validated results should be obtained before launching it as a commercial health drink. Proven dose-dependent functional activity of the beverage and its adjuncts from other non-tea sources creates need for carefully designing it as an herbal remedy of health hazards.

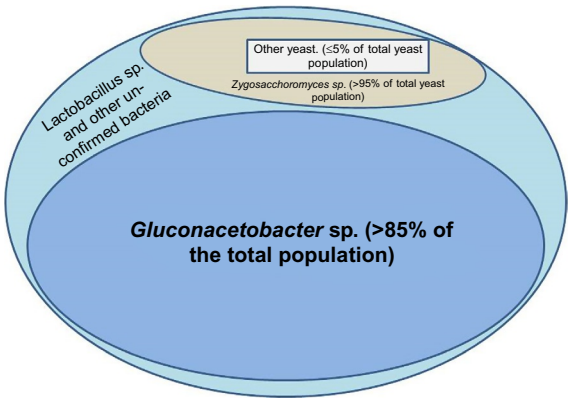


**Fig. 8.3** Distribution of microbial population in SCOBY and *kombucha* culture. Source: Marsh, A.J., O'Sullivan, O., Hill, C., Ross, R.P., Cotter, P.D., 2014. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. Food Microbiol. 38, 171–178.

## 8.7 Conclusion

*Kombucha* beverage is traditionally produced by home-scale fermentation technique involving conversion of sugar-dissolved black tea by a SCOBY. The fermented beverage has been claimed to have various health promoting and antimicrobial effects in different parts of the world since long time. Considering its long history of global consumption as a health supporting drink, *kombucha* is being continuously explored for its inherent properties and validated several facts of general believes associated with it. Some therapeutic and health promoting properties of *kombucha* beverage such as: antiinflammatory and anticancer, antihypertensive, antidiabetic, hepatoprotective, antimicrobial, etc. are already investigated and scientifically established.

Although various beneficial effects of *kombucha* drink on animal models have been experimentally confirmed, comparable mechanisms of its components and their role in physiological pathways of consumers along with their interactions in humans remain uncertain till date. Several possible health complications have also been witnessed and attributed to the drinking of *kombucha*. Despite of being a popular folk remedy to various diseases and ailments, the product requires scientific authentication.

The microbial composition of fermented drink as well as SCOBY is a least understood and studied entity till date. The microfloral community needs precise identification and characterization with details of each species including their proportion before using it for *kombucha* production. This will help in understanding the probable metabolites being produced by them and hence will open a window to map out its possible mechanism of action in physiological path ways of *kombucha* consumers. Profiling of all the *kombucha* metabolites, their formation and bioactive interactions should be thoroughly investigated and scientifically authenticated by conducting human trials.

With increase in consumer awareness toward natural and chemical free substances, popularity of herbal medicines and supplements like *kombucha* tea, etc. are also increasing worldwide. Subsequently, concerns for the risk and safety issues related to those are also gaining potential and a standardized production method as well as approved regulations for raw material, unit operations, quality control, packaging, storage, serving criteria, intake range, etc. from global recognized authorities are felt as a need of the hour. In this context, United States Food and Drug Administration (USFDA) conducted survey on commercial producers and have found no pathogenic bacteria or hygiene violations in *kombucha* tea. Furthermore, The Bureau of Food Safety and Laboratory Services under the Pennsylvania Department of Agriculture have given few guidelines for *kombucha* production and packaging. However,

authors feel that the available guidelines are not exhaustive and further studies are required to formulate a well-standardized protocol and safety guideline for the commercial production of *kombucha* beverage.

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