Chinese Herbal Medicines 15 (2023) 27–32

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Review

An overview of probiotic health booster-kombucha tea

Suriyapriya Selvaraj, Kalaichelvan Gurumurthy *

School of Biosciences and Technology, Vellore Institute of Technology, Tamil Nadu, Vellore 632014, India

A R T I C L E I N F O

Article history:
Received 15 January 2022
Revised 10 April 2022
Accepted 27 June 2022
Available online 16 December 2022

Keywords:
bio-tea
fermented beverage
fermentation
kombucha
probiotics
symbiotic culture of bacteria and yeast
tea fungus

A B S T R A C T

Traditional herbal medicine (THM) is a significant division of traditional Chinese medicine (TCM) that plays an important role in maintaining health and disease prevention. WHO has consistently highlighted the significance of traditional, complementary, and alternative medicine in human healthcare. Most people in Eastern Asia will start their day with a cup of tea. The tea provides a nourishing effect, and it has become an inevitable part of life. There are several types of tea, like black tea, green tea, oolong tea, white tea, and herbal tea. Besides the refreshments, it is important to consume beverages that benefit health. One such alternative is a healthy probiotic drink called kombucha, a fermented tea. Kombucha tea is aerobically fermented by infusing sweetened tea with a cellulose mat/ pellicle called SCOBY (symbiotic culture of bacteria and yeast). Kombucha is a source of bioactive compounds that include organic acids and amino acids, vitamins, probiotics, sugars, polyphenols, and antioxidants. Currently, studies on kombucha tea and SCOBY are gaining attention for their remarkable properties and applications in the food and health industries. The review gives an overview of the production, fermentation, microbial diversity, and metabolic products of kombucha. The possible implications for human health are also discussed.

Contents

1. Introduction

Probiotics are taken as dietary supplements to boost human immunity. The microbes in the gut are non-pathogenic and improve intestinal health. They also regulate nutrition, metabolism, and physiology. Fermented foods and drinks are traditional and trending probiotics that are available all around the world under different names. Fermented products are full of microbes that are responsible for conferring health benefits. Some of the fermented drinks include yogurt, Yakult, kefir, and kombucha. Kombucha is produced by fermenting starter culture; a mixed microbial consortium, with sweet tea infusions (Villarreal-Soto et al., 2019). In TCM, kombucha tea is known as the "Tea of Immortality" and the "Elixir of Life" (Soltani, Farshadfar, Shirvani, & Yaghotipoor, 2021). Kombucha is prepared by steeping black tea leaves in boiling water with the addition of some sugar (Fig. 1).

* Corresponding author.
E-mail address: kalaichelvan@gmail.com (K. Gurumurthy).

https://doi.org/10.1016/j.chmed.2022.06.010
1674-6384/

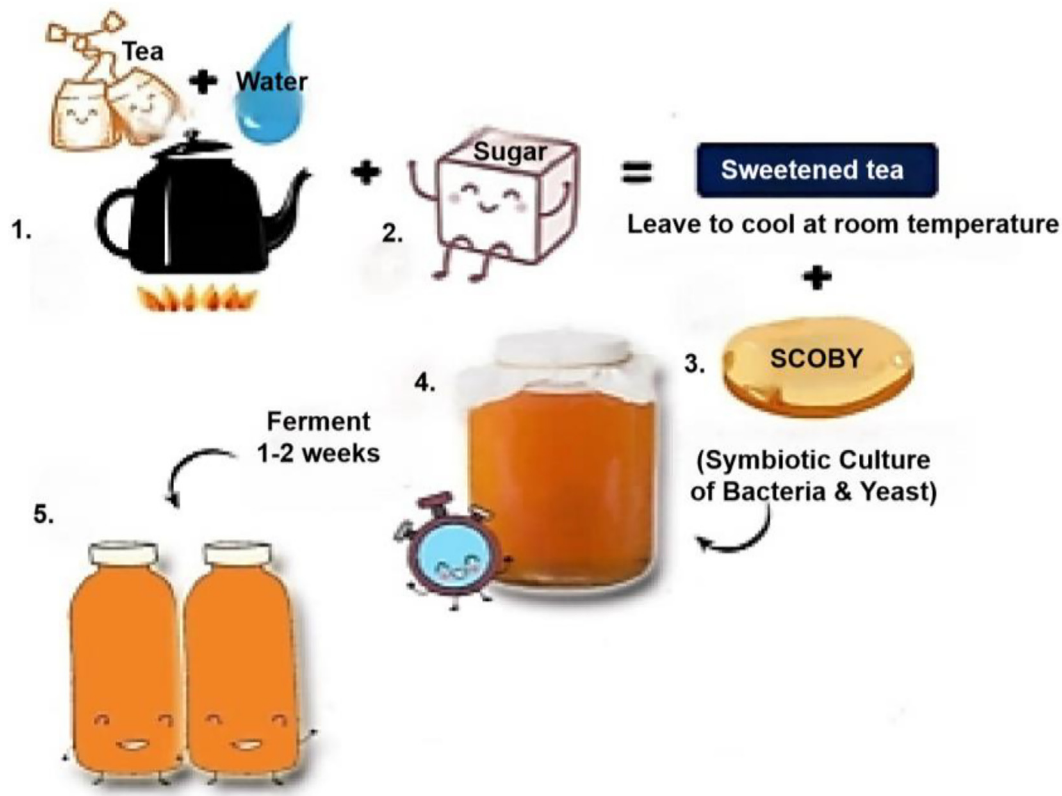

Fig. 1. Preparation and fermentation of kombucha tea (Generally it involves adding tea to the boiling water followed by the addition of sugar. The fermentation is initiated by inoculation of SCOBY layer. After two to three weeks, it can be stored and consumed).

Then the mother culture (symbiotic culture of bacteria and yeast, SCOBY) is placed into the mixture (Dufresne & Farnworth, 2000). The bacteria and yeasts in the SCOBY synthesize cellulose and form a cellulosic portion, and the fermented liquid is the tea portion (Vohra, Fazry, Sairi, & Babul-Airianah, 2019).

Kombucha is usually fermented in glass containers. To avoid contamination, the fermentation utensils must be sterilized. The opening of vessel should be wrapped in breathable fabric to forestall dirt and airborne mold. The incubation period of the culture is around 7 to 10 d at room temperature (18–30 °C). During fermentation, a gelatinous disc-shaped 'daughter SCOBY' is formed at the air–liquid interface. The biofilm formation depends upon the shape of container, and the thickness is determined by the duration of cultivation (Chan, Shin, & Jiang, 2018). The sugar and tea concentration and the fermentation time determine the colour and shade of kombucha. Due to the symbiotic action of the SCOBY, the pH diminishes when the acidity rises, which prompts gas bubble formation (Dufresne & Farnworth, 2000; Greenwalt, Steinkraus, & Ledford, 2000). After fermentation, the tea is converted into a probiotic beverage with improved health benefits. The tea seems to be an acidic beverage with a carbonated vinegar flavor. The end products can be stored in the refrigerator after straining it into a sterile jar bottle with a sealed lid. Chen et al. suggested that, as Kombucha is a living culture, it should be handled with care to avoid contamination (Chen & Liu, 2000). It can be easily brewed and well maintained in the home as well as in the laboratory. This ancient drink from China has been traditionally consumed for its therapeutic effects on the health of humans.

2. History

Kombucha tea originated in northeast China around 220BCE and was valued for its invigorating and detoxifying effects (Moreno-Jiménez et al., 2018). In 414 CE, Dr. Kombu used kombucha to cure the digestive problems of Emperor Inkyo, and by then kombucha had been brought from China to Japan. During World War II, the consumption of kombucha was extended beyond European countries, Russia, and North Africa (Blanc, 1996). After World War II, the cancer rates were lower in Kombucha-drinking regions of Russia than in nondrinking regions (Fontana, Franco, Lyra, De Souza, & De Souza, 1991). In the 1950s, Italian society reached its peak in the consumption of kombucha beverages (Dufresne & Farnworth, 2000; Hartmann, Burleson, Holmes, & Geist, 2000). In Germany in the 2000s, kombucha was devoured as a practical refreshment for the ailment, piles, and metabolic illness (Fu, Yan, Cao, Xie, & Lin, 2014; Pei et al., 2020). Currently, kombucha imbibing has spread across the world as it is a natural diuretic. It is packed with healthy probiotics and helps to break down foods and flush out waste and toxins more quickly.

3. Biological composition of kombucha

The composition of the tea beverage and symbiotic culture of yeast and bacteria (SCOBY) layer is based on locality, weather, bacterial and yeast strains, and the inoculum source (Goh et al., 2012). Kombucha tea is comprised of osmophilic strains of yeast like *Brettanomyces* spp., *Candida* spp., *Lachancea* spp., *Pichia* spp., *Saccharomyces* spp., *Schizosaccharomyces* spp., *Zygosaccharomyces* spp., and acetic acid bacteria like *Acetobacter* spp., *Gluconobacter* spp., *Gluconacetobacter* spp., *Komagataeibacter* spp. and *Lactobacillus* spp. (Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014; Teoh, Heard, & Cox, 2004). The SCOBY yield is high during the first 14 d of the fermentation, and then the growth of the microbes will gradually decrease and altogether cease owing to adverse growth conditions (Chen & Liu, 2000; Jayabalan, Malini, Sathishkumar, Swaminathan, & Yun, 2010). In kombucha fermentation, fructose and glucose are utilized as the carbon source. Glucose is the only monosaccharide that produces cellulose pellicle. Each pellicle has a unique microbial composition which either possesses yeast culture or bacterial culture dominantly. The baby SCOBY that is formed after the fermentation of the mother SCOBY will even have a variation in microbial composition and percentage of microbial domination. There is a mystery about the metabolism of the microbes involved in fermentation. The microbial composition of the fermented tea and SCOBY layer determines the properties of the cellulose that is produced during the fermentation process.

4. Properties and application of bacterial cellulose

Kombucha fermentation is influenced by numerous physicochemical and genetic factors that ought to be improved, like the hardness of water, carbon source, substrate, the concentration of the tea, the microbial and media composition, production of CO_2, surface area, the effect of pH, time and temperature and oxygen supply (Marsh, O'Sullivan, Hill, Ross, & Cotter, 2014; Wolfe & Dutton, 2015). Improper assessment of these parameters will have an impact on the nutritional quality, biological activities, organoleptic and physicochemical properties of the beverage. The cellulose mat is a byproduct of the kombucha tea fermentation process. It is a valuable raw material for nano-cellulose production. Bacteria produce an unbranched pellicle structure that chemically equals native cellulose (Rangaswamy, Vanitha, & Hungund, 2015). Bacterial cellulose has unique properties that include increased mechanical potential, elasticity, nutritious, antibacterial, uniformity, thermally stable, high purity, hydrophilic, ion exchange, adsorptive, translucent, crystalline, biodegradability, adaptability, non-poisonous, permeable, fibrous nanostructure matrix, and excessive water clasping limit, good light transmittance, enhanced cell upgrading, detachment, and escalation (Chawla, Bajaj, Survase, & Singhal, 2009; Costa, Almeida, Vinhas, & Sarubbo, 2017; Morán, Alvarez, Cyras, & Vázquez, 2008; Shade et al., 2011). Bacterial cellulose has a wide range of applications (Fig. 2) due to its favorable properties, which include the food industry, ecology, paper industry, cosmetics, textile industry, nano-composites, biomedical and biotechnology fields.

5. Metabolism of kombucha fermentation

Kombucha is bubbly, slightly sweet, slightly acidic, and is ingested throughout the world as a reviving refreshment (Sreeramulu, Zhu, & Knol, 2001). The bacteria and yeast in kombucha utilise substrates in different ways and are involved in many metabolic activities. During fermentation (Fig. 3), the yeast in the culture produces invertase and hydrolyses disaccharide sucrose into monosaccharide, glucose, and fructose, producing CO_2 and ethanol (Blanc, 1996). The acetic acid bacteria oxidize fructose into acetic acid and glucose into gluconic acid (Dufresne & Farnworth, 2000). The obligate anaerobic acetic acid bacteria in the culture oxidise ethanol and excrete acetic acid; thereby, the pH is lowered.

Tea is comprised of catechins, theaflavins, thearubigins, flavonols, flavonol glycosides, protein, amino acids, caffeine, carbohydrates, organic acids (Balentine, 1992; Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018). After the fermentation process, kombucha tea is a cocktail of numerous chemical compo-

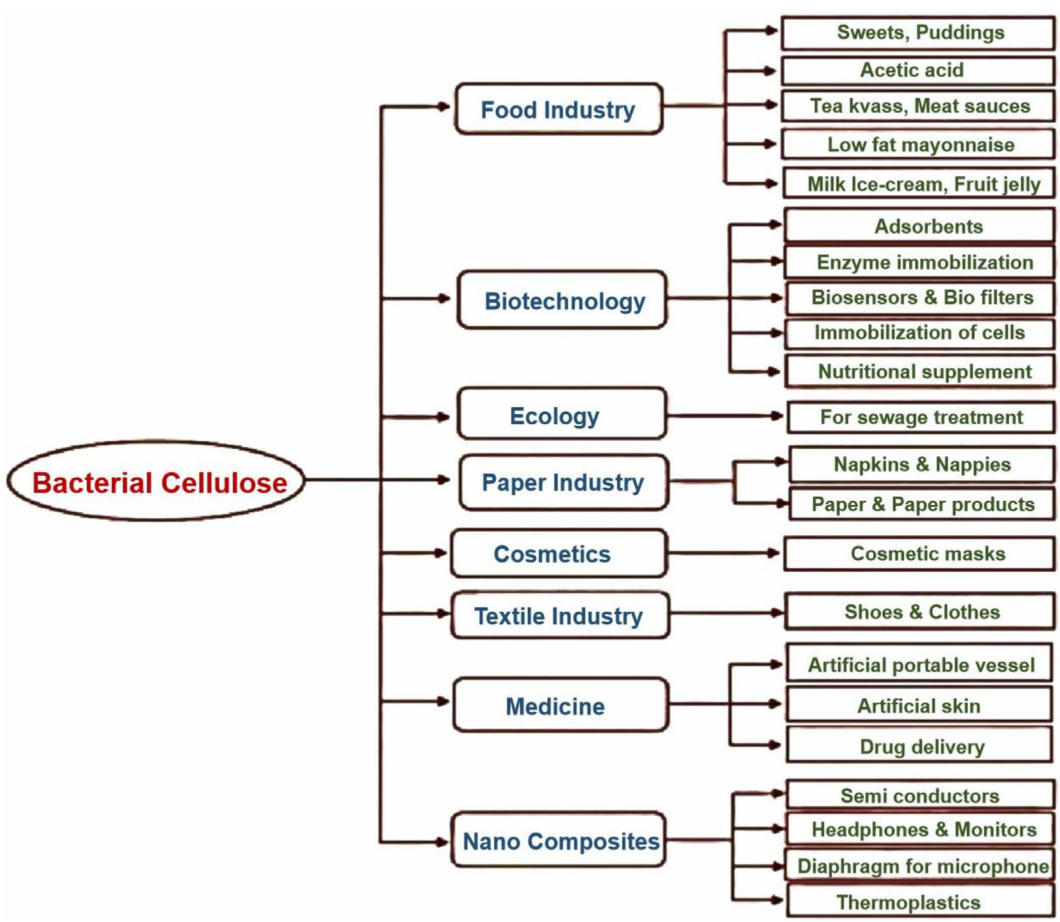

Fig. 2. Applications of bacterial cellulose (Niyazbekova, Nagmetova, & Kurmanbayev, 2018).

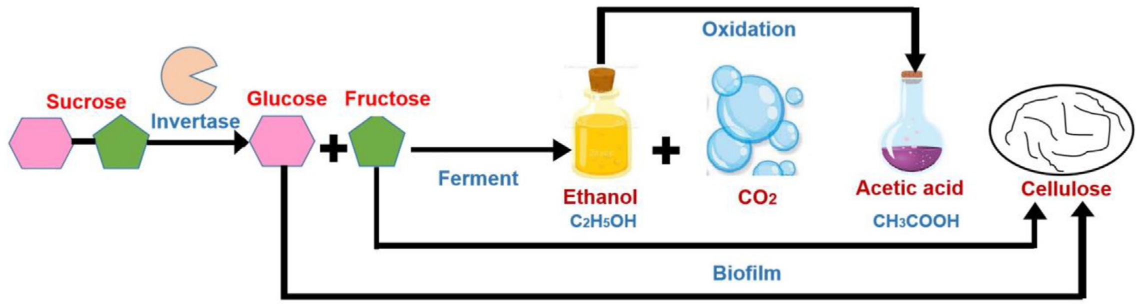

Fig. 3. Kombucha metabolism during fermentation process (May et al., 2019).

nents (Miranda, Lawton, Tachibana, Swartz, & Hall, 2016). *Acetobacter* species are commonly found to be abundant in kombucha culture and are highly remarked as cellulose-producing bacterial species. The biochemical pathway involves Kreb's cycle, Gluconeogenesis, and the Pentose Phosphate cycle, where the sugars are produced and converted into acids. At the end of the biochemical metabolism, the yeast and bacteria in the culture produce various beneficial metabolites.

6. Metabolites of kombucha fermentation

The metabolites of fermented tea consist of several acids such as acetic acid, gluconic acid, glucuronic acid, citric acid, L-lactic acid, (Malbaša, Lončar, Djurić, & Došenović, 2008; Malbaša, Lončar, & Kolarov, 2002) malic acid, tartaric acid, malonic acid, oxalic acid, succinic acid, pyruvic acid, saccharic acid (Chen & Liu, 2000), and D-saccharic acid-1,4-lactone (DSL) acid. DSL acid from D-glucaric acid has detoxifying and antioxidant properties (Bhattacharya, Gachhui, & Sil, 2013). The acetic acid concentration is inversely proportional to the fermentation time and also provides the taste of vinegar to the kombucha (Chen & Liu, 2000). Sreeramulu et al. has studied the antimicrobial effects of oxalic acid, saccharic acid, gluconic, succinic, and carbonic acids from kombucha, which helps to improve sleep (Sreeramulu, Zhu, & Knol, 2000). Glucuronic acid and malic acid from kombucha are helpful in the detoxification of the liver. It also beneficial in restricting lipid peroxidation (Jayabalan, Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008; Jayabalan et al., 2008). Kombucha yields monosaccharides such as glucose, fructose and also disaccharides like sucrose (Chen & Liu, 2000). Several vitamins are also obtained from kombucha fermentation, vitamin B1 with anti-ageing effect, vitamin B2 preventing arthritis and allergies, vitamin B12 aiding memory loss (Bauer-Petrovska & Petrushevska-Tozi, 2000), vitamin B6, which helps the body fight depression, stabilizes mood, and improves concentration, prevents stroke and obesity, and water-soluble vitamin C (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011) that suppresses the release of cortisol. Some trace amounts of alcohol (<0.5%) are usually produced in kombucha. The concentration of ethanol in kombucha is proportional to the fermentation time (Chen & Liu, 2000). The polyphenols from tea are active substances and powerful antioxidants (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011) are found in the kombucha beverage. The tea is widely known for its beneficial effects as it is rich in antioxidant content. Antioxidants play a role in preventing several diseases such as cancer, stress, neurodegenerative and cardiovascular diseases (Jayabalan, Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008; Jayabalan et al., 2008). Kombucha produced from green teas has higher radical scavenging activity than black tea (Chu & Chen, 2006). Essential metal elements like Na, K, Ca, Cu, Fe, Mn, Ni, Zn were found to be present in kombucha (Petrovic et al., 1999) Kumar et al. found the presence of anions such as F, CI, Br, I, NO_3, HPO_4 and SO_4 (Kumar, Narayan, & Hassarajani, 2008). Kombucha is widely consumed for the health benefits obtained from the numerous metabolites after the fermentation process.

7. Beneficial effects of kombucha

Kombucha tea is a functional food with several prophylactic and therapeutic benefits. The beneficial effects of this drink depend on the raw materials, type of sugar, fermentation duration, and composition of the starter culture. The biological activity of kombucha is determined by *in vitro* and *in vivo* studies using animal models and cell lines. Kombucha also possesses anti-inflammatory activity (Chakravorty et al., 2016; Vázquez-Cabral et al., 2017), antibacterial activity (Cardoso et al., 2020), anti-carcinogenic potential (Jayabalan et al., 2011), antimicrobial activity (Battikh, Bakhrouf, & Ammar, 2012; Sreeramulu, Zhu, & Knol, 2001), antioxidant activity (Chakravorty et al., 2016; Hartmann, Burleson, Holmes, & Geist, 2000; Jayabalan, Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008; Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011), and anti-proliferative activity (Cardoso et al., 2020; Deghrigue et al., 2013), which has been studied with *in vitro* experiments. Mice were used for the study of anti-oxidative effects of kombucha. It has an anti-oxidative stress against chromate (Ram et al., 2000), anti-oxidative stress against lead (Dipti et al., 2003), anti-stress activity against hypoxia and cold (Pauline et al., 2001), and protection against oxidative stress damage by induced alloxan (Bhattacharya, Gachhui, & Sil, 2013). The metabolites of kombucha prevent the leakage of myocardial tissues and provide protective cardiac effect (Lobo & Shenoy, 2015). It also protects against vascular and coronary heart disease (Isdadiyanto & Tana, 2019), and provides membrane stabilization in myocardial infarction (Lobo, Chandrasekhar Sagar, & Shenoy, 2017). Kombucha possesses anti-diabetic activity (Hosseini, Rasouli, Gorjian, & Yadollahpour, 2016; Zubaidah, Afgani, Kalsum, Srianta, & Blanc, 2019) and provides a renoprotective effect against diabetes (Chandrakala, Lobo, & Dias 2019). Drinking kombucha can help to prevent and maintain the weight loss of diabetic rats (Morshedi, Dashti, Mosaddegh, Rafati, & Salami, 2006). Kombucha provides an anti-lipidemic and anti-atherogenic effect against alloxan diabetics (Aloulou et al. 2012; Lobo, Chandrasekhar Sagar, & Shenoy, 2017), Kombucha provides protection against nephrotoxicity activity by ceasing lipid peroxidation (Gharib, 2010). Bhattacharya et al. have studied the anti-virulence activity of kombucha against *Vibrio cholerae* in rabbits and mice (Bhattacharya et al., 2020). Adriani et al. studied the effects of kombucha and its cholesterol-lowering activity with duck blood (Adriani, Mayasari, Angga, & Kartasudjana, 2011). Cytogenic activity (Mrdanović, Bognadović, Cvetković, Velićanski, & Četojević-Simin, 2007) and changes in chromosomal aberrations (Yapar, Cavusoglu, Oruc, & Yalcin, 2010) were also observed in human lymphocytes. The longevity of kombucha consumption, life span, and general health was studied using mice (Hartmann, Burleson, Holmes, & Geist, 2000). Shenoy proved the hypoglycemic activity of kombucha by monitoring the blood sugar level in mice after kombucha consumption (Shenoy, 2000). Banerjee et al. conducted a study and proved that kombucha has the ability to heal stomach ulcers in mice (Banerjee et al., 2010). Kombucha helps to cease phenol-induced cytotoxicity (Yapar, Cavusoglu, Oruc, & Yalcin, 2010). Četojević et al. used the CHO-K1 cell lines from hamsters to study the protection against genotoxic effects (Četojević-Simin et al., 2012). However, most of the health benefits are not scientifically proven in human models yet. Clinical trials are yet to be done to manifest the claimed health benefits.

8. Side effects and health risks of kombucha consumption

Despite the benefits, there are some side effects and health risks to kombucha consumption. *The Centers for Disease Control and Prevention* (1994) stated that consuming 4 ounces of kombucha a day does not risk a consumer's health. The US Food and Drug Administration conducted a biochemical and microbiological evaluation in 1995 that revealed kombucha consumption was secure in humans (Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014). Kombucha consumption can be harmful only if it is incorrectly prepared, and individuals with preexisting conditions should avoid it as it may lead to metabolic acidosis. As the kombucha culture has acetic acid and lactic acid, it possesses an acidic pH, which helps it protect itself by inhibiting the growth of harmful microorganisms (Chandrakala, Lobo, & Dias, 2019). The research rate on the stomach, skin, lactose intolerance, hyperlipidemia, and sensorimotor behavior is peaking continually (Martini, 2018). There have been substantial studies in animal models over the last decade on how probiotics and prebiotics regulate the metabolism of the host. These studies proved that intestinal flora modulates swelling, glucose metabolism, obesity, fullness, and energy consumption. In parallel to the emerging popularity and consumption of kombucha, there is a concern about its potential risks and safety issues. The major reasons for the beverage's contamination include unreliable raw materials, vessels, packages, and a lack of sanitation during the fermentation process. This may lead to exposure to toxic metabolites and anti-nutritional components. Therefore, some protocols and norms should be framed by health and nutrition organizations to standardize production methods, raw materials, and quality control.

9. Conclusion and prospects

Fermented products are trending as they are healthy, beneficial foods that are consumed either knowingly or unknowingly by humans in their daily routine. It provides nutrition, enhances metabolism, and regulates the gut microbiome. Kombucha is traditionally brewed at home and is also commercially available. This beverage can be taken as dietary probiotic supplement rich in bioactive compounds, antioxidants and has a quantifiable effect on intestinal flora. Many articles provide claimed beneficial effects, microbial composition, physical, chemical, and biological properties, various raw materials, different applications, and traditional beliefs of kombucha, but there is still a dispute as there is a lack of research on toxicity studies of kombucha, microbial content of SCOBY, biological properties, consumer preferences, safety and invulnerability in kombucha consumption, qualitative and quantitative properties, probiotic effects, fermentation kinetics, cellulose chemistry, quality control, and sensory evaluation. The biological activity of kombucha is being investigated in cell lines and animal models. This particular beverage is consumed all over the world for its health benefits, but it hasn't been involved or tested in any human trials. Human responses should be investigated to ethically claim the reported health benefits. A proper sensory evaluation study should be carried out to provide a clear view of the flavor profile, the consumer's desire, and acceptance. As the drink is easily accessible, consumers should learn and practice the basics of brewing, storage, and handling to avoid risk. Sucrose is commonly used as a carbon source in kombucha brewing, which can be replaced by alternate sugar sources as a cost-effective raw material. There is a lack of determining the metabolites and other content in the end products of the fermented tea, and the microbial evolution should also be explored. SCOBY is termed "vegan textile" as it is used as an alternate for leather in the fashion and textile industries. As there are major issues with the flexibility and water absorptive properties, the research gap is to alter this property of the pellicle and to utilize it effectively. The SCOBY layer of kombucha can replace the traditional cellulose and the cellulose chemistry of the biofilm can be uplifted. Despite scattered safety issues, this functional drink is safe and can replace carbonated beverages. To summarize, every part of this miracle drink has major benefits in various domains and should be considered a boon from China. The industrial application of eco-friendly SCOBY is increasing tremendously because of its high potential and unique properties. Combining kombucha with other beneficial materials may lead to profitable and feasible innovations. As kombucha in the fermentation industry peaks to gain popularity, the unexplored areas of this beverage should be scientifically validated to level up the beverage for the next generation. The information in this article is expected to help fill in the research gaps and provide value for this Chinese wonder drink.

Authors' contributions

Both the authors have read and approved the manuscript. The conception of work, drafting, revision and technical help were done by Kalaichelvan Gurumurthy. Data collection and material support were done by Suriyapriya S.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors sincerely thank and acknowledge the supports from Vellore Institute of Technology (VIT).

References

Adriani, L., Mayasari, N., & Kartasudjana, R. (2011). The effect of feeding fermented kombucha tea on HLD, LDL and total cholesterol levels in the duck bloods. *Biotechnology in Animal Husbandry, 27*(4), 1749–1755.

Aloulou, A., Hamden, K., Elloumi, D., Ali, M. B., Hargafi, K., Jaouadi, B., ... Ammar, E. (2012). Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. *BMC Complementary and Alternative Medicine, 12*(1), 1–9.

Balentine, D. A. (1992). Manufacturing and chemistry of tea. *Journal of the American Chemical Society, 8*, 102–117.

Banerjee, D., Hassarajani, S. A., Maity, B., Narayan, G., Bandyopadhyay, S. K., & Chattopadhyay, S. (2010). Comparative healing property of kombucha tea and black tea against indomethacin-induced gastric ulceration in mice: Possible mechanism of action. *Food & Function, 1*(3), 284–293.

Battikh, H., Bakhrouf, A., & Ammar, E. (2012). Antimicrobial effect of Kombucha analogues. *LWT-Food Science and Technology, 47*(1), 71–77.

Bhattacharya, D., Sinha, R., Mukherjee, P., Howlader, D. R., Nag, D., Sarkar, S., ... Gachhui, R. (2020). Anti-virulence activity of polyphenolic fraction isolated from Kombucha against *Vibrio cholerae*. *Microbial Pathogenesis, 140* 103927.

Bhattacharya, S., Gachhui, R., & Sil, P. C. (2013). Effect of Kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. *Food and Chemical Toxicology, 60*, 328–340.

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

Cardoso, R. R., Neto, R. O., dos Santos D'Almeida, C. T., do Nascimento, T. P., Pressete, C. G., Azevedo, L., ... & de Barros, F. A. R. (2020). Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International*, *128*, 108782.

Četojević-Simin, D. D., Velićanski, A. S., Cvetković, D. D., Markov, S. L., Mrđanović, J. Ž., Bogdanović, V. V., & Šolajić, S. V. (2012). Bioactivity of lemon balm kombucha. *Food and Bioprocess Technology, 5*(5), 1756–1765.

Chandrakala, S. K., Lobo, R. O., & Dias, F. O. (2019). Kombucha (bio-tea): An elixir for life?. In *Nutrients in beverages* (pp. 591-616). Academic Press.

Chan, C. K., Shin, J., & Jiang, S. X. K. (2018). Development of tailor-shaped bacterial cellulose textile cultivation techniques for zero-waste design. *Clothing and Textiles Research Journal, 36*(1), 33–44.

Chawla, P. R., Bajaj, I. B., Survase, S. A., & Singhal, R. S. (2009). Microbial cellulose: Fermentative production and applications. *Food Technology & Biotechnology, 47*(2), 107–124.

Chu, S. C., & Chen, C. (2006). Effects of origins and fermentation time on the antioxidant activities of kombucha. *Food Chemistry, 98*(3), 502–507.

Costa, A. F., Almeida, F. C., Vinhas, G. M., & Sarubbo, L. A. (2017). Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources. *Frontiers in Microbiology, 8*, 2027.

Deghrigue, M., Dellai, A., Akremi, N., Le Morvan, V., Robert, J., & Bouraoui, A. (2013). Evaluation of antiproliferative and antioxidant activities of the organic extract and its polar fractions from the Mediterranean gorgonian Eunicella singularis. *Environmental Toxicology and Pharmacology, 36*(2), 339–346.

Dipti, P., Yogesh, B., Kain, A. K., Pauline, T., Anju, B., Sairam, M., ... Selvamurthy, W. (2003). Lead induced oxidative stress: Beneficial effects of Kombucha tea. *Biomedical and Environmental Sciences, 16*(3), 276–282.

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

Fontana, J. D., Franco, V. C., Lyra, I. N., De Souza, A. M., & De Souza, S. (1991). *Nature of plant stimulators in the production of Acetobacter xylinum ({open_quotes} Tea fungas {close_quotes}) biofilm used in skin therapy* (No. CONF-900512-). Oak Ridge National Lab.(ORNL), Oak Ridge, TN (United States); Badger Engineers, Inc., Tampa, FL (United States); Solar Energy Research Inst., Golden, CO (United States).

Fu, C., Yan, F., Cao, Z., Xie, F., & Lin, J. (2014). Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage. *Food Science and Technology, 34*, 123–126.

Gharib, O. A. (2010). Does kombucha tea attenuate the hepato-nepherotoxicity induced by a certain environmental pollutant? *Egyptian Academic Journal of Biological Sciences, B Zoology, 2*(2), 11–18.

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012). Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal, 19*(1), 109–117.

Greenwalt, C . J., Steinkraus, K. H., & Ledford, R. A. (2000). Kombucha, the fermented tea: Microbiology, composition, and claimed health effects. *Journal of Food Protection, 63*(7), 976–981.

Hartmann, A. M., Burleson, L. E., Holmes, A. K., & Geist, C. R. (2000). Effects of chronic kombucha ingestion on open-field behaviors, longevity, appetitive behaviors, and organs in c57-bl/6 mice: A pilot study. *Nutrition, 16*(9), 755–761.

Hosseini, S. A., Rasouli, L., Gorjian, M., & Yadollahpour, A. (2016). A comparative study of the effect of Kombucha prepared from green and black teas on the level of blood glucose and lipid profile of diabetic rats. *International Journal of Pharmaceutical Research & Allied Sciences, 5*(2).

Isdadiyanto, S., & Tana, S. (2019). Effect of time fermentation kombucha tea on lipid profile of rats (*Rattus norvegicus* L.). *Journal of Physics: Conference Series, 1217*(1) 012158.

Jayabalan, R., Chen, P. N., Hsieh, Y. S., Prabhakaran, K., Pitchai, P., Marimuthu, S., ... Yun, S. E. (2011). *Effect of solvent fractions of kombucha tea on viability and invasiveness of cancer cells—characterization of dimethyl 2-(2-hydroxy-2-methoxypropylidine) malonate and vitexin*. Cambridge University Press.

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 538–550.

Jayabalan, R., Malini, K., Sathishkumar, M., Swaminathan, K., & Yun, S. E. (2010). Biochemical characteristics of tea fungus produced during kombucha fermentation. *Food Science and Biotechnology, 19*(3), 843–847.

Jayabalan, R., Marimuthu, S., Thangaraj, P., Sathishkumar, M., Binupriya, A. R., Swaminathan, K., & Yun, S. E. (2008). Preservation of kombucha tea effect of temperature on tea components and free radicalscavenging properties. *Journal of Agricultural and Food Chemistry, 56*(19), 9064–9071.

Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., & Swaminathan, K. (2008). Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry, 109*(1), 227–234.

Kumar, S. D., Narayan, G., & Hassarajani, S. (2008). Determination of anionic minerals in black and kombucha tea using ion chromatography. *Food Chemistry, 111*(3), 784–788.

Lobo, R. O., Sagar, B. C., & Shenoy, C. K. (2017). Bio-tea prevents membrane destabilization during isoproterenol-induced myocardial injury. *Journal of Microscopy and Ultrastructure, 5*(3), 146–154.

Lobo, R. O., & Shenoy, C. K. (2015). Myocardial potency of bio-tea against isoproterenol induced myocardial damage in rats. *Journal of Food Science and Technology, 52*(7), 4491–4498.

Malbaša, R., Lončar, E., Djurić, M., & Došenović, I. (2008). Effect of sucrose concentration on the products of Kombucha fermentation on molasses. *Food Chemistry, 108*(3), 926–932.

Malbaša, R., Lončar, E., & Kolarov, L. (2002). *L*-lactic, *L*-ascorbic, total and volatile acids contents in dietetic Kombucha beverage. *Romanian Biotechnological Letters, 7*(5), 891–895.

Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Čanadanović-Brunet, J. M. (2011). Influence of starter cultures on the antioxidant activity of kombucha beverage. *Food Chemistry, 127*(4), 1727–1731.

Marsh, A. J., O' Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2013). Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources. *PLoS One, 8*, e69371.

Martini, N. (2018). Potion or Poison? Kombucha. *Journal of Primary Health Care, 10*(1), 93–94.

May, A., Narayanan, S., Alcock, J., Varsani, A., Maley, C., & Aktipis, A. (2019). Kombucha: A novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. *PeerJ, 7*, e7565.

Miranda, B., Lawton, N. M., Tachibana, S. R., Swartz, N. A., & Hall, W. P. (2016). Titration and HPLC characterization of kombucha fermentation: A laboratory experiment in food analysis. *Journal of Chemical Education, 93*(10), 1770–1775.

Morán, J. I., Alvarez, V. A., Cyras, V. P., & Vázquez, A. (2008). Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose, 15*, 149–159.

Morshedi, A., Dashti, M. H., Rafati, A., Mosaddegh, M. H., & Salami, A. S. (2006). The chronic effect of Kombucha Tea consumption on weight loss in diabetic rats. *Journal of Medicinal Plants, 5*(17), 17–22.

Mrđanović, J., Bogdanović, G., Cvetković, D., Velićanski, A., & Četojević-Simin, D. (2007). The frequency of sister chromatid exchange and micronuclei in evaluation of cytogenetic activity of Kombucha on human peripheral blood lymphocytes. *Archive of Oncology, 15*(3–4), 85–88.

Niyazbekova, Z. T., Nagmetova, G. Z., & Kurmanbayev, A. A. (2018). An overview of bacterial cellulose applications. *Eurasian Journal of Applied Biotechnology, 2*. https://doi.org/10.11134/btp.2.2018.3.

Pauline, T., Dipti, P., Anju, B., Kavimani, S., Sharma, S. K., Kain, A. K., ... Selvamurthy, W. (2001). Studies on toxicity, anti-stress and hepato-protective properties of Kombucha tea. *Biomedical and Environmental Sciences, 14*(3), 207–213.

Pei, J., Jin, W., Abd El-Aty, A. M., Baranenko, D. A., Gou, X., Zhang, H., ... Yue, T. (2020). Isolation, purification, and structural identification of a new bacteriocin made by *Lactobacillus plantarum* found in conventional kombucha. *Food Control, 110* 106923.

Petrovic, S. E., Suturovic, Z. J., Loncar, E. S., & Malbasa, R. V. (1999). Potentiometric stripping analysis of certain metal ions in tea fungus beverage. *Nahrung (Weinheim), 43*(5), 345–346.

Rangaswamy, B. E., Vanitha, K. P., & Hungund, B. S. (2015). Microbial cellulose production from bacteria isolated from rotten fruit. *International Journal of Polymer Science, 2015*, 1–8.

Ram, M. S., Anju, B., Pauline, T., Prasad, D., Kain, A. K., Mongia, S. S., ... Selvamurthy, W. (2000). Effect of Kombucha tea on chromate (VI)-induced oxidative stress in albino rats. *Journal of Ethnopharmacology, 71*(1–2), 235–240.

Shade, A., Buckley, D. H., & Zinder, S. H. (2011). The kombucha biofilm: a model system for microbial ecology. *Final report on research conducted during the Microbial Diversity course. Marine Biological Laboratories, Woods Hole, MA.*

Soltani, M., Farshadfar, M., Shirvani, H., & Yaghotipoor, A. (2021). Evaluation of the antibacterial effect of a beneficial compound based on the probiotic kombucha and honey. *Honeybee Science Journal, 11*(21).

Shenoy, C. (2000). Hypoglycemic activity of bio-tea in mice. *Blood, 38*(3), 278–279.

Sreeramulu, G., Zhu, Y., & Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. *Journal of Agricultural and Food Chemistry, 48*(6), 2589–2594.

Sreeramulu, G., Zhu, Y., & Knol, W. (2001). Characterization of antimicrobial activity in Kombucha fermentation. *Acta Biotechnologica, 21*(1), 49–56.

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of Kombucha fermentation. *International Journal of Food Microbiology, 95*(2), 119–126.

Vázquez-Cabral, B. D., Larrosa-Pérez, M., Gallegos-Infante, J. A., Moreno-Jiménez, M. R., González-Laredo, R. F., Rutiaga-Quiñones, J. G., ... Rocha-Guzmán, N. E. (2017). Oak kombucha protects against oxidative stress and inflammatory processes. *Chemico-Biological Interactions, 272*, 1–9.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., Renard, T., Rollan, S., & Taillandier, P. (2019). Impact of fermentation conditions on the production of bioactive compounds with anticancer, anti-inflammatory and antioxidant properties in kombucha tea extracts. *Process Biochemistry, 83*, 44–54.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., & Taillandier, P. (2018). Understanding kombucha tea fermentation: A review. *Journal of Food Science, 83*(3), 580–588.

Vohra, B., Fazry, S., Sairi, F., & Othman, B. A. (2019). Effects of medium variation and fermentation time towards the pH level and ethanol content of Kombucha. In *AIP Conference Proceedings* (Vol. 2111, No. 1, p. 040008). AIP Publishing LLC.

Wolfe, B. E., & Dutton, R. J. (2015). Fermented foods as experimentally tractable microbial ecosystems. *Cell, 161*(1), 49–55.

Yapar, K., Cavusoglu, K., Oruc, E., & Yalcin, E. (2010). Protective effect of kombucha mushroom (KM) tea on phenol-induced cytotoxicity in albino mice. *Journal of Environmental Biology, 31*(5), 615.

Zubaidah, E., Afgani, C. A., Kalsum, U., Srianta, I., & Blanc, P. J. (2019). Comparison of *in vivo* antidiabetes activity of snake fruit Kombucha, black tea Kombucha and metformin. *Biocatalysis and Agricultural Biotechnology, 17*, 465–469.