Preface

The last few centuries have seen an increase in food production through modern agricultural technologies and techniques with a significant effect on the quality and quantity of food produced globally but at the same time has harmed the environment. The current food system, including production and postfarm operations such as processing and distribution, accounts for over a quarter (26%) of global greenhouse gas emissions, which pose a challenge for the coming decades. People across the globe are becoming increasingly concerned about the negative impact of global greenhouse gas emissions and are realizing that it is a result of their food choices. It is clear that eating less meat and dairy products will have a much more significant impact on carbon footprint than eating plant-based foods. Therefore, an increased understanding of the environmental impacts of food production and a growing preference for healthier vegetarian foods are driving the urgency to develop sustainable food systems. Plant-based food systems offer a practical solution to this sustainable goal, resulting in an increased global demand for plant-based meat and dairy alternatives. This book, ***Engineering Plant-Based Food Systems,*** provides a broad understanding of the various plant-based foods and the technologies used to create them.

The book introduces what plant-based food means, the current trends, and its potential as a sustainable food source for the future (Chapter 1), followed by consumer expectations, sensory perception, and attitude toward plant-based foods (Chapter 2). The general health and nutrition of plant-based foods and ingredients, including the antinutritional aspect of plant-based foods and their impact on human health, are also discussed. The book also includes sustainable plant protein sources and their extraction techniques (Chapter 3), the thermal and nonthermal processing techniques used to reduce food allergies caused by plant proteins (Chapter 4), and their functionality, including color, flavor, texture, wettability, dispersibility, solubility, rheological properties, emulsifying properties, and foaming properties (Chapter 5). The subsequent chapters in the book include popular commercially available plant-based foods such as plant-based beverages (Chapter 6), plant-based gels (Chapter 7), plant-based butter-like spreads (Chapter 8), plant-based meat analog (Chapter 9), plant-based imitated fish (Chapter 10), plant-based imitated seafood (Chapter 11), fermented plant-based beverage: Kombucha (Chapter 12), and fermented plant-based food (Chapter 13), the plant materials used to manufacture them using different processing techniques, and their sensory properties. The following two chapters discuss the various functional components obtained from plants and their extraction and incorporation in plant-based food (Chapter 14) and the processing techniques to improve their bioavailability and bioaccessibility (Chapter 15). The recent developments in 3D-printed plant-based foods using plant-based ingredients are reviewed in Chapter 16, followed by a concluding Chapter 17 discussing the future of plant-based foods.

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Chapter 12

Fermented plant-based beverage: kombucha

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12.1 Introduction

Fermentation is one of the most traditional methods of food preservation. Various fermented food products were originally known for their benefits to increase shelf-life, product safety, and improve organoleptic properties. Today more than ever, fermented foods are fast developing and have much higher functional health benefits. Fermentation results in the transformation of key substrates by microbes into bioactive metabolites. Many biochemical changes occur during fermentation and may improve bioavailability of essential nutrients and consequently enhance the properties of the final product, such as digestibility, utilization, and the bioactivity. Recently, this bioprocess has been applied for the production and extraction of bioactive ingredients from plants in food and beverage industries (Hur et al., 2014). Clinical trials have shown that fermented foods provide health benefits far beyond the starting ingredients. In addition, many fermented foods are rich in enzymes produced by microbes that assist with the digestive process and contain live microbes, which function as probiotics.

Some fermented foods have evidenced therapeutic benefits by virtue of the presence of live microbiome in them imparting probiotics or synbiotics properties. They are also termed: bio-foods, therapeutic foods, or nutraceutical foods and have been the subject of extensive research in the past few decades. The fermentation process generates new metabolites owing to the microbial activity, e.g., antioxidants and B vitamins [folic acid, riboflavin and vitamin B12, which is formed from nonvitamin compounds]. Some essential amino acids and their derivatives that either act as neurotransmitters such as gamma-aminobutyric acid or play an immunomodulatory function could also be produced during fermentation. Moreover, some of the proteins and exopolysaccharides created during fermentation are able to impair the attachment of disease-causing microbes to the intestinal wall and serve as cholesterol-lowering (hypocholesterolemic) agent. One of the fermented foods that have high functional properties is kombucha. The word "kombucha" comes from the Japanese words "seaweed" (Kombu) and "tea" (cha). Kombucha tea is also known as red tea fungus, Champignon de longue vie, Chainii grib, Ling zhi, kocha kinoko, and Chainii kvass (Amarasinghee et al., 2018).

12.2 Kombucha and its properties

Kombucha is a traditional tea beverage produced by fermenting sweet tea using Symbiotic Consortia of Bacteria and Yeast (SCOBY) (Filippis et al., 2018). SCOBY is a symbiotic community of acetic acid bacteria (AAB) Acetobacteraceae and osmophilic yeast (De Filippis et al., 2018). In general, kombucha is made using black or green tea as a raw material by symbiotic cultures of AAB (*Gluconobacter, Acetobacter*), lactic acid bacteria (*Lactobacillus, Lactococcus*), and yeast (*Saccharomycodes ludwiga, Zygosaccharomyces bailii,* etc.) (Villarreal-Soto et al., 2011). As a result of fermentation, the taste of kombucha will change from a fruity, sour, and carbonated taste to a mild vinegary flavor, thereby increasing consumer acceptance of the taste and other sensory aspects. The resulting kombucha tea will taste like apple cider, which is sour and slightly carbonated (Amarasinghee et al., 2018). Kombucha fermentation has traditionally been carried out from

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10 to 14 days (Zubaidah et al., 2018a, 2018b), but currently fermentation is generally carried out from 7 to 21 days (Ahmed et al., 2020; Amarasinghee et al., 2018).

Kombucha fermentation is a combination of three components: alcoholic, lactic, and acetic acid; this is because of the coexistence of several yeasts and bacteria in the medium, being initiated by osmotolerant microorganisms and ultimately dominated by acid-tolerant species (Villarreal-Soto et al., 2018). It also originated in China where the "Divine Che" was prized 220 BCE during the Tsin Dynasty for its detoxifying and energizing properties (Roche, 1998).

Various in vivo tests have proven the positive effects of kombucha on some, degenerative diseases, for instance, it is included in diabetes therapy, enhancing liver and pancreatic functions. Kombucha has been used as a therapeutic agent for hyperglycemia and dyslipidemia in diabetic animal models (Aloulou et al., 2012; Srihari et al., 2013). Kombucha is able to provide an inhibitory effect on alpha amylase and lipase activities in blood plasma and pancreas, so that it suppresses the surge in blood glucose levels (Aloulou et al., 2012). Thus, the health benefits thought to be associated with kombucha consumption are numerous and are attributed to the phenolic compounds, which rise during fermentation. In addition, there are organic acids, vitamins, amino acids, antibiotics, and various micronutrients produced during fermentation of kombucha, which are thought to have a role in health benefits (Vijayaraghavan et al., 2000). The literature outlined the multimechanisms through which kombucha assists in diabetes therapy through reduction of damage to pancreatic beta cells caused by oxidative stress so as to increase insulin production, lessen glucose uptake from the digestive system, and improve cellular glucose uptake (Aloululo et al., 2012; Bhattacharya et al., 2011; Srihari et al., 2013). In addition, kombucha is rich in enzymes that are beneficial for digestion, as well as potential as a probiotic drink. Kombucha was illustrated in Fig. 12.1.

Kombucha is known to have several health benefits such as immune response boosting and liver detoxification (Chakravorty et al., 2016) due to the genesis of key metabolites during the fermentation process (Dufresne & Farnworth, 2000) such as acetic acid, gluconic acid, and other compounds such as sugar, ethyl gluconate, oxalate, saccharic, lactate, keto-gluconate, amino acids, water soluble vitamins, tea components (catechins, teaflavins, flavonols), and hydrolytic enzymes (invertase, amylase) (Velićanski et al., 2014). In vitro study by Vázquez-Cabral et al. (2017) showed that kombucha fermented for 28 days has remarkable antioxidant properties that can fight oxidative damage by H_2O_2 in macrophages and can significantly decrease the levels of proinflammatory cytokines IL-6, tumor necrosis factor (TNF)-α, and nitric oxide (NO). (Zubaidah et al., 2020a, 2020b) reported that kombucha tea and snake fruit kombucha were able to significantly enhance the immune response in mice infected with *Salmonella typhi*.

12.3 Microbiology of kombucha

Microbial colonies in kombucha depend on fermentation conditions such as temperature, time, type of microbe selected, raw materials, and the source of sugar used. Hence, there are no unique or standardized kombucha microbes, but some of them are identified as species of AAB, lactic acid bacteria or LAB, and yeast (Morales, 2020). Kombucha consists of two components, namely, the cellulose layer and liquid kombucha. The cellulose layer is also called a biofilm, where the diameter corresponds to the diameter of the fermentation vessel. Microbes that produce cellulose, namely, the type of

FIGURE 12.1 Liquid kombucha and cellulose layer (Zubaidah et al., 2018a, 2018b).

Acetobacter with the dominant species *Acetobacter xylinum*. The latter was reclassified as *Gluconacetobacter xylinus*, then reclassified again as *Komagataeibacter xylinus* (Yamada et al., 2012; Villarreal-Soto et al., 2018). Particularly that the biochemical activity of these bacteria is the oxidation of glucose to gluconic acid (*found in the liquid phase*), then another specific metabolic pathway synthesizes microbial cellulose into biofilms on the surface of the liquid. The composition of microbial colonies in kombucha tea is yeast and AAB, which grow symbiotically, have synergistic activity, and complement each other in the fermentation process.

12.3.1 Yeast

Yeast is facultative anaerobic, which means it can grow well, with or without oxygen. During kombucha fermentation, yeast plays a role in producing alcohol. There are many yeasts genus and species in kombucha culture; a broad spectrum has been reported including species of Zyg*osaccharomyces, Candida, Kloeckera/Hanseniaspora, Torulaspora, Pichia, Brettanomyces/Dekkera, Saccharomyces, Lachancea, Saccharomycoides, Schizosaccharomyces,* and *Kluyveromyces* (Chakravorty et al., 2016; Coton et al., 2017; Marsh et al., 2014). *Saccharomyces* sp. being one of the most common types of yeast is found in kombucha. Saccharomyces strains found in kombucha include *Saccharomycodes apiculatus*, *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe*, *Zygosaccharomyces*, and *Saccharomyces cerevisiae*. Another strain is Brettanomyces sp., which also grows in both aerobic and anaerobic conditions and produces alcohol or acetic acid. *Zygosaccharomyces kombuchaensis* is a yeast strain in kombucha that produces alcohol, undergoes carbonation, and also contributes to the formation of SCOBY (Harler, 1964).

12.3.2 Acetic acid bacteria

The acetic acid bacteria that grow on kombucha tea are aerobic, which means that their growth requires oxygen. The main AAB identified in kombucha tea is *Acetobacter*, which produces acetic acid and gluconic acid. The Acetobacter strain is almost found in kombucha and contributes to the formation of the SCOBY fungus. The strains of Acetobacter that are most commonly found in kombucha are *Acetobacter xylinoides* and *Acetobacter ketogenum* (Harler, 1964). *Gluconacetobacter kombuchae* is an anaerobic bacterial strain that was identified from kombucha tea; the bacteria utilizes nitrogen in tea to produces acetic acid and gluconic acid and contributes to the formation of SCOBY (Harler, 1964). *Komagataeibacter xylinus* is also one of the strains of aerobic bacteria, which is known for its ability to produce cellulose (Römling & Galperin, 2015).

12.3.3 Lactic acid bacteria

In some kombucha tea, it was identified that lactic acid bacteria came from the Lactobacillus (aerobic) strain, a type of strain that produces lactic acid and mucus (Harler, 1964). Some lactic acid bacterial strains could be found in kombucha tea or be added to kombucha to increase its antioxidant and antimicrobial capacity as well as increasing the production of glucuronic acid. There are many strains of AAB and yeast, which have been identified in kombucha.

The identified microbial species in kombucha are shown Table 12.1.

During fermentation, yeast hydrolyzes sucrose to glucose and fructose. The emerged glucose and fructose will be fermented by yeast into ethanol, carbon dioxide, and glycerol (Gaggia et al., 2019). Ethanol is then oxidized by AAB to acetic acid so that the pH in kombucha will be eventually decreased (Saichana et al., 2015). These AAB are obligate aerobes (i.e., requiring oxygen for the fermentation process) (Saichana et al., 2015). In static conditions, a cellulose biofilm will be produced on the kombucha surface (Mao et al., 2019). This cellulose has a strong structure and can be used as protection from pathogenic bacteria (Mao et al., 2019).

During the fermentation process, further metabolites are also produced. AAB (*Gluconobacter species*) converts glucose into gluconic acid and glucuronic acid (Jayabalan et al., 2017, pp. 965−978). Meanwhile, yeast will stimulate the production of glucuronic acid by lactic acid bacteria (Nguyen et al., 2015). Other metabolites e.g., 1,4-lactone D-saccharide acid, lactic acid, quinic acid, oxalic acid, malic acid, and citric acid (Laureys et al., 2020) are also generated in the medium.

During kombucha fermentation, a symbiotic relationship between microbes and a selective environment is formed. Ethanol produced by yeast (Pfeiffer & Morley, 2014) and production of organic acids by yeast and bacteria will reduce the pH of kombucha to 2.0−4.0 (Chakravorty et al., 2016; Neffe-Skocinska et al., 2017); such selective medium inhibits the growth of certain microbes.

TABLE 12.1 Microbial species identified in kombucha beverages.

Microorganism	Genus	Species	Source
AAB	*Acetobacter*	*Acetobacter lovaniensis*	Coton et al. (2017)
		Acetobacter peroxydans	Coton et al. (2017)
	Gluconobacter	*Gluconobacter liquefaciens*	Coton et al. (2017)
		Gluconobacter europaeus	Coton et al. (2017)
	Tanticharoenia	*Tanticharoenia sakaeratensis*	Coton et al. (2017)
LAB	*Lactobacillus*	*Lactobacillus nagelli*	Coton et al. (2017)
Yeast	*Candida*	*Candida Boidinii*	Coton et al. (2017)
	Dekkera	*Dekkera anomala*	Coton et al. (2017)
	Hanseniaspora	*Hanseniaspora valbyensis*	Coton et al. (2017)
	Pichia	*Pichia anomala*	Coton et al. (2017)
		Pichia membranifaciens	Coton et al. (2017)
	Torulaspora	*Torulaspora microellipsoides*	Coton et al. (2017)
	Saccharomyces	*Saccharomyces uvarum*	Coton et al. (2017)
	Zygotorulaspora	*Zygotorulaspora florentina*	Coton et al. (2017)
	Zygosaccharomyces	*Zygosaccharomyces bailii*	Coton et al. (2017)
Yeast	*Candida*	*Candida parapsilosis*	Chakravorty et al. (2016)
	Eremothecium	*Eremothecium ashbyii*	Chakravorty et al. (2016)
	Hansemiaspora	*Hanseniaspora vineae*	Chakravorty et al. (2016)
	Kluyveromyces	*Kluyveromyces marxianus*	Chakravorty et al. (2016)
	Pichia	*Pichia mexicana*	Chakravorty et al. (2016)
	Saccharomyces	*Saccharomyces cerevisiae*	Chakravorty et al. (2016)
	Sporopachydermia	*Sporopachydermia lactativora*	Chakravorty et al. (2016)
	Zygowilliopsis	*Zygowilliopsis californica*	Chakravorty et al. (2016)

AAB = Acetic acid bacteria; *LAB* = Lactic acid bacteria.
Source: Morales (2020).

12.4 Kombucha processing

(Zubaidah et al., 2018a, 2018b), explained the process of making kombucha (Fig. 12.2). It starts by boiling water, adding 10% sugar, and 0.8%−1% of tea leaves. After the tea and sugar are evenly mixed, they are allowed to boil for 10 min and then poured into a jar, which is then tightly closed and cooled to room temperature. The jar used is presterilized to prevent contamination from other microbes that can hinder the fermentation process. The tea is then inoculated with the kombucha starter 10% and covered with a cloth to form an aerobic atmosphere. The fermentation process is carried out at a temperature of 20 to 30°C with optimal temperatures in the range of 23 to 28°C. The fermentation vessel should be placed on a level surface to maximize the formation of new SCOBY. Several days later, SCOBY will form and begin to float forming a thin, clear gel-like membrane on the entire surface, located above the parent SCOBY. The fermentation process takes between 10 and 14 days.

The tea leave used as a raw material for making kombucha could be replaced with other ingredients. Some of the ingredients developed for making kombucha include Jerusalem artichoke, echinacea flowers, mint leaves, eucalyptus leaves (Ayed et al., 2017; Vázquez-Cabral et al., 2016; Jayabalan et al., 2014; Lobanova et al., 2016; Yavari et al., 2010, 2011), and other leaves, which are high in antioxidants, e.g., guava leaves, tailings leaves, coffee leaves, and betel leaves can also be used (Zubaidah & Wistiani, 2015). Other ingredients for making kombucha include cherries, grapes, oranges, black currants, apples, dragon fruit and snake fruit (Zubaidah et al., 2018a, 2018b), dates and turmeric (Zubaidah, et al., 2020a, 2020b).

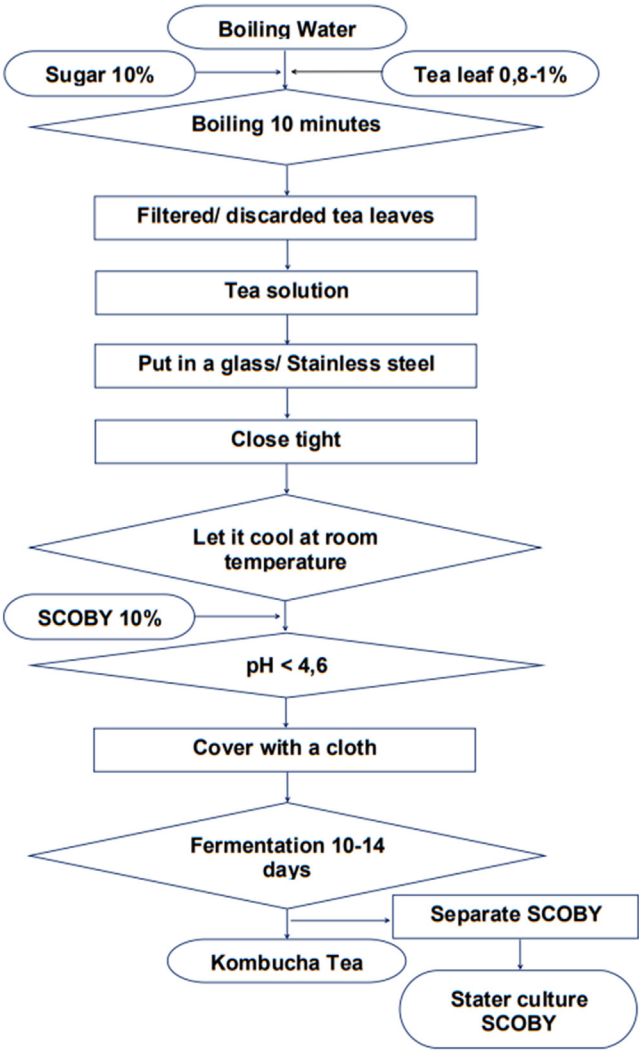

FIGURE 12.2 The process of making kombucha tea (Zubaidah et al., 2018a, 2018b).

12.4.1 Preparation of fermentation medium

In a liter of boiling water, 8−10 g of tea and 10% sucrose sugar are added, and then the tea is allowed to boil for 10 min. Afterward the tea is poured into a fermentation container. The fermentation container used could be made from glass or stainless steel that has been presterilized, tightly closed, and is at room temperature.

12.4.2 Inoculation

The inoculation process is carried out after the tea has cooled to room temperature. Kombucha culture, which is a symbiosis of bacteria and yeast (SCOBY), is added and as much as 10% (v/v) of the kombucha liquid from the previous fermentation. Sheet-shaped "tea mushrooms" can be added as much as 3% (w/v) (Jayabalan et al., 2008). Malbaša et al. (2006) revealed that the kombucha fermentation is faster with the addition of liquid starter compared to simply adding "tea mushroom." According to Ma et al. (2008), adding pure culture can be done to replace mixed cultures, with a combination of *Saccharomyces cerevisiae* 108 CFU/mL, *Gluconacetobacter* sp 108 CFU/mL, and *Lactobacillus plantarum* 108 CFU/mL with a ratio of 1: 1: 1.

12.4.3 Fermentation

The fermentation container is tightly closed with a breathable cloth, then left for 10−14 days. The length of kombucha fermentation depends on the ambient temperature and also the desired acidity level. The fermentation temperature ranges

from 20 to 30°C. The longer the fermentation process, the higher the organic acids and phenols content (Leal et al., 2018). However, if the fermentation is continued for a longer time, the acidity level will increase and could cause potential harm if consumed. Fermentation can be stopped by separating the kombucha culture from the medium and then transferring the kombucha liquid into a closed bottle container (Greenwalt et al., 2000). During the fermentation process, microbes will produce primary and secondary metabolites, including acetic acid, glucuronic acid, alcohol, and nata/cellulose (Villarreal et al., 2018). The main acids present are acetic, gluconic, tartaric, malic, and in less proportion, citric acid. All these acids are responsible for its characteristic sour taste (Jayabalan et al., 2007). The composition and concentration of kombucha metabolite compounds are influenced by the source of the inoculum (Nguyen et al., 2015), the concentration of tea and sugar (Fu et al., 2014; Watawana et al., 2017), and the length of fermentation time (Zubaidah et al., 2018a, 2018b).

As outlined earlier, in static conditions, a cellulose biofilm will be produced on the kombucha surface (May et al., 2019). AAB synthesize and polymerize cellulose chains to form fibril tissue in SCOBY. The following is a reaction to the formation of glucuronic acid in kombucha (Prescott & Dunn, 1959; Wood, 1998). Synthesis and polymerization of cellulose chains to form fibril networks begins with the synthesis of uridine diphospho-glucose (UDPGlc), which is a precursor to cellulose and polymerization of β-1,4-glucan chains by single cell acetobacter bacteria. The production of cellulose in this way provides the advantage that bacteria can grow rapidly under controlled conditions and can produce cellulose from a variety of carbon sources including glucose, ethanol, sucrose, and glycerol (Villarreal-Soto et al., 2018).

In the first stage, cellulose-producing bacteria increase the population by utilizing dissolved oxygen and synthesizing cellulose membranes in the liquid phase. The thickness of the membrane continues to increase to form a solid structure in the culture medium. The development of the membrane together with hydrogen and C−H bonds will continue and then stop when the membrane begins to grow covering the entire surface and trapping all bacteria, causing the bacteria to no longer be active due to insufficient oxygen supply (Villarreal-Soto et al., 2018). These bacteria will reactivate when used as an inoculum in fermentation (Villarreal-Soto et al., 2018). The cellulose formed varies depending on many factors: the strain used, culture time, and chemical additives added to the fermentation medium (Villarreal-Soto et al., 2018). Several factors that need to be controlled to maximize cellulose yield, e.g., volume of inoculated media, incubation time, and surface area (Villarreal-Soto et al., 2018).

12.4.4 Composition of kombucha

Kombucha consists of two components, namely, the cellulose layer and liquid kombucha. Some of the compounds, which have been identified in kombucha, include organic acids, sugars (sucrose, glucose, and fructose), water-soluble vitamins, amino acids, biogenic amines, purines, pigments, lipids, proteins, hydrolytic enzymes, ethanol, carbon dioxide, polyphenols, minerals (Mn, Fe, Ni, Co, Zn, Cu, Cd, Pb), anions (fluorides, chlorides, bromides, iodides, nitrates, phosphates, and sulfates), D-saccharic acid-1,4-lactone, and metabolic products from yeast and bacteria (Leal et al., 2018). During the fermentation process of sugared tea infusion, disaccharides undergo decomposition into monosaccharides under the influence of enzymes and acids, i.e., simple sugars. Afterward, the role of yeasts in the fermentation process is to convert sugar into alcohol and carbon dioxide. Concurrently, AAB lives on the cellulose part of the kombucha starter culture. They play a vital role in the fermentation process because they are responsible for creating new cellulose layers, and moreover, they metabolize alcohol produced by yeasts into organic acids. Apart from organic acids, the basic metabolites in the fermentation process of kombucha are simple sugars, carbon dioxide, and ethanol (Chakravorty et al., 2016). According to Chen and Liu (2000), Lončar, et al. (2000), Malbaša et al. (2002) and Jayabalan et al. (2007), acetic, glucuronic and gluconic acids are the main healthy products of kombucha drink, which is produced from sucrose and black tea in a controlled and optimized fermentation process. According to Nguyen et al. (2015), a right choice of fermentation conditions and selection of kombucha starter culture enables production of beverages with the highest content of healthy organic acids, including glucuronic acid. Apart from acetic acid, it is one of the main organic acids resulting from fermentation (Jayabalan et al., 2007).

Kombucha has a total organic acid content of 2.5 g/L, dominated by acetic acid (1.55 g/L) tartaric (0.23 g/L) and citric acid (0.05 g/L) (Ivanišova et al., 2019). Acetic acid is a chemical compound that plays a role in the smell and sour taste of kombucha. This compound is produced by AAB through the conversion of sucrose to ethanol, glucose and fructose (Spedding, 2015). The amount of acetic acid is strongly influenced by the length of fermentation.

Jayabalan et al. (2007) showed that the acetic acid value in kombucha black tea ranged from 0.22 g/L (fermentation on day 3) to 9.51 g/L (fermentation on day 15). Oxalic, malic, lactic, tartaric and glucuronic acids are also found in kombucha. Glucuronic acid is considered as one of the key components in kombucha tea because it has the ability to detoxify by conjugation (Jayabalan et al., 2007).

Organic acids have the ability to capture reactive oxygen species (ROS) (Sentjurc et al., 2003) and increase the biological availability of phenolic compounds (van den Berg et al., 2003). Apart from that, organic acids can also be used as antimicrobials (Mau et al., 2002). Several studies have shown that organic acids can capture free radical compounds and have an antioxidant effect because they have the ability to chelate so they can deactivate reduced cations (Mau et al., 2004).

12.5 Functionality of kombucha

There are many reports that kombucha may help prevent chronic diseases and has beneficial properties for human health such as antihyperglycemic, antimicrobial, antioxidant, anti-carcinogenic and antihyperlipidemic; however, most of the benefits were stated in models (Chakravorty et al., 2016; Dufresne & Farnworth, 2000; Mo et al., 2008). Kombucha consists of the following favourable components: organic acids, vitamins, polyphenols, amino acids, antibiotics and microelements (Jayabalan et al., 2014; Marsh et al., 2014). Some scientific publications confirm induction of synthesis of vitamin C, B vitamins (e.g., folic acid) during the fermentation process of kombucha (Jayabalan et al., 2014; Malbaša et al., 2002). The most important organic acids produced during fermentation are glucuronic, gluconic, lactic, malic, citric, tartaric, folic, malonic, oxalic, succinic, pyruvic and usnic acids. Glucuronic acid deserves special attention: It is the result of a microbiological process of glucose oxidation and it is one of the most valuable, healthy kombucha components. Being produced by the liver in a human body, it exhibits detoxifying effects. Glucuronic acid has an ability to bind with xenobiotics, including phenols present in the liver, allowing these substances to be excreted by kidneys more efficiently. Glucuronic acid is also a precursor in the biosynthesis of vitamin C (Jayabalan et al., 2014; Nguyen et al., 2015).

12.5.1 Antioxidant activity

During fermentation there is an increase in antioxidant activity. The increase in antioxidant activity is followed by an increase in phenol levels. The increase in polyphenol compounds is also triggered by several enzymes produced during fermentation by yeast such as vinyl phenol reductase, ferulic acid reductase, vinyl phenol reductase enzymes, which are enzymes that catalyze the reaction of 4-vynilphenol + NAD + + 3H + to 4-ethylphenol + NADH and are commonly found in *Brettanimyces bruxellensis* (Tchobanov et al., 2008). The ferulic acid reductase enzyme is known as a phenol-forming enzyme through the decarboxylation of cinnamic acid and ferulic acid produced by bacteria. Cinnamic acid was identified as a phenol compound while firulic acid was identified as a derivative of cinnamic hydroxy acids. Both are able to act as antioxidants. The production of compounds that have radical scavenging properties depends on the culture period and the origin of the starter from which they determine which metabolites to produce (Watawana et al., 2015). The total phenol value in kombucha increases linearly during the fermentation process. According to research by Ivanišova et al. (2019), the total polyphenols in kombucha are 412 mg GAE/L, this value is higher than sweet tea (180.17 mg GAE/L). This is in accordance with Aidoo's research (2015) which shows that the total phenol content in kombucha is higher than unfermented tea. During the kombucha fermentation process, thearubigin depolymerization occurs which causes an increase in the total amount of phenol (Chu & Chen, 2006), besides that, polyphenols also undergo transformation and structural damage by microorganisms, which causes the color change in kombucha to become brighter (Chu & Chen, 2006).

Kombucha has a higher antioxidant content than unfermented tea (Fu et al., 2017) because it contains polyphones and metabolite compounds such as vitamins and organic acids (Essawet et al., 2015). Research by Fu et al. (2013) using green tea, black tea, and commercial tea showed that black tea had the highest DPPH binding ratio (95.30%), while green tea was 38.7%. However, the DPPH binding ratio in black tea can increase to 70% within 15 days of fermentation (Chu & Chen, 2006). Research by Jayabalan et al. (2008) showed that among the kombucha samples of green tea, black tea, and commercial teas analyzed, green tea had the highest ability to bind DPPH (88%) on fermentation on the 18th day.

The ability of kombucha to scavenge free radicals is also very diverse, depending on the microorganism and its metabolic process (Liu et al., 1996; Mayser et al., 1995) and its value increase 1.7 times higher after fermentation (Chu & Chen, 2006). This increase in ability to fight against DPPH radicals is ascribed to kombucha significant capacity to reverse chromate (VI) or compounds that cause oxidative damage (Dipti et al., 2003; Ram et al., 2000).

Chu and Chen's research (2006) showed that kombucha that has been fermented for 15 days has 1.4 times higher antioxidant level compared to regular tea, where the length of fermentation time has a positive correlation with the antioxidant yield. The effect of scavenging or free radical binding by catechins in tea varies widely, depending on the type of radical compounds, as well as antioxidant properties such as polarity, ionization level, and steric inhibition (Rice-Evans et al., 1996).

The difference in the composition of kombucha can be caused by the use of different starters, resulting in variability of microflora in each sample (Chen & Liu, 2000; Jayasekera et al., 2011; Reiss, 1994); this causes differences in antioxidant activity on each substrate (Malbaša et al., 2011).

12.5.2 Antibacterial activity

Kombucha is a fermented drink that has antibacterial activity against pathogenic bacteria. Various studies have demonstrated this antibacterial property against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Agrobacterium tumefaciens, Salmonella choleraesuis, and Helicobacter pylori* (Rinihapsari & Catur, 2008). By virtue of the fermentation process, the produced acetic acid destroys a number of Gram-positive and Gram-negative bacteria. Acetic acid acts as an antibacterial by penetrating the cell wall and denature cell's plasma proteins (Sreeramulu et al., 2000). During the fermentation process, kombucha culture, which is a symbiosis between bacteria and yeast, will metabolize the substrates resulting antibacterial activity, which increases with the longer kombucha fermentation time. According to Dufresne and Farnworth (2000), kombucha has the activity of inhibiting the growth of pathogenic microbes such as *Escherichia coli* (causes diarrhea), *Helicobacter pylori* (causes stomach ulcers), *Entamoeba cloacae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermis, Agrobacterium tumefaciens, Bacillus cereus, Aeromonas salmonella enter, Salmonella, Shigella sonnei, Leuconostoc monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, and Candida albicans*. According to Jayabalan et al. (2014), kombucha's antibacterial activity is supported by low pH because kombucha contains acetic acid and several types of organic acids and catechins that support bacterial inhibitory properties.

Frank (1991) reported that kombucha tea is antibacterial against *Helicobacter pylori*, a bacteria that can cause gastritis. This drink contains vitamins B1, B2, B6, B12, folic acid, and glucuronic acid. These acidic compounds form bonds with unused metabolic waste, with drugs and poisons, thus helping the detoxification process. In general, the kombucha fermentation process occurs symbiosis between *Acetobacter xylinum* and the yeast *Saccharomyces cerevisiae*. This symbiosis produces acids and alcohols that block the growth of foreign microbes that do not come from the kombucha tea fungus (Blanc, 1996; Cvetkovic & Markov, 2002).

Kombucha contains 7 g/L (0.7%) acetic acid. Acetic acid or ethanoic acid (CH 3COOH) is a colorless liquid that has a sharp aroma and a sour taste. Acetic acid ionizes weakly in dilute solutions and has long been used as a preservative because of its antimicrobial effect. Acetic acid in kombucha has in vitro antibacterial activity against *Salmonella choleraesius*, serotype typhimurium, and *Agrobacterium tumifaciens* (Naidu et al., 2000).

12.5.3 Antidiabetes activity

Various in vivo tests have proven the positive effects of kombucha on degenerative diseases, including diabetes therapy, improving liver, and pancreatic function. kombucha was used as a therapeutic agent for hyperglycemia and dyslipidemia in diabetic animal models (Aloulou et al., 2012; Srihari et al., 2013). Kombucha has the capacity to provide an inhibitory effect on alpha amylase and lipase activity in blood plasma and pancreas so that it can suppress the increase in blood glucose levels (Aloulou et al., 2012). The health benefits thought to be associated with kombucha consumption are numerous and are attributed to the phenolic components, which increase during fermentation. In addition, there are organic acids, vitamins, amino acids, antibiotics, and various micronutrients produced during fermentation of kombucha, which are thought to have a role in health benefits (Vijayaraghavan et al., 2000). Several studies have shown that kombucha can be used as a diabetes therapy agent through several mechanisms to reduce damage to pancreatic beta cells caused by oxidative stress so as to increase insulin production, reduce glucose uptake from the digestive system, and increase cellular glucose uptake (Aloululo et al., 2012; Bhattacharya et al., 2011; Srihari et al., 2013).

12.5.3.1 Fasting plasma glucose level reduction

Zubaidah et al. (2019) compared snake fruit and black tea kombucha, and metformin as diabetes therapy agents. The research investigated the antidiabetic activity of snake fruit kombucha, black tea kombucha, and metformin in streptozotocin-induced diabetic rats. Snake fruit kombucha, black tea kombucha, and metformin were orally administered to the diabetic rats daily for 28-day experiment. The blood plasma was analyzed, and pancreas immunohistochemical study and β-cells quantification were carried out.

Changes in the FPG levels before and after the treatment are presented in Table 12.2. The FPG levels among the DM rat groups (P1, P2, P3, and P4) on day 0 were not significantly different ($P > .05$), while there were significant differences

TABLE 12.2 Effect of the kombucha and metformin administration on indices of the fasting plasma glucose (FPG) levels in the rats (Zubaidah et al., 2019).

Treatment	Fasting plasma glucose (FPG) levels (mg/dL)	
	Day 0	Day 28
P0 (normal rat)	119.5 ± 8.9 a	104.2 ± 3.4 a
P1 (diabetes mellitus)	463.7 ± 36.6 b	413.2 ± 3.8 c
P2(DM + kombucha tea)	473.7 ± 58.7 b	154.0 ± 54.4 b
P3(DM + snake fruit kombucha)	453.0 ± 12.9 b	110.2 ± 2.8 a
P4 (DR + metformin)	417.2 ± 59.3 b	104.0 ± 3.5 a

Note: 1. Different notations show significant differences ($\alpha = 0.05$).

($P < .05$) within the normal rats (P0) group. At the end of the 28-day treatment, the normal and DM rats appeared to show constant FPG levels, whereas the FPG levels of the DM groups P2, P3, and P4 decreased. The DM group with the KS (P3) and metformin (P4) administration showed lower FPG levels than the DM group with the KT (P2).

Metformin is an antidiabetic drug, and it works by increasing the sensitivity of the liver and peripheral tissues to insulin without affecting insulin secretion and increasing glucose uptake in the peripheral tissues to reduce insulin resistance (Iida et al., 2003). The snake fruit kombucha decreased the level of FPG because of its high content of antioxidant compounds such as phenolic, tannin, and other bioactives, as well as some other organic acids, such as citric, lactic, butyric, and propionic acids (Östman et al., 2005; Zubaidah et al., 2018a). Antioxidant compounds decrease FPG levels through increasing cellular glucose uptake. Increased insulin secretion will have implications for the body's ability to utilize blood glucose for normal metabolism. Glycolysis, glycogenesis, and lipogenesis are some of the metabolisms of insulin-regulated glucose (Dufresne & Farnworth, 2000; Aloulou et al., 2012). Antioxidant compounds can also inhibit glucose absorption in the small intestine by decreasing glucose-conducting activity, such as sodium-glucose transport protein 1 (GLUT1), glucose transporter 5 (GLUT 5), and glucose transporter 2 (GLUT 2). The gross effect of these is to reduce the glucose that enters the bloodstream (Kwon et al., 2007). Consequently, it can be inferred that the snake fruit kombucha had a comparable effect as the metformin and a better effect on FPG than the black tea kombucha.

12.5.3.2 Pancreas immunohistochemistry study

The result of IHC staining is shown in Figs. 12.3 and 12.4 (Zubaidah et al., 2019). The results showed increasing Langerhans island structures and insulin secretions in the three treated (kombucha and metformin) groups (Fig. 12.3). The size and shape of the structures from the DM group were irregular and smaller than those of the normal group (P0) and the three groups of KT, KS, and metformin. In addition, the DM group showed a very low immunoreactive response (brown color) to anti-insulin, which indicated low levels of insulin production. The DM rat groups with the kombucha and metformin improved in the Langerhans island structures, and in the size, shape, distributions, and numbers of the β-cells, as well as the high intensity of brown color when compared with the DM rats (Fig. 12.3).

The DM group with metformin (P4) had a high number of pancreatic β-cells, which is not significantly ($P > .05$) different from the snake fruit kombucha. Metformin has adenosine monophosphate-activated protein kinase (AMPK) enzyme that plays a role in repairing HbA1c, the main parameter of blood glucose. In addition, it can reduce hepatic glucose production, lower LDL and triglyceride levels, increase HDL levels, decrease platelet aggregation, increase fibrinolytic activity and improve weight, reduce the risk of hypoglycemia, and increase insulin sensitivity with a concomitant improvement of pancreatic performance (Gunton et al., 2003). The DM group with the snake fruit kombucha treatment had a pancreatic β-cells higher than the black tea kombucha, possibly due to its higher antioxidant compounds as discussed above that can protect and repair pancreatic β-cells and increase insulin secretions (Zubaidah et al., 2018a, 2018b).

12.5.4 Potential of kombucha as an immunomodulator

The immune system is a vital mechanism used to protect the body against foreign objects, microbes (e.g., bacteria, fungi, and parasites), viruses, cancer cells, and toxins that can cause a decrease in immune system and cause disease (Mao et al., 2019).

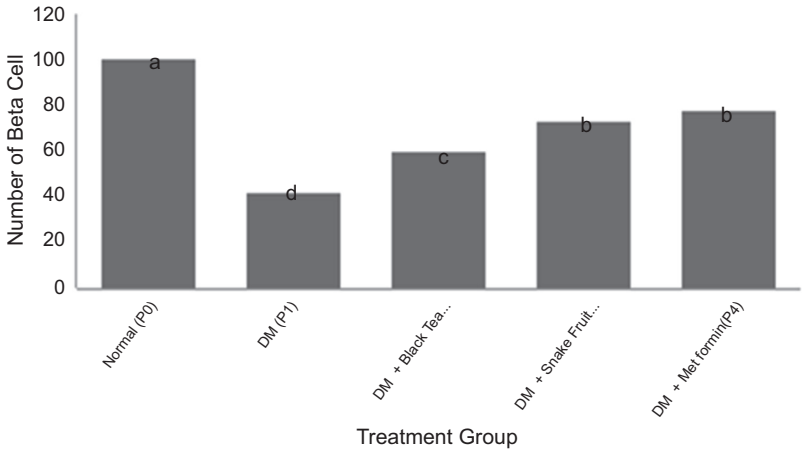

FIGURE 12.3 Effect of the kombucha and metformin administration on the number of pancreatic β-cells (Zubaidah et al., 2019) Values are mean ± SD of β = cells of five Langerhans islands. The same letter indicates no significant difference ($P > .05$). DM: diabetes mellitus.

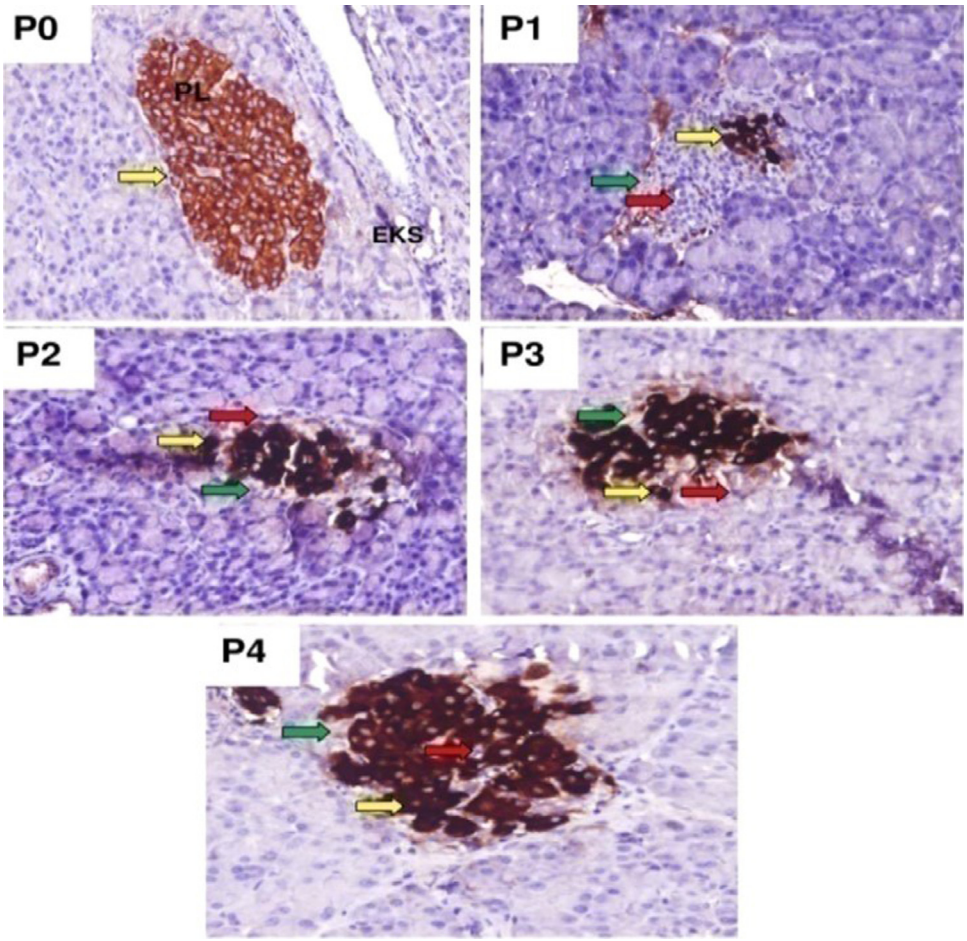

FIGURE 12.4 Effect of the kombucha and metformin administration on the pancreatic cells in rats evaluated by the IHC staining (400× magnification) (Zubaidah et al., 2019). PL: Langerhans Island, EKS: Exocrine glands (acini). Yellow arrow (light gray in print): Pancreatic β-cells, which have immunoreactivity to anti-insulin. Green arrow (gray in print): Endocrine cells, which are not immunoreactive to anti-insulin. Red arrow (dark gray in print): Empty space by necrosis, DM: Diabetes Mellitus, KT: black tea Kombucha, KS: snake fruit Kombucha, BW: Body Weight.

The immune system consists of two [2] types, namely.

1. the innate immune system (innate). It is the main defense against attack by foreign microorganisms (e.g., pathogens), has a fast response and does not show antigen in its working system. Innate immune cells include granulocytes, mast cells, macrophages, and dendritic cells (DCs)
2. the adaptive immune system has a slow response for days but develops to form immunological memory (Turvey & Broide, 2010; Bonilla & Oettgen, 2010; Murphy et al., 2007).

Immunomodulatory mechanisms are enhancing the immune system by means of stimulation (immunostimulants) and normalizing abnormal immune reactions (immunosuppressants). Immunomodulators are associated with increased nonspecific (innate) and specific (adaptive) immune responses, which are expected to restore a compromised immune system (Mao et al., 2019).

Several natural immunomodulatory compounds can enhance the immune system by affecting immune cell function or influencing the secretion of antibodies to control infection and maintain homeostatic conditions (Ortuño-Sahagún et al., 2017).

Research by Sofiakmi et al. (2015) showed that kombucha has immunomodulatory activity due to the presence of polyphenols, flavonoids, and organic acids. The most immunomodulatory activity is shown by acetic acid, glucuronic acid, lactic acid, and ascorbic acid. The increase in these compounds can induce the immune system as it escalates the response of T lymphocytes, improves the immune system, and stimulates lymphocyte cell proliferation (Arnas, 2009). In addition to some of the compounds above, probiotic bacteria in kombucha can also prevent infection because of the presence of T helper cells (Th1, Th2, Th17, and T regulator cells), affecting the production of various cytokines by DCs and macrophages, especially interleukin-12 (Kawashima et al., 2011).

Immunostimulants are substances that raise the body's immune system against foreign bodies, infectious diseases, tumors, and immunodeficiency. Immunostimulants can improve the immune system by using substances that can stimulate the immune system (Bascones-Martinez et al., 2014). Immunosuppressants are used to suppress the immune response, especially in the organ transplant process to prevent rejection reactions, and to treat autoimmune diseases such as pemphigus, lupus, or allergies (Lee et al., 2010).

Flavonoids such as quercetin and epigallocatechin 3-gallate (EGCG) in black tea increase the body's defense system through the increased mechanism of Interleukin-6 (IL-6) and IL-10, which affects the activation of T and B lymphocytes (Neiman et al., 2009). In addition, flavonoids can also modulate the immune system by inhibiting Th1, Th2, TNF-α, IL-2, IFN-γ, IL-12, and IL-6 cells. The inhibition of TNF-α and IL-12 can affect the activity of phagocytes and NK cells (Peluso et al., 2015).

Vitamin C in kombucha supports innate and adaptive immunity by supporting the function of the epithelial barrier against pathogens so that it can prevent pathogens. Vitamin C increases the differentiation and proliferation of B cells and T cells. Vitamin C deficiency results in impaired immunity and susceptibility to infection (Carr & Maggini, 2017).

Innate immunity is a specific immune system response due to the entry of pathogenic microorganisms and other foreign substances through phagocytosis by neutrophils and monocytes (macrophages), chemical barriers through internal and external secretions (lysozymes in mucus tissue, tears, lactoperxidase in saliva) and blood proteins (interferon, kinin system, complement), and natural killer (NK) cells (Fitzsimmons et al., 2014).

(Zubaidah et al., 2020a) conducted an investigation to compare the potential of kombucha tea Arabica coffee leaves and kombucha black tea as immunomodulators in mice infected with *Salmonella typhi*. Immune response analyses were carried out on the adaptive immune response of T cells with CD4 +, CD8 +, IFN-γ +, and TNF-α + cytokines are presented in Table 12.3.

Mice infected with *S. typhi* bacteria triggered an innate and adaptive immune response. Upon entering the body, the bacteria will phagocyte so that it stimulates macrophages so that it will increase interleukin 12 to activate NK cells. With the activation of NK cells, interferon-gamma is produced, which functions as a bacterial eliminator (Agnello et al., 2003; Mosser, 2003)

The mechanism of increasing CD4 +, CD8 +, CD4 + IFNɣ, CD4 + TNFα, CD8 + IFNɣ, and CD8 + TNFα. Cells took place due to the presence of phenolic compounds found in kombucha arabica coffee leaf tea and kombuchas black tea such as flavonoids and tannins. These bioactive compounds can increase the production of interleukin-2, which can stimulate a Th1 response by activating CD4 cell differentiation. The Th1 response increases the production of gamma interferon, which activates macrophages by releasing reactive O_2 compounds, which are toxic to microbes (Abbas & Lichman, 2011, pp. 113−121; Cheeke, 2000; Hoffmann et al., 2006; Lyu & Park, 2005; Qureshi et al., 2012).

Flavonoid compounds have been shown to increase IL-2 and lymphocyte proliferation (Nopitasari, 2006a, 2006b). The administration of kombucha black tea can increase the proliferation of lymphocyte cells of Falur BALB/c mice in vitro. In

TABLE 12.3 Immunomodulatory activity of kombucha black tea and kombucha tea arabica coffee leaves (Zubaidah et al., 2020a).

Group	$CD4^+IFN\ \gamma^+$ (%)	$CD4^+TNF\ \alpha^+$ (%)	$CD8^+IFN\ \gamma^+$ (%)	$CD8^+TNF\ \alpha^+$ (%)
Negative control - healthy mice (P0)	$0.26^{c} \pm 0.04$	$0.47^{c} \pm 0.01$	$0.43^{d} \pm 0.17$	$0.70^{d} \pm 0.17$
Healthy mice with kombucha arabica coffee leaf tea (P1)	$0.42^{c} \pm 0.10$	$0.65^{bc} \pm 0.05$	$0.67^{cd} \pm 0.27$	$1,19^{d} \pm 0.28$
Healthy mice with black tea kombucha (P2)	$0.53^{c} \pm 0.06$	$0.75^{bc} \pm 0.05$	$0.78^{c} \pm 0.20$	$1.34^{cd} \pm 0.51$
Positive control - infection mice (P3)	$0.62^{bc} \pm 0.10$	$0.79^{bc} \pm 0.17$	$1.39^{b} \pm 0.06$	$1.96^{bc} \pm 0.03$
Mice infection with kombucha arabica coffee leaf tea (P4)	$0.97^{b} \pm 0.34$	$1.75^{ab} \pm 0.22$	$1.89^{a} \pm 0.01$	$2.49^{ab} \pm 0.51$
Mice infection with kombucha black tea (P5)	$1.84^{a} \pm 0.22$	$2.13^{a} \pm 1.30$	$2.07^{a} \pm 0.02$	$3,15^{a} \pm 0.03$

Note: Different notations show significant differences ($\alpha = 0.05$) 2. Data obtained from the mean of four replications ± SD.

this study, the researchers outlined the effect of giving kombucha arabica coffee leaf tea and kombucha black tea on the increase in CD4 +, CD8 +, CD4 + IFNɣ, CD4 + TNFα, CD8 + IFNɣ, and CD8 + TNFα. cell. A significant increase occurred in the black kombucha group. The increase in TNF alpha is due to an ongoing infectious process (Sofiakmi et al., 2015).

The kombucha mechanism provides immunomodulatory effects such as mitogens that attach to the surface of lymphocyte cells. Bioactive compounds in this case such as flavonoids are thought to be ligands that will bind to the surface of the T and B cell receptors so that signal transduction activation occurs through second messengers including IP3 (Inositol triphosphate), which results in stimulation of Ca2 + to the cytoplasm so that the concentration increases. This increase will stimulate the activation of protein kinase C. This activation of protein kinase C will trigger cell-level gene expression such as the expression of interleukin 2 products, which causes the activation of B cells and T cells for proliferation (Erniati, 2018; Gomez-Florez et al., 2008; Schechter, 2010; Subaryono et al., 2017).

12.6 Summary and future direction

Currently there is a lot of information about the health benefits of kombucha based on people's experiences. However, research focusing on the in vivo effects of kombucha on human health is still unfolding. Along with the development of kombucha, which is currently not only made from tea leaves, but also based on various fruits.

In vivo research opportunities for the health effects of kombucha, both tea-based and fruit-based, are still possible. Nevertheless, research on possible side effects of consumption of kombucha has not been reported.

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