Jurnal
Teknologi

Full Paper

SUBSTRATES AND METABOLIC PATHWAYS IN SYMBIOTIC CULTURE OF BACTERIA AND YEAST (SCOBY) FERMENTATION: A MINI REVIEW

Nurliyana Sofiya Zailani, Azila Adnan*

Faculty of Science and Marine Environment, UMT, 21030, Kuala Nerus, Terengganu, Malaysia

Article history
Received
14 April 2022
Received in revised form
25 June 2022
Accepted
4 July 2022
Published Online
21 August 2022

*Corresponding author
azila.adnan@umt.edu.my

Graphical abstract

Abstract

Kombucha is a fermented beverage that is prepared traditionally by fermenting Symbiotic Culture of Bacteria and Yeast (SCOBY) with sugar and black/green tea, which is known as *Camellia sinensis* leaves. The previous study analyses the microbial composition that can be obtained in Kombucha production. Study shows that yeast species and acetic acid bacteria (AAB) species are the microorganisms that involve in the fermentation process of Kombucha. Some studies emphasize the chemical composition that was obtained from the production of Kombucha drinks such as organic acids, sugars, ethanol, and polyphenols. However, further review and elucidation regarding the substrates used and metabolic activity in Kombucha fermentation is necessary. Thus, the objective of this study is to review the metabolic pathway and substrates involve in Symbiotic Culture of Bacteria and Yeast (SCOBY) fermentation. This review also collected information related to the symbiosis of fermentation by yeast and AAB pathway in Kombucha fermentation. Several pharmaceutical effects of Kombucha were also discussed to prove the health benefits of Kombucha. To produce good quality and high yield of Kombucha that can provide various health benefits to consumers, it is crucial to understand the connection between the metabolic activity with Symbiotic Culture of Bacteria and Yeast (SCOBY) during the fermentation process of Kombucha. By conducting this review work, it could provide an insightful overview and better understanding of metabolic pathways and substrates involved in SCOBY and Kombucha fermentation.

Keywords: Kombucha, fermented beverages, SCOBY, acetic acid bacteria, metabolic pathway

Abstrak

Kombucha ialah minuman yang ditapai yang disediakan secara tradisional melalui proses penapaian oleh Kultur Simbiotik Bakteria dan Ragi (SCOBY), berserta gula dan teh hitam/hijau *Camellia sinensis*. Kajian lepas menganalisis komposisi mikrob yang boleh diperolehi di dalam penghasilan Kombucha. Kajian menunjukkan bahawa spesies yis dan spesies Bakteria Asid Asetik (AAB) adalah mikroorganisma yang terlibat dalam proses penapaian Kombucha. Beberapa kajian melaporkan komposisi kimia yang diperoleh daripada penghasilan minuman Kombucha adalah seperti asid organik, gula, etanol, dan polifenol. Walaubagaimanapun, penerangan mengenai substrat yang digunakan dan aktiviti metabolik dalam proses penapaian Kombucha memerlukan sorotan literatur lanjut. Oleh itu, objektif kertas ulasan ini adalah untuk memberi penerangan mengenai laluan metabolik dan substrat yang terlibat dalam proses penapaian SCOBY. Kertas ulasan ini juga mengumpul maklumat berkaitan simbiosis penapaian oleh yis dan tapak jalan AAB dalam penapaian Kombucha. Beberapa kesan farmaseutikal Kombucha juga dibincangkan untuk menjelaskan manfaat kesihatan Kombucha. Untuk menghasilkan Kombucha yang berkualiti serta yang boleh memberikan pelbagai manfaat kesihatan kepada pengguna, adalah amat penting untuk memahami perkaitan di antara aktiviti metabolik dengan Kultur Bakteria dan Ragi Simbiosis (SCOBY) semasa proses penapaian Kombucha. Dengan melaksanakan kertas ulasan ini, ia boleh memberikan gambaran keseluruhan yang lebih baik tentang laluan metabolik dan substrat yang terlibat dalam proses penapaian SCOBY dan Kombucha.

Kata kunci: Kombucha, minuman yang ditapai, SCOBY, bakteria asetik asid, laluan metabolic

© 2022 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

Nowadays, Kombucha receives a lot of attention because of its potential in improving gut health as a probiotic drink. Probiotics are microorganisms that provide health benefits to the host when given in sufficient concentrations [1]. As reported by Mazraedoost and Banaei [2], Kombucha has the ability to increase liver detoxification, enhance the immune system, and lower cholesterol levels, anti-diabetic, antioxidant, and anti-bacterial properties. Kombucha is a fermented beverage that is prepared traditionally by fermenting Symbiotic Culture of Bacteria and Yeast (SCOBY) with sugar and black/green tea, which is known as *Camellia sinensis* leaves [3]. It is known as a non-alcoholic beverage. Due to its high carbonation level, replacing soft drink or sparkling wines with Kombucha make it a healthier option. Kombucha is available in non-alcoholic and low-alcohol varieties. The low-alcoholic version of Kombucha drinks has the percentage of alcohol less than 0.5 percent by volume [4]. Even though Kombucha received its attention recently, however, in the field of food biotechnologies, Kombucha is not recently discovered.

Kombucha is said to have originated from China during the 220 BC of the Chin dynasty. The tea fungus, which is known as divine "Ling-tche" or "Che", is used to treat Japanese monarch Inkyo's intestinal problem by a Korean doctor named Kombu in AD 414, which is believed have been popularized later in Japan, which the name of divine "Che" was popularized as Kombucha [5]. Kombucha is also known with various other names such as red tea fungus, Chainii grib, Champignon de longue vie, Chainii kvass, kinoko, Lingzhi, and kocha [6]. Kombucha also was known as, Kombuchaschwamm in Germany, Mu-Go in Russian, Cendawan Mekah in Malay and Finkochinese in Italian. This beverage is also known as "the ultimate health drink" because of its large number of health benefits [7].

In general, fermentation is a slow degradation process of complex organic components into simpler compounds induced by microorganisms such as yeasts, molds, and bacteria or enzymes. These microorganisms play important role in converting carbohydrates to alcohols or organic acids as well as gaseous by-products [8]. Fermentation is the main process in obtaining Kombucha, which involves a symbiosis between the bacteria and yeast. Symbiotic Culture of Bacteria and Yeast (SCOBY) consists of acidophilic yeast and Acetic Acid Bacteria (AAB) that work strictly in aerobic conditions. These microorganisms are the ones that respond to work together in producing the floating biofilm known as microbial cellulose layer or tea mushroom [9] (Figure 1). SCOBY is a type of bacterial cellulose (BC), which similar to others single strain that produce BC. Several properties such as its basic structure, the pathway of exopolysaccharide synthesis and the bacterium biosynthesis pathway are identical. Although SCOBY is a symbiotic culture between acidophilic yeast and Acetic Acid Bacteria (AAB), the cellulose layer that is formed are solely produced by the bacterial species. The biofilm form by the bacteria is important in cell attachment and also it protect against unfavorable condition. For example, cellulose biofilm maintaining an aerobic environment for the bacteria, which is necessary for fermentation [10]. The liquid phase, which is the Kombucha by-product consists of organic acids such as acetic acid, gluconic acid, glucuronic acid, and ethanol [11]. According to Laavanya *et al*. [10], the liquid phase also consists of various chemical compositions such as amino acids, water-soluble vitamins, amines, purines, polyphenol, hydrolytic enzymes, minerals such as iron, nickel, cobalt, cadmium, manganese, zinc, and copper and anions such as fluoride, sulfate, nitrate, iodine, bromide, phosphate, and chloride.

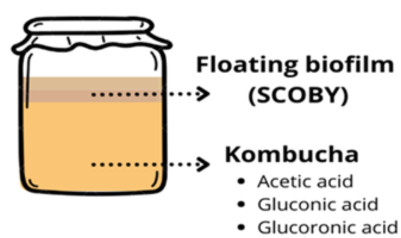

Figure 1 Main component in Kombucha

According to Mousavi *et al*. [12], different tastes and flavors are produced due to differences in the fermentation period. The fermentation duration can vary by up to two months. Kombucha, for example, has a slight vinegar flavor after 60 days of fermentation. The concentration of ingredients in such beverages can be influenced by a variety of circumstances. The concentration of components in this complex beverage is influenced by the initial content of sucrose and substrates, which is tea leaves, the time required for fermentation, and the nature of the Kombucha culture [13]. The final product also can be influenced by factors such as pH and incubation temperature. Temperature changes might affect the antioxidant action of substances derived from plants, such as the formation of phenol-containing compounds.

Substrates used in Kombucha production also affect the yield of Kombucha and cellulose production. Nutrients, which are tea leaves as nitrogen sources, while sucrose as carbon sources are needed by SCOBY in the fermentation process., while sucrose as carbon sources are needed by SCOBY in the fermentation process. *Camellia sinensis* leaves, which are known as black tea or green tea are the most common substrates used in fermenting Kombucha. The caffeine in its compositions makes it suitable as a substrate for Kombucha production [4]. Nonetheless, few studies proved that Kombucha can also be produced using substrates that do not have caffeine in their compositions. For example, fermented coconut water and grape juices have potential as substrates in Kombucha fermentation [14]. Molasses can also be used as carbon sources as it is a cheaper alternative to sucrose [10].

Al-Mohammadi *et al*. [15] stated that weight loss, therapy of metabolic illnesses, arthritis, indigestion, cancer, and acquired immunodeficiency syndrome (AIDS) were among the potential preventative effects of Kombucha. Kombucha beverages have been claimed to have anti-carcinogenic, anti-diabetic, anti-microbial, and anti-oxidative properties, which are linked to acids obtained in tea and metabolites produced by bacteria and yeast fermentation, such as acetic acid and glucuronic acid, as well as the presence of gut microbiota (probiotics) in Kombucha cultures. Fermentation of this beverage has been used to turn items into value-added products for the development of new functional foods via microbial fermentation [16].

The previous study analyzes the microbial composition that can be obtained in Kombucha production. Study shows that yeast species and Acetic Acid Bacteria (AAB) species are the microorganisms that involve in the fermentation process of Kombucha. There are also studies that emphasize the chemical composition that was obtained from the production of Kombucha drinks. However, less elucidation regarding the connections between the metabolic activity with Symbiotic Culture of Bacteria and Yeast (SCOBY) in Kombucha fermentation. Additionally, there is also less focus on the substrates that have the ability in replacing the conventional Kombucha production substrates.

To produce good quality and high yield of Kombucha that can provide various health benefits to consumers, it is crucial to understand the connection between the metabolic activity with Symbiotic Culture of Bacteria and Yeast (SCOBY) during the fermentation process of Kombucha. By conducting this review work, it could provide an insightful overview and better understanding of metabolic pathways and substrates involved in Kombucha fermentation. Thus, the objective of this study is to review the pathway and substrates used involve in Symbiotic Culture of Bacteria and Yeast (SCOBY) fermentation.

2.0 PRODUCTION OF KOMBUCHA

Kombucha was made using black or green tea as substrates and sugar, mainly sucrose, as a carbon source. According to Jafari et [17], after autoclaving the containers at 121°C, the carbon source (typically sucrose) and substrates (commonly black tea or green tea leaves) are combined in boiling water and allowed to infuse. The excess tea leaves were then filtered off with sterile filter paper, and the liquid was left to cool at room temperature, away from direct sunlight. To begin the fermentation process, the cooled tea is added to a sterile glass jar and inoculated with the freshly grown starter culture (bacterial cellulose). This lowers the pH of the fluid, making it less favorable to the growth of undesirable microorganisms. The glass container must have a wide bottleneck to allow access and sufficient surface area for the oxygen exchange from the environment [18]. Finally, the glass jar is covered with a linen towel and left to incubate at room temperature. Fermentation takes place at a temperature of 20°C to 22°C, which takes about 7–8 days at room temperature resulting in newly produced layer cellulose culture formed on the surface of the glass jar and creating a transparent thin pellicle, which reaches thickness from 8 to 12 mm in around 14 days [19] (Figure 2).

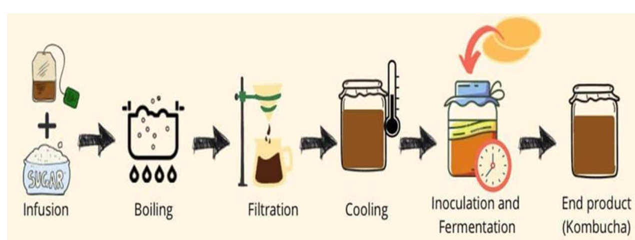

Figure 2 Production of Kombucha

3.0 METABOLIC PATHWAYS IN SCOBY FERMENTATION

Fermentation by Yeast

There are certain microbial compositions of yeast species that can be found in the Kombucha fermentation process. Yeast species that can be used to produce Kombucha are *Candida boidinii, Zygosaccharomyces rouxii, Dekkera bruxellensis, and Saccharomyces cerevisiae* [20] (Table 1). The most common species of yeast used in Kombucha fermentation are *Zygosaccharomyces* as the predominant yeast with 84.1% of the relative percentage of abundance and Dekkera and Pichia species with 6% and 5%, respectively [14]. Yeast undergoes its fermentation in anaerobic conditions as SCOBY keeps the acetic acid bacteria on top of the surface, which places the yeasts at the bottom part of the film causing insufficient oxygen [4].

Table 1 Yeast species in Kombucha

Genus	Species	References
Candida	*Candida boidinii, Candida famata, Candida guilliermondii, Candida obtusa*	[4]
Dekkera	*Dekkera bruxellensis*	[31]
Saccharomyces	*Saccharomyces cerevisiae, Saccharomyces bisporus*	[5], [4], [31]
Zygosaccharomyces	*Zygosaccharomyces rouxii, Zygosaccharomyces bailii*	[5], [4]

Yeast initiates the fermentation process of Kombucha by hydrolyzing sucrose using invertase into fructose and glucose through the glycolysis pathway under anaerobic conditions [21]. According to Melkonian and Schury [22], Hexokinase will be phosphorylated glucose into glucose 6-phosphate. This step requires a phosphate group and one molecule of ATP. Phosphohexose isomerase will isomerize glucose-6-phosphate into Fructose 6-phosphate, which then will be phosphorylated into Fructose-1,6-biphosphate by phosphofructokinase, which uses one ATP molecule. Aldolase will split Fructose-1,6-biphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Triosephosphate isomerase will isomerize dihydroxyacetone phosphate forming second glyceraldehyde-3-phosphate.

Then, glyceraldehyde-3-phosphate will then be phosphorylated to form 1,3-biphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase, which NAD+ as a cofactor will be reduced to NADH. Phosphoglycerate kinase will convert 1,3-biphosphoglycerate into 3-phosphoglycerate, which involves the transfer of a phosphate molecule to ADP forming 1 ATP. 3-phosphoglycerate rearranges to form 2-phosphoglycerate by phosphoglycerate mutase. 2-phosphoglycerate will dehydrate into phosphoenolpyruvate by enolase, which is then converted into pyruvate by pyruvate kinase. The conversion of Phosphoenolpyruvate into pyruvate involves the transfer of a phosphate to ADP which forms 1 ATP [23]. The final step in yeast is the decarboxylation of pyruvate into acetaldehyde by enzyme pyruvate decarboxylase, which later leads to the reduction of acetaldehyde into ethanol and carbon dioxide [24]. The fermentation in yeast achieved the redox balance in which the NAD^+ is regenerated as alcohol dehydrogenase reduced acetaldehyde into ethanol [25] (Figure 3).

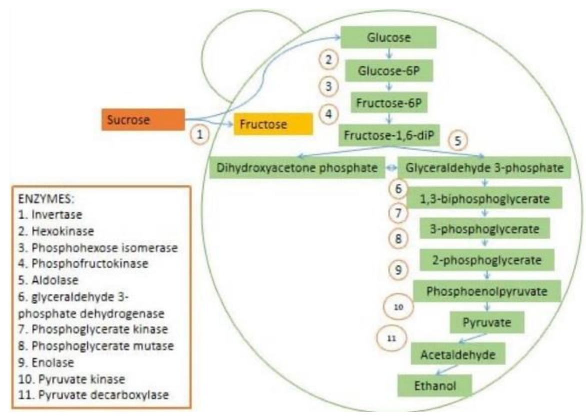

Figure 3 Fermentation Metabolic Pathway of Yeast

Acetic Acid (AAB) Pathway

Acetic Acid Bacteria (AAB) are aerobic Gram-negative bacteria with rod-shaped cells that belong to the Acetobacteraceae family, which is capable of oxidizing ethanol to acetic acid strictly in aerobic conditions [26]. There are currently eighteen genera in the acetous group, with *Gluconacetobacter, Acetobacter, Gluconobacter*, and *Komagataeibacter* as the most important genera in terms of fermented foods [27]. Some of the identified AAB species are *Bacterium gluconicum*, *Gluconobacter oxydans, Gluconacetobacter hansenii, Komagataeibacter xylinus*, and *Acetobacter aceti* [28] (Table 2). Acetic acid bacteria that function strictly in aerobic conditions are able to oxidize glucose and fructose into acids such as gluconic acids, glucuronic acids, and acetic acids. The acetic acid bacteria also have the ability to oxidize the ethanol produce by yeast and form the floating cellulose biofilm [20].

Table 2 Acetic Acid Bacteria (AAB) in SCOBY

Genus	Species	Sources
Komagataeibacter	*Komagataeibacter xylinus* *Komagataeibacter kombuchae*	[14], [31]
Acetobacter	*Acetobacter aceti* *Acetobacter xylinum (reclassified as Gluconacetobacter xylinus)* *Acetobacter xylinoides*	[18], [15], [5]
Bacterium	*Bacterium gluconicum*	[18]
Gluconobacter	*Gluconobacter oxydans*	[4], [20]
Gluconacetobacter	*Gluconacetobacter Hansenii* *Gluconacetobacter kombuchae* *Gluconacetobacter europaeus* *Gluconacetobacter xylinus*	[18], [4], [20], [29]

Acetic acid will boost yeast to produce more ethanol, which later will be oxidized by the bacteria to produce more acetic acid using enzyme alcohol dehydrogenase [4]. The production of more acetic acid and ethanol prevents contamination of pathogenic microbes as it acts as an antimicrobial agent [30]. Acetic acid bacteria use the glucose molecules that have been breakdown from sucrose by invertase in yeast as substrate to undergo oxidation by glucose oxidase producing gluconic acid and then converted to glucuronic acid [31]. First, the glucokinase transforms glucose into glucose-6-phosphate. Then, glucose-6-phosphate will transform into glucose-1-phosphate by phosphoglucomutase which later will be catalyzed by uridine diphosphate (UDP)-glucose pyrophosphorylase to form uridine diphosphoglucose (UDPG). UDPG is the precursor of cellulose molecule, which lastly transform into units of UDP glucose by cellulose synthase (Figure 4) [10].

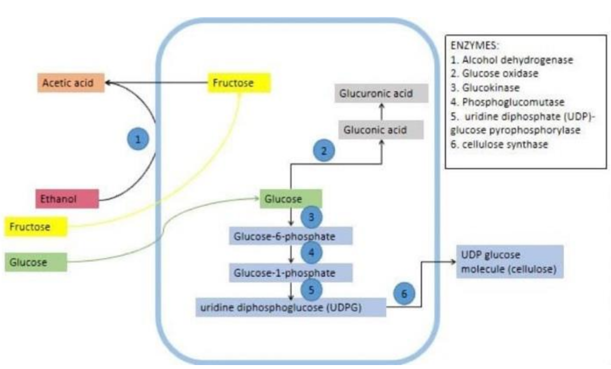

Figure 4 Pathway involves in Kombucha and SCOBY Fermentation

Substrates Used in Kombucha Fermentation

Camella sinensis Leaves

It is well known that Kombucha was traditionally prepared using black tea, green tea, or oolong tea from *C. sinensis* leaves. According to Prasanth *et al*. [32], the variation of *C. sinensis* tea depends on the degree of fermentation and the level of antioxidants present in it. Coelho *et al*. [4] stated that crushing leaves and exposure to extreme humidity yield black tea. The enzyme polyphenol oxidase oxidizes the polyphenols because of these steps. Aromatic compounds were released as a result of enzymatic oxidation, transforming phenolic compounds into more substantial, dark-colored complexes. Steam is used to produce green tea from fresh leaves. The heat from the steam inactivates the enzymes that prevent the leaves from oxidizing. These processes lead to some differences in the compositions of teas.

According to Naveed *et al*. [33], black tea consists of components such as flavonoids (thearubigins (TRs), theaflavins (TFs) and catechins), methylxanthines such as caffeine, amino acids such as theanine and phenolic acids (caffein acid (CA), gallic acid (GA), cauramic acid and chlogenic acids (CGA)). Black tea also consists of carbohydrates, proteins, lipids, volatile compounds, β-carotene, and vitamins (C, K, and A). According to Dubey *et al*. [4], green tea consists of carbohydrates (cellulosic fiber and pectin), xanthic (caffeine and theophylline), polyphenols, which are flavonoids that consists of epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epicatechin (EC), amino acid (y-N-ethyl glutamine), fatty acid (a-linolenic acids), organic acids (malic acid and oxalic acid), sterols (stigmasterol) and vitamins (B, C, and E). Minerals and trace elements (Zn, F, Se, Ca, Cr, Mn, Fe, and Mg) are available in different concentrations depending on the fermentation process, size, and age of the leaves. Pigments (chlorophyll and carotenoids) and volatile compounds as alcohols, lactones, hydrocarbons, aldehydes, esters, and others present as minor constituents in tea infusions which play role in the development of aroma. The variations in phenolic component concentration and type may affect the bioactive qualities of Kombucha made from green or black tea [35].

Green tea contains the most abundant polyphenols, which are flavonoids [36]. Green tea has a higher polyphenol concentration than black and oolong teas and hence has more health-promoting effects due to the presence of EGCG, one of the strongest antioxidants in Kombucha [31]. Prasanth *et al*. [32] stated that caffeine content in tea leaves varies between 2 and 5%, depending on the age of the leaf, with younger leaves having a greater caffeine content. Caffeine in tea compounds is an important source of nitrogen that can promote the development of the microorganisms involved in Kombucha production.

Green tea has a higher caffeine content than black tea, which allows it to produce more Kombucha [4].

Tea leaves are known for their potential as anti-allergic, anticancer, anti-obesity, antimutagenic, anti-apoptotic, neuroprotective, hyperglycemic and antihyperglycemic, antioxidant, antimicrobial, and anti-inflammatory effects. These are due to the presence of bioactive compounds such as caffeine and theobromine [37]. Tea is classified as a functional food because, in addition to its nutritional value, it can provide a variety of physiological benefits. Tea leaves, which are high in antioxidants, serve in the proper functioning of the cardiovascular system, the decrease of body mass, and even the prevention of cancer and neurological illnesses. Its antioxidant properties make it a key regulator in the fight against free radicals, which is useful in medicine [32].

Coffee

Many studies produce Kombucha from other alternative substrates instead of using black tea or green tea. Coffee is one of the substrates that can be used to produce Kombucha. According to Bueno *et al*. [38], phenolic compounds (chlorogenic acids, cafestol, and kahweol), alkaloids (caffeine and trigonelin), diterpenes (cafestol and kahweol), and other secondary metabolites are some of the key bioactive compounds in coffee. Replacing tea with coffee in the fermentation of Kombucha exchanges compounds such as polyphenols and flavors. Even so, carbohydrate source and sucrose are the same and it is also fermented with the same SCOBY species that are used in common Kombucha fermentation such as *A. xylinum* and the genera of yeast such as *Brettanomyces* and *Saccharomyces*.

The caffeine in the coffee extract also activated the cellulogenic complexes, which stimulate the cellulose production. Vitamins and additional nutrients stimulated bacterial cells as a result of yeast cells' death and autolysis [10]. Coffee contains a lot of phenolic chemicals, which have high antioxidant activity and are good for health. The study also shows that Kombucha prepared from coffee leaves had a higher total phenolic content than Kombucha made from tea leaves [39]. According to Kusdiana *et al*. [40], coffee based Kombucha has catechins, acid content, caffeine, and alcohol, which can be drunk as a healthy beverage similar to Kombucha tea.

Molasses

Traditionally, molasses from beets and canes are widely utilized in animal feeding and are produced as a by-product of sugar extraction all over the world. They are fed to ruminants as an energy source due to their composition [41]. However, molasses is also widely used as a source of carbon in fermentation processes such as in ethanol production as it is cheaper compared to other carbon sources [42]. This brings to study, which develop Kombucha from molasses substrates. According to Varee *et al*. [43], molasses is a brown and thick syrup that is obtained from the final by-product of sugar refinement from sugar beet (*Beta vulgaris* var. *saccharifera*) or sugar cane (*Saccharum L*.).

There are a few types of molasses (light molasses, dark molasses, blackstrap molasses, fancy molasses, unsulphured molasses, sulphured molasses), which are categorized based on consistency, sugar content, color, and flavour [44]. Molasses is mostly made up of fermentable carbohydrates (glucose, fructose, sucrose) and water, with a lot of sucrose and organic as well as inorganic non-sugars compositions. Amino acids, betaine, phenolic compounds, lactic acid, vitamins, trace elements like Ca^{2+}, and Na^{+}, and color-forming chemicals including caramel compounds, melanoidines, and melanins, are all found in molasses [43, 45]. Molasses can be used as a source of carbon for yeast strains' growth and metabolism, making it a good candidate for ethanol production. Molasses, which contains a lot of sugars, is a cheap feedstock for making value-added bioproducts through bioconversion when compared to glucose and molasses [46].

Honey

According to Majtan *et al*. [47], honey is high in antioxidants, which have been shown to help against diseases such as cancer, aging, cardiovascular disease, inflammatory disorders, and neurological deterioration.

Monosaccharides, glucose, and fructose are found in honey, followed by disaccharides, sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, kojibiose, and trisaccharides maltotriose, and melezitose, according to Gaglio *et al*. [48]. Carbon sources for SCOBY can be found in these carbohydrate compositions. Mărgăoan *et al*. [49] fermented SCOBY with pollen gathered from honey bees (BCP). The addition of honey bee collected pollen (BCP) enhanced the proportion of LAB in the total number of SCOBY as well as the concentration of bioactive substances including polyphenols and short-chain fatty acids, which is larger in fermented pollen.

Fruit-based Kombucha

The growing demand and health-conscious population for Kombucha beverages have prompted the development of additional varieties made from diverse plants and fruits. Fruit-based beverages are among the most ancient and traditional fermented foods. Fruits have a high sugar content, making them an ideal raw material for alcoholic fermentation as well as other fermentations such as acetic or lactic acid fermentation [50]. There are few studies that develop Kombucha from fruits such as red grape, snake fruit, fermented coconut water, and soursop.

Vitis vinifera L. known as red grape or grapevine contains sugars that are the principal metabolites transported into berry from leaves. They offer carbon skeletons for the synthesis of organic acids and nitrogenous compounds. Nonflavonoid and flavonoid phenolics are the two types of phenolics found in grapes. Benzoic, cinnamic acids, plus stilbenes are classified as non-flavonoid while flavonoids are flavonols, anthocyanins, and proanthocyanidins. Anthocyanins, for example, are pigments found in the skins of grape berries [51]. According to Ayed *et al*. [52], the nutritional characteristics of grape juice Kombucha beverage can be improved as early as six days of fermentation which can reduce the synthesis of undesirable components such as organic acids, which give the beverage a vinegary taste. In this process, beneficial chemicals were produced, giving the beverage a strong antioxidant profile and having antibacterial action against the Gram-positive and Gram-negative pathogenic microorganisms.

There is also a study that developed Kombucha from snake fruits. In Malaysia and Indonesia, the snake fruit (*Salacca zalacca*) is known as "salak," and in Thailand, it is known as "sala." The pulp of the snake fruit contains a high level of phenolic compounds. Furthermore, the most common phenolic acids found in snake fruit are caffeic acid, p-coumaric acid, and ferulic acid [53]. According to Zubaidah *et al*. [54], snake fruit is the potential to be used as substrates. The formation of the thin layer of cellulose produced during the fermentation process shows that snake fruits can be used as Kombucha substrates. This might be due to the phenolic and sugar contents in the fruits. Snake fruit juices were fermented for 14 days with the Kombucha consortium containing yeasts and acetic acid bacteria. Acetic acid or vinegar, the major organic acid of the fermented product showed an enhanced antibacterial activity by inhibiting Gram-positive, which is *Staphylococcus aureus* and Gram-negative, which is *Escherichia coli* bacteria. Thus, it is shown that snake fruits can be an option for substrates to develop Kombucha beverages.

The tropical plant *Annona muricata L*. (Annonaceae), popularly known as soursop, can be found in areas of the Americas, Asia, Australia, and Africa. Antimicrobial, anti-inflammatory, anti-protozoan, antioxidant, and anticancer properties have been demonstrated in plant extracts [55]. The fruit of the soursop has been found to be high in bioactive substances such as alkaloids, acetogenins, and phenolic compounds. Soursop Kombucha also contained gluconic acid, acetic acid, and malic acid in its compositions. Acetic acid levels in soursop Kombucha samples rose from days 7 to 21 when kept at room temperature. Malic acid levels, on the other hand, remained consistent throughout the storage duration and under various settings in all soursop Kombucha. Sucrose levels dropped considerably, whereas glucose levels rose significantly. The findings demonstrated that soursop Kombucha has the ability in improving the metabolic contents and its quality when storing for 21 days [28].

Pharmaceuticals Effects of Kombucha

Antimicrobial Activity of Kombucha Tea

Many antimicrobial metabolites have been discovered in Kombucha, including organic acids, such as acetic acid and catechins, which are proved to show action against Gram-positive and Gram-negative bacteria [15] (Table 3). It is due to the compositions of acetic acid in Kombucha, which is associated with the presence of phenolic chemicals and organic acids, which cause the cytoplasmic pH to drop. By inhibiting glycolysis and preventing active transport, bacterial cytoplasm acidification can inhibit growth [52]. According to Mizuta *et al*. [56], Kombucha has antimicrobial activity against bacteria such as *Salmonella typhimurium, Staphylococcus epidermidis, Helicobacter pylori*, *S. aureus, Bacillus cereus, S. enteritidis, Escherichia coli,* and *C. glabrata*. It was discovered that both black and green tea Kombucha demonstrated antimicrobial activity against the pathogens examined, with green tea Kombucha having the most antibacterial potential. Kombucha has antifungal properties against a variety of pathogenic *Candida* species. Kombucha tea also includes strong water-insoluble bacteriocins that have antimicrobial activity against a wide range of infectious illnesses, including influenza, typhoid, paratyphoid fever, and dysentery [19].

Table 3 Pathogenic microorganisms causing food-borne diseases (Soares *et al*., 2021)

Gram-positive bacteria	Gram-negative bacteria	Yeast
Staphylococcus aureus	*Klebsiella pneumoniae*	*Candida glabrata*
Staphylococcus epidermidis	*Pseudomonas aeruginosa*	*Candida tropicalis*
Enterococcus faecalis	*Escherichia coli*	*Candida sake*
Bacillus cereus	*Helicobacter pylori*	*Candida dubliniensis*
Listeria monocytogenes	*Shigella sonnei*	*Candida albicans*

Kombucha receives the attention of its antimicrobial activity against food-borne pathogens. A "food-borne disease" is a term described as a sickness caused by polluted and uncleaned food. Food-borne illness is frequent and has become a public health issue in recent years which has an impact on millions of people all around the world

[57]. Nowadays, the development of natural antimicrobial approaches receives its attention in reducing food-borne diseases and are preferable compared to synthetic antimicrobial because the natural antimicrobials can be obtained in natural sources such as fruits, vegetables, herbs, and spices. Study shows that Kombucha can fight against foodborne pathogens because of the low pH value of acetic acids contains in Kombucha.

The acetic acid in Kombucha could penetrate Gram-positive bacteria cells easily compared to Gram-negative bacteria due to its lipophilic features. For example, acetic acid inhibits microbial growth against *E. coli and S. aureus*, with the highest percentage of inhibition of microbial growth being 99.83% and 100%, respectively [58]. The gut microbiota creates a hostile habitat for pathogens, produces antimicrobial compounds, and boosts the human body's defences. It also promotes peristalsis, which causes the contents of the gut to flow more swiftly, making it more difficult for infections to establish themselves [59].

Antioxidant Properties of Kombucha Tea

The antioxidant activity of Kombucha beverages is also linked to its health advantages. It is said that Kombucha is related to antioxidant activity since most of Kombucha is made from green tea or black tea as their substrates. Tea leaves are known for the presence of tea polyphenol, D-saccharic acid-1,4-lactone (DSL), and vitamin C, which contributes to the antioxidant activity [27]. According to Jayabalan and Waisundara [6], tea is recognized to have antioxidant effects on its own as it contains several chemicals with radical-scavenging characteristics, which are produced from the tea leaves during the Kombucha fermentation. Fermentation duration can also affect the amounts of total polyphenols and flavonoids in Kombucha tea. According to Coelho *et al*. [4], kombucha has also been shown in rats and albino mice to have hepatoprotective properties against numerous poisonous and carcinogenic substances in the liver. The antioxidant activity of kombucha is likely to be responsible for the liver's detoxifying capability and hepatoprotection. DSL (D-saccharic acid-1, 4-lactone), generated in the drink by *Gluconacetobacter* sp., is primarily responsible for these protective benefits.

According to Kaewkod *et al*. [60], DSL, likewise showed great anti-oxidative features, with the capacity to reduce oxidative damage to cellular biomolecules. Green tea Kombucha revealed the highest level of phenolic content as green tea has an abundance of polyphenol compared to black tea. The potential of the bacteria and yeast to free enzymes like phytase, which could break down the cellulose backbone of the tea leaves to release polyphenol chemicals, resulted in a rise in overall phenolic content during Kombucha fermentation. This is due to yeast in SCOBY, which is known to demonstrate antioxidant activity due to the high quantity of -glucans and wall proteins in their cell walls [52].

Sinir *et al*. [19] examine the impacts of various cultures on the antioxidant capacity of Kombucha made from black and green tea with AAB and *Zygosaccharomyces* sp. culture. Result shows that the antioxidant capacity of Kombucha tea tested against tertiary butyl hydroperoxide induced cytotoxicity in murine hepatocytes. It is also found to regulate oxidative stress-induced apoptosis in hepatocytes, which could be useful in the treatment of oxidative stress-related liver diseases. It is due to changes of tea polyphenols by enzymes created during fermentation and the formation of several low molecular weight metabolites which exhibits stronger antioxidant activity than unfermented tea.

Anticancer Activity of Kombucha Tea

It is crucial to obtain anticancer agents from sources that are focusing on killing cancer cells, which do not poisonous to the normal cells [61]. Some compositions in fermented food can lower the risk of cancer [62]. Kombucha compositions have also been investigated in vitro as antitumoral and antiproliferative agents, with encouraging results (Morales, 2020).

According to Kauwkod *et al*. [60], the presence of additional organic acids such as glucuronic, gluconic, DSL, ascorbic, acetic, and succinic acid was also observed to induce toxicity activities against cancer cells. The acetic acid found in Kombucha was toxic to Caco-2 cancer cells. DSL inhibited the activity of glucuronidase enzymes, such as those that degraded glucuronides and generated harmful aglycones. According to Mousavi *et al*. [12], the anticancer properties of tea polyphenols found in Kombucha have been determined to prevent gene alteration, induce cancer cell apoptosis, suppress cancer cell proliferation, and the ability to complete metastasis as possible functions. Indeed, for cancer patients whose blood pH is 7.56 or higher during illness, Kombucha tea can be an effective strategy to re-equilibrate blood pH.

The traditional black tea kombucha beverage was found to prevent prostate cancer growth and minimize the risk of metastasis [63]. According to Lynch *et al*. [27], a study on the cytotoxicity and anti-invasive properties of Kombucha fractions extracted by different solvents against cancer cell lines such colon adenocarcinoma (HT-29), cervical epithelial carcinoma (HeLa), human osteosarcoma (U2OS), human renal carcinoma (786-O) cells, as well as human lung cancer (A549) cells, resulting in a reduction in cell invasion and motility. It is demonstrating that dimethyl malonate 2-(2-hydroxy-2-methoxypropylidene) and vitexin are the possible compounds responsible for the anticancer properties of kombucha. The presence of polyphenols in Kombucha was also found to contribute to anticancer bioactivity [4].

Recent SCOBY Induced Food Products

Since SCOBY is a cellulosic gelatinous pellicle or layer formed at the air–liquid interface, it is well known in food industry such as nata de coco making, which involved the fermented from coconut water [64]. Since cellulosic is considered "generally recognized as safe" (GRAS) by the Food and Drug Administration (FDA), it possess great potential in food products due to its various textures and easy production process, several recent food products are good to be kept in view, as cellulosic SCOBY can be applied food applications (Table 4).

Table 4 Cellulosic SCOBY Food Applications

Food Products	Role	References
Meat sausage	Meat-replacer	[65]
Tofu	Food additive	[66]
Kamaboko (Surimi-based food)	Food additive	[66]
Ice cream	Thickener	[67]
Low-fat sausage	Dietary food	[68]

The cellulosic SCOBY, which associated with bacterial cellulose fibrils secrete a unique hydrogel-like substance with attributes that are being used in the numerous food industries. In spite of the interest shown in food applications, SCOBY can be a great potential as a sustainable substitute in fashion and automobile device industries. Additionally, cellulosic sheets could be synthesized with straight edges, without scars, marks, and other defects; which could lead to reducing waste.

Conclusion and Future Prospects

Based on the outlined review, SCOBY can be considered a potentially expanding biopolymer for applications in food and biomaterial industries, which makes attention on this product even more crucial. Reviewing and investigating the substrates utilized, microbial ecosystem involved, the metabolic pathways, substrates utilized, and the effects on biological reactions enable researchers to gain further information about Kombucha and SCOBY functionality. SCOBY biofilm has the potential to fulfil demands in biomaterial applications. There are interesting applications for biopolymer using cellulosic polymer, however, many of these applications have not been largely explored. SCOBY could be one of the sustainable materials that opens advances in biomaterial and food biotechnology industries to produce value-added products.

Acknowledgement

The authors acknowledged Universiti Malaysia Terengganu for carrying out this review at the Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Malaysia.

References

[1] Bergström, H. 2018. The Effect of the Fermented Tea Beverage Kombucha on the Gut Microflora. Master's Thesis. Lund University, Lund, Sweden. 3-31.

[2] Mazraedoost, S. and Banaei, N. 2020. Biochemical Composition Properties of Kombucha SCOBY: Mini Reviews. *Advances in Applied NanoBio-Technologies*. 1(4): 99-104.

[3] Kapp, J. M. and Sumner, W. 2019. Kombucha: A Systematic Review of the Empirical Evidence of Human Health Benefit. *Annals of Epidemiology*. 30: 66-70. DOI: https://doi.org/10.1016/j.annepidem.2018.11.001.

[4] Coelho, R. M. D., Almeida, A. L. de, Amaral, R. Q. G. do, Mota, R. N. da and Sousa, P. H. M. de. 2020. Kombucha: Review. *International Journal of Gastronomy and Food Science*. 22: 100272. DOI: https://doi.org/10.1016/j.ijgfs.2020.100272.

[5] Dutta, H. and Paul, S. K. 2019. 8 - Kombucha Drink: Production, Quality, and Safety Aspects. In A. M. Grumezescu & A. M. Holban (Eds.). Production and Management of Beverages, Woodhead Publishing. 259-288.

[6] Jayabalan, R. and Waisundara, V. Y. 2019. 12—Kombucha as a Functional Beverage. In A. M. Grumezescu & A. M. Holban (Eds.). Functional and Medicinal Beverages. Academic Press. 413-446.

[7] Zhen-jun Zhao, Y. S., Wu, H., Zhou, C. and Xian-chun Hu, J. Z. 2018. Flavour Chemical Dynamics during Fermentation of Kombucha Tea. *Emirates Journal of Food and Agriculture*. 732-741. DOI:https://doi.org/https://doi.org/10.9755/ejfa.2018.v30.i9.1794.

[8] Malakar, S., Paul, S. K. and Jolvis Pou, K. R. 2020. 1 - Biotechnological Interventions in Beverage Production. In: Grumezescu, A. M., Holban, A. M. (Eds.). Biotechnological Progress and Beverage Consumption. Academic Press. 1-37.

[9] Amarasekara, A. S., Wang, D. and Grady, T. L. 2020. A Comparison of Kombucha SCOBY Bacterial Cellulose Purification Methods. *SN Applied Science*. 2: 240. DOI: https://doi.org/10.1007/s42452-020-1982-2.

[10] Laavanya, D., Shirkole, S. and Balasubramanian, P. 2021. Current Challenges, Applications and Future Perspectives of SCOBY Cellulose of Kombucha Fermentation. *Journal of Cleaner Production*. 295: 126454. DOI: https://doi.org/10.1016/j.jclepro.2021.126454.

[11] May, A., Narayanan, S., Alcock, J., Varsani, A., Maley, C. and Aktipis, A. 2019. Kombucha: A Novel Model System for Cooperation and Conflict in a Complex Multi-species Microbial Ecosystem. *PeerJ*. 7: e7565. DOI: https://doi.org/10.7717/peerj.7565.

[12] Mousavi, S. M., Hashemi, S. A., Zarei, M., Gholami, A., Lai, C. W., Chiang, W. H., Omidifar, N., Bahrani, S. and Mazraedoost, S. 2020. Recent Progress in Chemical Composition, Production, and Pharmaceutical Effects of Kombucha Beverage: A Complementary and Alternative Medicine. *Evidence-Based Complementary and Alternative Medicine*. 2020: e4397543. DOI: https://doi.org/10.1155/2020/4397543.

[13] Talebi, M., Frink, L. A., Patil, R. A. and Armstrong, D. W. 2017. Examination of the Varied and Changing Ethanol

Content of Commercial Kombucha Products. *Food Analytical Methods.* 10: 4062-4067.
DOI: https://doi.org/10.1007/s12161-017-0980-5.

[14] Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., Renard, T., Rollan, S. and 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.
DOI: https://doi.org/10.1016/j.procbio.2019.05.004.

[15] Al-Mohammadi, A. -R., Ismaiel, A. A., Ibrahim, R. A., Moustafa, A. H., Abou Zeid, A. and Enan, G. 2021. Chemical Constitution and Antimicrobial Activity of Kombucha Fermented Beverage. *Molecules*. 26: 5026.
DOI: https://doi.org/10.3390/molecules26165026.

[16] Sharifudin, S. A., Ho, W. Y., Yeap, S. K., Abdullah, R. and Koh, S. P. 2021. Fermentation and Characterisation of Potential Kombucha Cultures on Papaya-based Substrates. *LWT*. 151: 112060.
DOI: https://doi.org/10.1016/j.lwt.2021.112060.

[17] Jafari, R., Naghavi, N. S., Khosravi-Darani, K., Doudi, M. and Shahanipour, K. 2020. Kombucha Microbial Starter with Enhanced Production of Antioxidant Compounds and Invertase. *Biocatalysis and Agricultural Biotechnology*. 29: 101789.
DOI: https://doi.org/10.1016/j.bcab.2020.101789.

[18] Soares, M. G., de Lima, M. and Reolon Schmidt, V. C. 2021. Technological Aspects of Kombucha, Its Applications and the Symbiotic Culture (SCOBY), and Extraction of Compounds of Interest: A Literature Review. *Trends in Food Science & Technology*. 110: 539-550.
DOI: https://doi.org/10.1016/j.tifs.2021.02.017.

[19] Sinir, G. Ö., Tamer, C. E. and Suna, S. 2019. 10 - Kombucha Tea: A Promising Fermented Functional Beverage. In: Grumezescu, A. M., Holban, A. M. (Eds.). Fermented Beverages. Woodhead Publishing. 401-432.

[20] Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., Fall, A., Daube, G. and Coton, E. 2017. Unraveling Microbial Ecology of Industrial-scale Kombucha Fermentations by Metabarcoding and Culture-based Methods. *FEMS Microbiology Ecology*. 93.
DOI: 10.1093/femsec/fix048.

[21] Ahmed, R. F., Hikal, M. S. and Abou-Taleb, K. A. 2020. Biological, Chemical and Antioxidant Activities of Different Types Kombucha. *Annals of Agricultural Sciences*. 65: 35-41.
DOI: https://doi.org/10.1016/j.aoas.2020.04.001.

[22] Melkonian, E. A. and Schury, M. P. 2021. Biochemistry, Anaerobic Glycolysis. in: StatPearls. StatPearls Publishing, Treasure Island (FL).

[23] Chaudhry, R. and Varacallo, M. 2021. Biochemistry, Glycolysis. In: StatPearls. StatPearls Publishing, Treasure Island (FL).

[24] Leonard, W., Zhang, P., Ying, D., Adhikari, B. and Fang, Z. 2021. Fermentation Transforms the Phenolic Profiles and Bioactivities of Plant-based Foods. *Biotechnology Advances*. 49: 107763.
DOI: https://doi.org/10.1016/j.biotechadv.2021.107763.

[25] Zentou, H., Zainal Abidin, Z., Yunus, R., Awang Biak, D. R., Abdullah Issa, M. and Yahaya Pudza, M. 2021. A New Model of Alcoholic Fermentation under a Byproduct Inhibitory Effect. *ACS Omega*. 6: 4137-4146.
DOI: 10.1021/acsomega.0c04025.

[26] Gomes, R. J., Borges, M. de F., Rosa, M. de F., Castro-Gómez, R. J. H. and Spinosa, W. A. 2018. Acetic Acid Bacteria in the Food Industry: Systematics, Characteristics and Applications. *Food Technology and Biotechnology.* 56: 139-151.
DOI: 10.17113/ftb.56.02.18.5593.

[27] Lynch, K. M., Zannini, E., Wilkinson, S., Daenen, L. and Arendt, E. K. 2019. Physiology of Acetic Acid Bacteria and their Role in Vinegar and Fermented Beverages. *Comprehensive Reviews in Food Science and Food Safety*. 18: 587-625.
DOI: https://doi.org/10.1111/1541-4337.12440.

[28] Tran, T., Grandvalet, C., Verdier, F., Martin, A., Alexandre, H. and Tourdot-Maréchal, R. 2020. Microbiological and Technological Parameters Impacting the Chemical Composition and Sensory Quality of Kombucha. *Comprehensive Reviews in Food Science and Food Safety*. 19: 2050-2070.

[29] De Filippis, F., Troise, A.D., Vitaglione, P. and Ercolini, D. 2018. Different Temperatures Select Distinctive Acetic Acid Bacteria Species and Promotes Organic Acids Production During Kombucha Tea Fermentation. *Food Microbiology*. 73: 11-16.
DOI: 10.1016/j.fm.2018.01.008.

[30] Kumar, V. and Joshi, V. K. 2016. Kombucha: Technology, Microbiology, Production, Composition and Therapeutic Value. *International Journal of Food and Fermentation Technology*. 6: 13.
DOI: 10.5958/2277-9396.2016.00022.2.

[31] Antolak, H., Piechota, D. and Kucharska, A. 2021. Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). *Antioxidants*. 1: 1541.
DOI: 10.3390/antiox10101541.

[32] Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C. and Tencomnao, T. 2019. A Review of the Role of Green Tea (*Camellia sinensis*) in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy. *Nutrients.* 11: 474.
DOI: 10.3390/nu11020474.

[33] Naveed, M., BiBi, J., Kamboh, A. A., Suheryani, I., Kakar, I., Fazlani, S. A., FangFang, X., kalhoro, S. A., Yunjuan, L., Kakar, M. U., Abd El-Hack, M. E., Noreldin, A. E., Zhixiang, S., LiXia, C. and XiaoHui, Z. 2018. Pharmacological Values and Therapeutic Properties of Black Tea (*Camellia sinensis*): A Comprehensive Overview. *Biomedicine & Pharmacotherapy*. 100: 521-531.
DOI: 10.1016/j.biopha.2018.02.048.

[34] Dubey, K. K., Janve, M., Ray, A. and Singhal, R. S. 2020. Chapter 4 - Ready-to-drink Tea. In: Galanakis, C.M. (Ed.). Trends in Non-Alcoholic Beverages. Academic Press. 101-140.

[35] Cardoso, R. R., Neto, R. O., dos Santos D'Almeida, C. T., do Nascimento, T. P., Pressete, C. G., Azevedo, L., Martino, H. S. D., Cameron, L. C., Ferreira, M. S. L. and Barros, F. A. R. de. 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.
DOI: https://doi.org/10.1016/j.foodres.2019.108782.

[36] Saeed, M., Naveed, M., Arif, M., Kakar, M. U., Manzoor, R., Abd El-Hack, M. E., Alagawany, M., Tiwari, R., Khandia, R., Munjal, A., Karthik, K., Dhama, K., Iqbal, H. M. N., Dadar, M. and Sun, C. 2017. Green Tea (*Camellia sinensis*) and l-theanine: Medicinal Values and Beneficial Applications in Humans—A Comprehensive Review. *Biomedicine & Pharmacotherapy*. 95: 1260-1275.
DOI: 10.1016/j.biopha.2017.09.024.

[37] Azevedo, R. S. A., Teixeira, B. S., Sauthier, M. C. da S., Santana, M. V. A., dos Santos, W. N. L. and Santana, D. de A. 2019. Multivariate Analysis of the Composition of Bioactive in Tea of the Species *Camellia sinensis*. Food Chemistry, 8th Brazilian Workshop of Chemometrics: *Application of Chemometrics techniques In Food Chemistry*. 273: 39-44.
DOI: https://doi.org/10.1016/j.foodchem.2018.04.030.

[38] Bueno, F., Chouljenko, A. and Sathivel, S. 2021. Development of Coffee Kombucha Containing *Lactobacillus rhamnosus* and *Lactobacillus casei*: Gastrointestinal Simulations and DNA Microbial Analysis. *LWT- Food Science and Technology*. 142: 110980.
DOI: https://doi.org/10.1016/j.lwt.2021.110980.

[39] Fibrianto, K., Zubaidah, E., Muliandari, N. A., Wahibah, L. Y., Putri, S. D., Legowo, A. M. and Al-Baarri, A. N. 2020.

Antioxidant Activity Optimisation of Young Robusta Coffee Leaf Kombucha by Modifying Fermentation Time and Withering Pre-treatment. *IOP Conference Series: Earth Environmental. Science*. 475: 012029. DOI: 10.1088/1755-1315/475/1/012029.

[40] Kusdiana, R. N., Ferdi, V., Kusumawardhana, I. and Levyta, F. 2020. Hedonic Test of Kombucha Coffee. *IOP Conference Series: Materials Science and Engineering*. 924: 012005. DOI: :10.1088/1757-899X/924/1/012005.

[41] Palmonari, A., Cavallini, D., Sniffen, C. J., Fernandes, L., Holder, P., Fagioli, L., Fusaro, I., Biagi, G., Formigoni, A. and Mammi, L. 2020. Short Communication: Characterization of Molasses Chemical Composition. *Journal of Dairy Science*. 103: 6244-6249. DOI: 10.3168/jds.2019-17644.

[42] Luo, J., Guo, S., Wu, Y. and Wan, Y. 2018. Separation of Sucrose and Reducing Sugar in Cane Molasses by Nanofiltration. *Food Bioprocess Technology*. 11: 913-925.

[43] Varaee, M., Honarvar, M., Eikani, M.H., Omidkhah, M. R. and Moraki, N. 2019. Supercritical Fluid Extraction of Free Amino Acids from Sugar Beet and Sugar Cane Molasses. *The Journal of Supercritical Fluids*. 144: 48-55. DOI: https://doi.org/10.1016/j.supflu.2018.10.007.

[44] Jamir, L., Kumar, V., Kaur, J., Kumar, S. and Singh, H. 2021. Composition, Valorization and Therapeutical Potential of Molasses: A Critical Review. *Environmental Technology Reviews*. 10: 131-142.

[45] Djordjević, Mi., Šereš, Z., Maravić, N., Šćiban, M., Šoronja-Simović, D. and Djordjević, M. 2021. Modified Sugar Beet Pulp and Cellulose-based Adsorbents as Molasses Quality Enhancers: Assessing the Treatment Conditions. *LWT-Food Science and Technology*. 150: 111988. DOI: https://doi.org/10.1016/j.lwt.2021.111988.

[46] Zhang, S., Wang, J., and Jiang, H. 2021. Microbial Production of Value-added Bioproducts and Enzymes from Molasses, a By-product of Sugar Industry. *Food Chemistry*. 346: 128860. DOI: 10.1016/j.foodchem.2020.128860.

[47] Majtan, J., Bucekova, M., Kafantaris, I., Szweda, P., Hammer, K. and Mossialos, D. 2021. Honey Antibacterial Activity: A Neglected Aspect of Honey Quality Assurance as Functional Food. *Trends in Food Science & Technology*. 118: 870-886.

[48] Gaglio, R., Alfonzo, A., Francesca, N., Corona, O., Di Gerlando, R., Columba, P. and Moschetti, G. 2017. Production of the Sicilian Distillate "Spiritu re fascitrari" from Honey By-products: An Interesting Source of Yeast Diversity. *International Journal of Food Microbiology*. 261: 62-72. DOI: 10.1016/j.ijfoodmicro.2017.09.004.

[49] Mărgăoan, R., Cornea-Cipcigan, M., Topal, E. and Kösoğlu, M. 2020. Impact of Fermentation Processes on the Bioactive Profile and Health-promoting Properties of Bee Bread, Mead and Honey Vinegar. *Processes*. 8: 1081.

[50] Keșa, A. -L., Pop, C. R., Mudura, E., Salanță, L. C., Pasqualone, A., Dărab, C., Burja-Udrea, C., Zhao, H. and Coldea, T. E. 2021. Strategies To Improve the Potential Functionality of Fruit-based Fermented Beverages. *Plants*. 10: 2263.

[51] Koundouras, S. 2018. Environmental and Viticultural Effects on Grape Composition and Wine Sensory Properties. *Elements*. 14: 173-178.

[52] Ayed, L., Ben Abid, S. and Hamdi, M. 2017. Development of a Beverage from Red Grape Juice Fermented with the Kombucha Consortium. *Annals of Microbiology*. 67: 111-121.

[53] Mazumdar, P., Pratama, H., Lau, S.-E., Teo, C. H., Harikrishna, J. A. 2019. Biology, Phytochemical Profile and Prospects for Snake Fruit: An Antioxidant-rich Fruit of South East Asia. *Trends in Food Science & Technology*. 91: 147-158. DOI: https://doi.org/10.1016/j.tifs.2019.06.017.

[54] Zubaidah, E., Dewantari, F. J., Novitasari, F. R., Srianta, I. and Blanc, P. J. 2018. Potential of Snake Fruit (*Salacca zalacca* (Gaerth.) Voss) for the Development of a Beverage through Fermentation with the Kombucha Consortium. *Biocatalysis and Agricultural Biotechnology*. 13: 198-203. DOI: https://doi.org/10.1016/j.bcab.2017.12.012.

[55] Gyesi, J. N., Opoku, R. and Borqueye, L. S. 2019. Chemical Composition, Total Phenolic Content, and Antioxidant Activities of the Essential Oils of the Leaves and Fruit Pulp of *Annona muricata L.* (Soursop) from Ghana. *Biochemistry Research International*. 2019: 4164576. DOI: https://doi.org/10.1155/2019/4164576.

[56] Mizuta, A. G., de Menezes, J. L., Dutra, T. V., Ferreira, T. V., Castro, J. C., da Silva, C. A. J., Pilau, E. J., Machinski Junior, M. and Abreu Filho, B. A. de. 2020. Evaluation of Antimicrobial Activity of Green Tea Kombucha at Two Fermentation Time Points Against *Alicyclobacillus* spp. *LWT*. 130: 109641.

[57] Wiwanitkit, V. 2018. Chapter 2 - Important Emerging and Reemerging Tropical Food-borne Diseases. In: Holban, A. M., Grumezescu, A. M. (Eds.). Foodborne Diseases, Handbook of Food Bioengineering. Academic Press. 33-55.

[58] Hou, J., Luo, R., Ni, H., Li, K., Mgomi, F. C., Fan, L. and Yuan, L. 2021. Antimicrobial Potential of Kombucha against Foodborne Pathogens: A Review. *Quality Assurance and Safety of Crops & Foods*. 13: 53-61.

[59] Bhatt, A., Kothari, D., Kothari, C., Kothari, R. 2021. Probiotic: An Uprising Human Health Concept. *IntechOpen*.

[60] Kaewkod, T., Bovonsombut, S. and Tragoolpua, Y. 2019. Efficacy of Kombucha Obtained from Green, Oolong, and Black Teas on Inhibition of pathogenic Bacteria, Antioxidation, and Toxicity on Colorectal Cancer Cell Line. *Microorganisms*. 7: 700. DOI: 10.3390/microorganisms7120700.

[61] Lichota, A. and Gwozdzinski, K. 2018. Anticancer Activity of Natural Compounds from Plant and Marine Environment. *International Journal of Molecular Sciences*. 19: 3533.

[62] Tasdemir, S. S and Nevin Sanlier, N. 2020. An Insight into the Anticancer Effects of Fermented Foods: A Review. *Journal of Functional Foods*. 75: 2020, 10428. DOI: https://doi.org/10.1016/j.jff.2020.104281.

[63] Vitas, J. S., Cvetanović, A. D., Mašković, P. Z., Švarc-Gajić, J. V. and Malbaša, R. V. 2018. Chemical Composition and Biological Activity of Novel Types of Kombucha Beverages with Yarrow. *Journal of Functional Foods*. 44: 95-102. DOI: https://doi.org/10.1016/j.jff.2018.02.019.

[64] Amarasekara, A. S., Wang, D. and Grady, T. L. 2020. A Comparison of Kombucha SCOBY Bacterial Cellulose Purification Methods. *SN Applied Sciences*. 2: 240. https://doi.org/10.1007/s42452-020-1982-2.

[65] Marchetti, L., and Andrés, S. C. 2021. Use of Nanocellulose in Meat Products. *Current Opinion in Food Science*. 38: 96-101. http://dx.doi. org/10.1016/j.cofs.2020.11.003.

[66] Choi, S. M., Rao, K. M., Zo, S. M., Shin, E. J. and Han, S. S. 2022. Bacterial Cellulose and Its Applications. *Polymers*. 14: 1080. https://doi.org/10.3390/polym14061080.

[67] Guo, Y., Zhang, X., Hao, W., Xie, Y., Chen, L., Li, Z. and Zhu, B., Feng, X. 2018. Nano-bacterial Cellulose/soy Protein Isolate Complex Gel as Fat Substitutes in Ice Cream Model. *Carbohydrate Polymers*. 198: 620-630. https://doi: 10.1016/j.carbpol.2018.06.078.

[68] Kwon, H. C., Shin, D. M., Yune, J. H., Jeong, C. H., and Han, S. G. 2021. Evaluation of Gels Formulated with Whey Proteins and Sodium Dodecyl Sulfate as a Fat Replacer in Low-fat Sausage. *Food Chemistry*. 337: 127682. http://dx.doi.org/10.1016/j.foodchem.2020.127682.