



Remiern

Microbial Composition, Bioactive Compounds, Potential Benefits and Risks Associated with Kombucha: A Concise Review

Abidemi Oluranti Ojo * and Olga de Smidt

Centre for Applied Food Sustainability and Biotechnology (CAFSaB), Central University of Technology, Bloemfontein 9300, South Africa; odesmidt@cut.ac.za

* Correspondence: aojo@cut.ac.za

Abstract: Kombucha is a fermented tea beverage containing bioactive compounds from tea and vital compounds such as acetic acid, D-saccharic acid-1,4-lactone, and glucuronic and gluconic acids produced from the metabolic activities of bacteria and yeasts, which benefit human health. Kombucha contains a symbiotic culture of bacteria and yeast (SCOBY), which actively ferments sugar. Kombucha microbial compositions vary due to environmental conditions and the starter culture. Saccharomyces sp., Schizosaccharomyces pombe, Schizosaccharomyces sp., and Brettanomyces sp. (yeasts) and Acetobacter aceti, Komagataeibacter xylinum (formerly known as Gluconacetobacter xylinum), Gluconobacter oxydans, and Acetobacter pasteurianus (acetic acid-producing bacteria) are commonly found in kombucha. This review focused on the microbial compositions of kombucha and their functionality. Aspects discussed include: (i). developments in kombucha, (ii). microbial compositions of kombucha, (ii). microbial production of kombucha cellulose, (iv). factors influencing kombucha microbial compositions, (v). tea type and kombucha bioactive compounds, (vi). kombucha health benefits, and (v). potential risk factors of kombucha consumption. Current gaps, recommendations, and prospects were also discussed. Kombucha production using rooibos as the tea base is recommended, as rooibos is caffeine-free. Upcycling kombucha wastes, mainly SCOBY, for producing cellulose filters improving food flavors and as a substrate in food fermentations is touched on.

Keywords: microbial compositions; kombucha; fermented tea; acetic acid-producing bacteria; yeasts

Citation: Ojo, A.O.; de Smidt, O. Microbial Composition, Bioactive Compounds, Potential Benefits, and Risks Associated with Kombucha: A Concise Review. *Fermentation* **2023**, *9*, 472. https://doi.org/10.3390/fermentation9050472

Academic Editor: Michela Verni

Received: 14 April 2023 Revised: 02 May 2023 Accepted: 11 May 2023 Published: 13 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Kombucha is a carbonated, non- or low-alcoholic beverage derived from tea, sugar, and a metabolically active symbiotic community of bacteria and yeast (SCOBY) in static fermentation under aerobic conditions at an ambient temperature [1,2]. Kombucha (fermented sweetened tea), known as a tea fungus, originated in northeast China (Manchuria) as early as 220 B.C., where it was first prized for its detoxification and energizing properties [3–5]. In 414 A.D., Doctor Kombu from Korea brought kombucha to Japan to cure emperor Inkyo's digestive problems [6,7], and it was introduced into Russia (as Cainii kvass, Jsakvasska, Kambucha, and Cainiigrib), then into Eastern Europe, and in the 1950s, kombucha arrived in France and North Africa [6,8,9].

The preparation of kombucha was described by [6,10–12]; the standard protocol involves stirring 50 g of sucrose in boiling water (1 L) and adding tea, which is filtered after 5 min. The sweetened tea is allowed to cool at 20 °C and inoculated with 24 g of SCOBY. This is transferred into an autoclaved beaker (1 L), and the pH is lowered by adding previously fermented kombucha (0.2 L) to inhibit the growth of unwanted microorganisms [6]. Kombucha preparation results in a cocktail of molecules such as lactic acid, ethanol,

Fermentation 2023, 9, 472 2 of 13

acetic acid, carbon dioxide, and gluconic and glucuronic acids (Figure 1). Sweetened tea fermentation involves the cleavage of sucrose by the invertase enzyme produced by the yeast; this produces fructose and glucose, which are then metabolized into ethanol and carbon dioxide via the glycolysis pathway [13]. In turn, acetic acid bacteria oxidize ethanol into acetic acid under aerobic conditions [13–15]. The generation of acetic acid reduces the pH and contributes to kombucha's sour taste [14,16], accompanied by ethanol production, which also inhibits the growth of possible pathogenic microorganisms [4,11]. During fermentation, acetic acid bacteria (e.g., *Komagataeibacter* sp.) utilize fructose and glucose to produce a network of cellulose as a secondary metabolite of fermentation [17], and enzymatic oxidation of glucose at carbon 1 and 6 by acetic acid bacteria results in gluconic acid and glucuronic acid production [18,19].

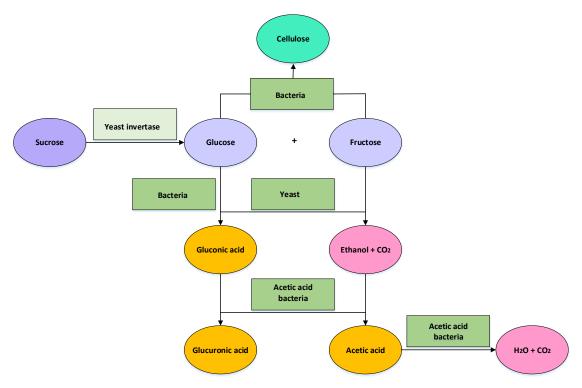


Figure 1. Main metabolic pathway in kombucha production and the generated metabolites (adapted from Markov et al. (2003) [20]).

1.1. Developments in Kombucha

There is a growing interest in kombucha production and fermentation processes, bacterial cellulose studies, microbial analysis of kombucha, and applications of SCOBY in some food products [17]. A VOSviewer analysis (a software for constructing and visualizing bibliometric networks) of published records on kombucha formed six clusters (Figure 2). Cluster 1 contains 'kombucha tea' (483 hits) connected to 'production' (206 hits), 'bacterial cellulose' (130 hits), 'kombucha tea fermentation' (329 hits), 'Komagataeibacter' (54 hits), and 'preparation' (44 hits); cluster 2 contains 'application' (128 hits) linked to 'yeasts' (253 hits), 'bacteria' (309), 'kombucha SCOBY' (93 hits), 'SCOBY' (92 hits), and 'use' (390 hits); in cluster 3, 'kombucha beverage' (295 hits) is connected to 'antioxidant activity' (208 hits), 'chemical composition' (54 hits), and 'biological activity' (49 hits); cluster 4 contains 'beverage' (295 hits) linked to 'kombucha fermentation' (404 hits), and 'antimicrobial activity' (81 hits); cluster 5 contains 'fermentation' (404 hits) linked to 'kombucha culture' (230 hits) and 'microorganism' (85 hits); and cluster 6 contains 'kombucha' (665 hits) linked to 'tea' (483 hits) and 'fermentation time' (112 hits).

Fermentation 2023, 9, 472 3 of 13

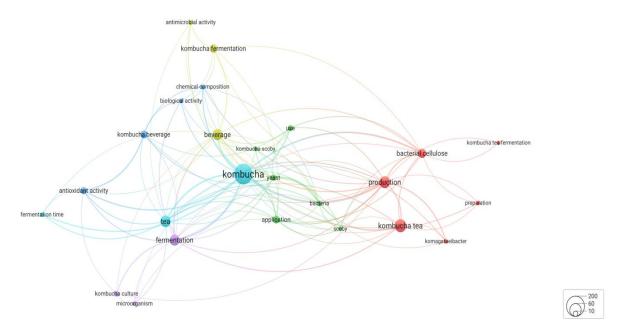


Figure 2. Co-occurrence analysis using Web Science data for kombucha records. The bubble size represents the number of published articles in the database, and the bubble proximity represents the frequency of co-occurrence of phrases in the same articles (Source: authors, 2023).

Studies on the microbial composition of kombucha using next-generation sequence analysis have been conducted [3,21]. However, nucleic acid-based microbial diversity studies of kombucha SCOBY often end after assessing microbial compositions, and few studies on the isolation of pure culture from kombucha have been reported [22]. A few studies have reported possible kombucha microbial metabolic functionality and a link between microbiota and bioactive compounds in kombucha using Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) and metabolomic analysis [3,23]. Still, to date, extensive studies on microbial functionality in kombucha production to establish the roles of individual microorganisms in metabolic processes during production and testing of the postulated functionality of kombucha cultures are yet to be performed.

1.2. Microbial Compositions of Kombucha

The broad spectrum of kombucha microorganisms has been reported [18]. Microbial compositions may vary depending on the source of SCOBY (starter culture) and the environmental conditions during fermentation [18,24]. A microbial community analysis on several kombucha cultures confirmed the presence of dominant yeasts and bacteria. Common yeasts in kombucha cultures reported include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Brettanomyces bruxellensis*, *Brettanomyces intermedius*, *Brettanomyces lambicus*, *Brettanomyces custersii*, *Zygosaccharomyces sp.*, *Zygosaccharomyces kombuchaensis*, *Zygosaccharomyces rouxii*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Pichia sp.*, *Pichia membranaefaciens*, *Torulaspora sp.*, *Torulaspora delbrueckii*, *Candida sp.*, and *Zygosaccharomyces bailii* [18,25–28].

The reported bacterial compositions in kombucha cultures include acetic acid-producing bacteria such as *Acetobacter aceti, Komagataeibacter xylinum* (recently reclassified from *Gluconacetobacter xylinum* [18,29]), *Gluconobacter oxydans*, and *Acetobacter pasteurianus* [6,18,21,25,30]. Other bacteria (*Acetobacter nitrogenifigens* and *Gluconacetobacter kombuchae* sp. nov.) have also been identified [31,32], while in kombucha cultures from Ireland, lactic acid bacteria were abundant [21]. However, lactic acid bacteria may not be essential to the kombucha microbial compositions because they are not always found in SCOBY [1]. The additional presence of *Leuconostoc* sp., *Bifidobacterium* sp. [33], *Bacterium gluconicum* [34], *Propionibacterium* sp., *Allobaculum* sp. [12], *Bacillus coagulans*, and *Lactobacillus nagelii* [35]

Fermentation 2023, 9, 472 4 of 13

in kombucha cultures have also been reported. Nonetheless, the dominant microorganisms in respective kombucha cultures are the core drivers of sweetened tea fermentation [1,36].

1.3. Kombucha Microbial Interactions

Although in-depth information on kombucha microbial functionality is still needed, it is essentially clear that kombucha microorganisms are involved in various activities during kombucha production. Kombucha acetic acid bacteria convert acetaldehyde into ethanol and acetaldehyde hydrate into acetic acid by acetaldehyde dehydrogenase, while kombucha yeasts ferment sugar to ethanol [18,30]. A symbiotic relationship among kombucha microorganisms was reported. For instance, ethanol produced by yeast helped bacteria to produce acetic acid, while the produced acetic acid stimulated yeast production of ethanol [37]. The concurrent production of these two compounds prevents the growth of other competitive microbes. Hence, kombucha can be used as a model to evaluate the evolution of synergetic relationships and conflict in diverse multispecies systems.

1.4. Microbial Production of Kombucha Cellulose

Microbial cellulose, a natural polymer, has unique structural and chemical properties compared to plant cellulose [38]. Cellulose ($C_6H_{10}O_5$)_n is a homopolymer that composes β-D-glucopyranose units linked by β-1,4-glycosidic bonds [39]. Bacteria belonging to *Aerobacter, Rhizobium, Agrobacterium, Achromobacter, Sarcina, Azotobacter, Salmonella,* and *Escherichia* produce cellulose [40], but bacteria mostly reported in synthesizing cellulose during kombucha production belong *Komagataeibacter xylinum* [18,38,41]. In general, capable bacteria can produce two types of cellulose: cellulose I and cellulose II [42]. Cellulose I is a ribbon-shaped polymer with bundles of microfibrils, and cellulose II (a thermodynamically stable amorphous polymer) is a mercerized bacterial cellulose [43,44].

Kombucha cellulose, the jelly-like membrane known as zooglea biofilm, is formed by symbiotic yeast and bacteria adhering together [26]. The mode of zoogleal biofilm formation is by the adhesion of bacteria to the surface of the aqueous environment and the excretion of a polysaccharide matrix that holds the biofilm together [45]. The synthesis of kombucha cellulose by bacteria such as Komagataeibacter xylinum involves the formation of uridine diphosphoglucose, a direct cellulose precursor and glucose unit polymerization to form β -1 \rightarrow 4 glucan chain and a chain that forms ribbon-shaped cellulose chains [42]. Uridine diphosphoglucose is synthesized by glucose phosphorylation by glucose kinase into glucose-6-phosphate, followed by isomerization of glucose-6-phosphate into glucose-1-phosphate by phosphoglucomutase. Uridine diphosphoglucose pyrophosphorylase then converts glucose-1-phosphate into uridine diphosphoglucose (Figure 3). Polymerization of glucose units by cellulose synthase results in cellulose synthesis in the form of fibers with β -1 \rightarrow 4 glucan chains assembled, forming a ribbon-like structure of cellulose chains from thousands of cellulose chains that are extruded outside the cell and assembled into macrofibrils [42-44]. The bacterial biofilm allows cell adhesion and constant bacteria exposure to aerobic environments, which is vital for fermentation [46]. The biofilm's mechanical, chemical, and biological structure protects bacteria from harsh environmental conditions [46].

Fermentation 2023, 9, 472 5 of 13

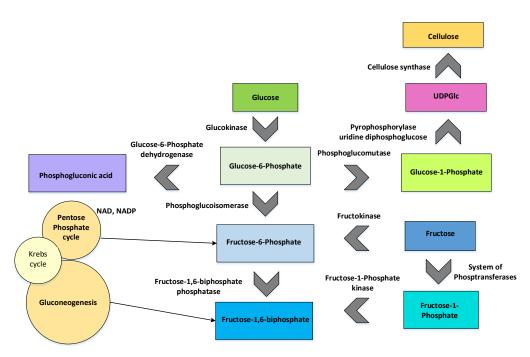


Figure 3. Biochemical pathway of cellulose synthesis by *Komagataeibacter xylinum*. NAD: Nicotinamide adenine dinucleotide; NADP: Nicotinamide adenine dinucleotide phosphate (adapted from Chawla et al. (2009) [42]).

1.5. Factors Influencing Microbial Compositions of Kombucha

Several factors influencing kombucha microbiota diversity include substrates, tea type, pH values, fermentation time, and temperature [2]. Substrates such as carbon and nitrogen sources are vital for the growth of almost all microorganisms [47]. The growth medium of kombucha microbiota should contain nitrogen sources obtained from the tea extract and a carbon source such as sucrose (mostly used), fructose, glucose, ethanol, or mannitol [48]. The type of tea used influences microbial yield. Green tea leaves had a higher SCOBY yield than black tea and tea waste [49]. Acetic acid bacteria growth was inhibited when the tea leave concentration exceeded 6 g L^{-1} [50], while the optimum sugar concentrations frequently used for kombucha SCOBY are within the range of 6-12 g L⁻¹ [21,47,51–54]. High sugar concentrations inhibited microbial growth, and the optimum fermentation time ranged from 6 to 14 days [55]. It was reported that the bacterial growth increased rapidly for the first 6 days and gradually decreased until the end of fermentation, while viable yeast counts initially increased with incubation time (6-14 days) and then decreased gradually until the end of fermentation [56]. The increase in fungal tea biomass up to 14 days and a decreased biomass after day 14 is due to bacteria and yeast in the stationary phase and a decreased pH, causing acid-sensitive cell death [49]. Maintaining the optimum temperature during fermentation is vital to achieving optimum microbial growth for microbial enzymatic activities [11].

1.6. Tea Types and Bioactive Compounds in Kombucha

Different types of tea have been used in kombucha production, and green and black teas are commonly used in preparing kombucha. Green tea is produced by immediately heating *Camellia sinensis* leaves after picking to prevent enzymatic oxidation. In contrast, black tea is produced by exposing *Camellia sinensis* leaves to air for oxidation and enzymatic reactions [57,58]. Rooibos tea, which is not frequently used in kombucha production, is produced by placing the shredded *Aspalathus linearis* sp. *Linearis* tea shoots in a fermentation heap and bruising by late afternoon, followed by night fermentation (oxidation) and sun drying the heap the following morning [59].

Fermentation 2023, 9, 472 6 of 13

Tea components and the metabolic activities of microorganisms in kombucha tea are the sources of the bioactive compounds in kombucha that give the beverage nutraceutical properties. The major bioactive compounds in green, black, or rooibos tea are polyphenols that give them medicinal properties [57,60], while bioactive compounds obtained from microbial metabolic activities during kombucha tea fermentation include enzymes, vitamins, bacteriocins, D-saccharic acid-1,4-lactone, acetic, and gluconic and glucuronic acids [6,61,62].

1.7. Kombucha Health Benefits

Kombucha, classified as a nutraceutical, is a natural product developed to improve human mental and physical health. The beneficial effects of kombucha result from the presence of D-saccharic acid-1,4-lactone, gluconic acid, glucuronic acid, tea polyphenols, vitamins, amino acids, various micronutrients, and antibiotics [6,61,62]. The antioxidant and antimicrobial properties of kombucha are due to bioactive compounds [5,18,63]. D-saccharic acid-1,4-lactone (a derivative of D-glucaric acid), known for its antioxidant and detoxifying properties, was reported to protect against hyperglycemia-induced hepatic apoptosis in diabetic rats [64]. D-saccharic acid-1,4-lactone also inhibited glucuronidase activity indirectly related to cancers [61]. Glucuronic acid is one of the vital components because of its detoxifying properties [65]. Glucuronic acid participates in glucuronidation, eliminating toxic by-products (metabolites) or toxins from the body, thereby supporting liver detoxication function [66]. In the glucuronidation process, glucuronyltransferase catalyzes the binding of glucuronic acid salt (glucuronate ion) to a toxin to form glucuronide, a water-soluble molecule that is then excreted from the body [66].

Several in vivo and in vitro studies have reported the health benefits of kombucha consumption concerning the beneficial effects of liver detoxification and acting against oxidative stress. Kombucha tea reverted carbon tetra chloride-induced hepatoxicity in male albino rats [67]. The protective effects of kombucha tea against acetaminophen-induced hepatoxicity were reported in a kombucha tea-treated group where severe glycogen storage in hepatocytes, hepatocellular degeneration, and necrosis were reduced [68]. Kombucha tea modulated oxidative stress-induced apoptosis in murine hepatocytes due to its antioxidant activity, which functions through mitochondria-dependent pathways that have been demonstrated [69]. Kombucha boosts the anticancer activity of the doxorubicin agent [70] and reportedly can give long life after continuous consumption due to its ability to reverse aging processes [50]. Kombucha possibly has medicinal effects against arthritis, metabolic diseases, and some types of cancer [2,4,66]. Furthermore, kombucha exhibits hypoglycaemic and antilipidemic properties with high probiotics and is a better suppressor of a high blood glucose level [71]. It also has a better inhibitory effect on α amylase and lipase activities in the plasma and pancreas [71]. It is important to note that much of the scientific results on the potential health effects of kombucha pertain to animal models such as rats and may not accurately reflect the human model [36,72,73].

1.8. Potential Risk Factors of Kombucha Consumption

Mechanisms linked to the toxic and adverse effects of consuming kombucha are unclear. The concerns about the toxicity of kombucha and its damage to health after consumption are mostly due to human error during production, resulting in poor product quality. Most reported kombucha toxicity cases are related to individuals with previous illnesses such as human immunodeficiency virus, acute kidney failure, acidosis vulnerability, and pregnant women with hypersensitivity to some kombucha compounds [12]. For instance, the reported cases of nausea, jaundice, headache, allergy, and dizziness were linked to underlining illnesses [6].

Fermentation 2023, 9, 472 7 of 13

1.9. Toxicity of Kombucha Due to Human Error

The human error that commonly introduces toxic effects into kombucha is associated with biological and chemical hazards and excessive kombucha consumption. Microbial composition, pH values, storage temperature, and fermentation containers could contribute to these toxic effects [8]. Biological hazards associated with kombucha consumption include foodborne pathogens such as *Salmonella* spp., *Listeria monocytogenes, Bacillus* spp., *Staphylococcus aureus*, and *Clostridium botulinum* [74]. Mold contamination with organisms such as *Penicillium* and *Aspergillus* can also occur in kombucha [26]; these microorganisms produce mycotoxins linked to carcinogenicity and aflatoxicosis in humans [75]. The biological hazards are usually attributed to poor hygiene practices [76]. The chemical hazards associated with kombucha consumption originally involved 'lead poisoning' because kombucha was prepared in a ceramic pot coated in a lead-based glaze; over time, the acids produced during kombucha production due to low pH leached the lead from the enamel of the ceramic pot into kombucha [77]. Excessive consumption of kombucha could result in toxicity, which could cause chemical acidosis due to excessive organic acids in the blood, resulting in life-threatening acidosis in individuals susceptible to acidosis [73].

1.10. Possible Toxicity Due to Product Quality

Kombucha pH values for production and consumption range from 2.5 to 4.2 [78]. The only product quality that could result in kombucha toxicity is in a scenario where organic acids (acetic, lactic, gluconic, and glucuronic acids) produced are in very high concentrations, which results in very low pH values. Consumption of this type of kombucha can lead to acidosis in susceptible individuals.

1.11. Alleviating Some Potential Risk Factors

Biological hazards, e.g., food contamination by pathogens, can be avoided with proper hygiene, such as hand sterilization by washing hands with soap before kombucha preparations and sterilizing utensils and apparatus. The pH must be kept within the value that prohibits the growth of mold and food pathogens. Chemical hazards can be avoided by using a glass container for kombucha tea fermentation [73].

There is little information on the daily recommended consumption of kombucha [79]. The bioactive compounds in kombucha should be quantified, daily kombucha consumption should be determined and recommended, and the recommended daily kombucha consumption should be specified on kombucha bottles. The microbial contents and pH values of kombucha should be determined before making the beverage available for consumption. Adding preservatives to kombucha after production would keep kombucha at its normal pH value, thereby preventing acidosis in individuals susceptible to acidosis.

1.12. Current Gaps, Recommendations, and Prospects

Extensive studies have been done on black and green tea-prepared kombucha microbial composition. Still, more information is needed on the microbial composition of kombucha produced from rooibos tea. Microbial evaluation of kombucha prepared with rooibos tea and a comparison of microbial dynamics of kombucha prepared using black, green, and rooibos tea will shed light on the effects of tea types on kombucha microbial compositions during fermentation. It was shown that 16S rDNA and ITS-targeted metagenomics were useful tools for assessing microbial compositions and structures of kombucha cultures. However, more focus is needed on assessing kombucha microbial community metabolic functionalities. Extensive research on the metabolic functionalities of kombucha microbes, testing, and confirmation of each microbe's metabolic functionality will assist in preparing kombucha of the desired flavors.

Fermentation 2023, 9, 472 8 of 13

1.13. Rooibos Tea for Kombucha Production

Camellia sinensis teas (black and green tea) have been extensively used to produce kombucha, but more research is needed using rooibos tea in kombucha production. Black and green teas have caffeine; using these Camellia sinensis teas for preparing kombucha may not be suitable for individuals with bleeding disorders, anxiety disorders, and heart problems [80]. Rooibos has unique properties that could be useful in producing kombucha [22]. Rooibos tea (Aspalathus linearis) has a low tannin content and is caffeine-free [81]; the tea has unique phenolic compounds, i.e., aspalathin and aspalalinin [82,83]. Rooibos is one of the only three known sources of nothofagin; it contains a rare C-C linked hydroxydihydrochalcone glucoside (nothofagin); the other known nothofagin sources include the heartwood of Nothofagus fusca [84] and Schoepfia chinesis (a Chinese medicinal plant bark) [85]. Rooibos tea also contains flavone (orientin and iso-orientin), vitexin, isovitexin, flavanone (dihydro-orientin and dihydroiso-orientin), lignans and coumarins, and phenolic acids [82,86,87].

Rooibos tea is known to relax the body as it is caffeine-free and reduces stress and anxiety due to the presence of aspalathin and nothofagin [88]. The biological activities of rooibos tea make it safe as a base tea for kombucha production since several studies on the safety and toxicity of rooibos tea consumption revealed rooibos tea's protective roles [89,90]. Animal studies from several laboratories have shown that processed (fermented) or unprocessed (unfermented) rooibos tea consumption as the sole source of drinking fluid over various lengths of time did not cause any adverse effect in animals [89,91,92].

Rooibos tea can be used as adjuvant support for preventing and treating some diseases, such as diabetic vascular complications [93]. Studies have shown that rooibos tea extracts exhibited antimutagenic activity in mice and Chinese hamster ovary cells [94]. Rooibos tea prevented 2-acetylaminofluorene and aflatoxin B₁-induced mutagenesis [90] and rooibos extract attenuation of lipopolysaccharide-induced liver injury was reported [95]. A study on the regeneration of rat liver intoxicated by carbon tetrachloride showed a reduction in fibrotic tissue in groups treated with rooibos tea compared to the group that received water [91]. As such, using rooibos tea as a co-adjuvant for liver disease therapy and prevention is recommended [91]. A human intervention study showed scientific proof of safety for humans consuming rooibos tea, as no adverse effect was reported, while the clinical pathology results were within the reference range [89].

1.14. Upcycling Kombucha Waste Products

In recent years, the focus has been on using renewable sources, including living biological systems, to develop sustainable biomaterials. Kombucha SCOBY is one of the best biomaterials, and its use in many applications has started gaining more attention. Kombucha SCOBY has been used in the recycling of industrial wastes (SCOBY was used in the chemoorgano-heterotrophic leaching process and recycling of rare earth elements (REEs)) [96] and food packaging (dried biofilm sheets were made as covers to store vegetables for a long time without any nutritive quality degradation) [97]. It has also been used in the textile industry [98], as a biosorption material for metal removal [99], and in animal feed as an extra ingredient, which provides crude fiber, protein, amino acids, and minerals to broiler chick feed [100]. Fermented milk drinks using kombucha SCOBY have been developed for human consumption to improve health, and some of these fermented milk drink products were developed with lactose-free variants and traditional milk [101]. Furthermore, kombucha SCOBY has been used in tissue engineering as a nerve conduit grown in Schwann cells in vitro [102] and in medicine as silver nanoparticles of fungal extract tested on human breast cancer cells [103].

Kombucha is an excellent system for scientific exploration since it is easily propagated, non-pathogenic, and inexpensive. Kombucha SCOBY can be applied in water technology to produce cellulose filters of specific pore sizes, which could be used in wastewater filtration. The SCOBY can be applied in food technology to improve food

Fermentation 2023, 9, 472 9 of 13

flavors and as a food fermentation and brewing substrate. Kombucha SCOBY can also be used in making edible straws, cutlery, and plates and can be applied in medicine to produce probiotics and antimicrobial ointments.

2. Conclusions

Kombucha consumption has been in existence for some decades due to its healthpromoting properties. Bioactive compounds from tea and metabolic products of microorganisms in kombucha contribute to kombucha nutraceutical properties. Microbial compositions of kombucha vary due to environmental factors (e.g., pH and temperature) and the starter cultures. However, Saccharomyces sp., Schizosaccharomyces pombe, Schizosaccharomyces sp., and Brettanomyces sp. (yeasts) and Acetobacter aceti, Komagataeibacter xylinum, Gluconobacter oxydans, and Acetobacter pasteurianus (acetic acid-producing bacteria) were reported to be present in nearly all kombucha SCOBY. A symbiotic relationship exists among kombucha microbiota as ethanol produced by yeast helps bacteria to produce acetic acid while acetic acid stimulates yeast production of ethanol. Several factors influencing the diversity of kombucha microbiota include substrates (carbon and nitrogen sources), tea type, pH values, fermentation time, and temperature. Kombucha exhibits hypoglycaemic, antilipidemic, antibacterial, antioxidant, and detoxification properties. Possible risk factors of kombucha consumption can be alleviated using proper hygiene practices and maintaining the kombucha's optimum pH value to prohibit mold and food pathogens growth. Kombucha production using rooibos as the tea base is highly recommended since rooibos is caffeine-free and its consumption is safe for individuals with bleeding and anxiety disorders. Research on the metabolic functionalities of kombucha microbes, testing, and confirming each microbe's metabolic functionality, remains to be explored. Kombucha SCOBY has numerous prospects as it can be used to produce waterfilter cellulose, improve food flavors, and manufacture probiotics and antimicrobial ointments.

Author Contributions: All authors contributed to the development and writing of this article. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no specific grant from funding agencies.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Laureys, D.; Britton, S.J.; De Clippeleer, J. Kombucha Tea Fermentation: A Review. *J. Am. Soc. Brew. Chem.* **2020**, 78, 165–174. https://doi.org/10.1080/03610470.2020.1734150.

- 2. De Filippis, F.; Troise, A.D.; Vitaglione, P.; Ercolini, D. Different Temperatures Select Distinctive Acetic Acid Bacteria Species and Promotes Organic Acids Production during Kombucha Tea Fermentation. *Food Microbiol.* **2018**, 73, 11–16. https://doi.org/10.1016/j.fm.2018.01.008.
- 3. Arıkan, M.; Mitchell, A.L.; Finn, R.D.; Gürel, F. Microbial Composition of Kombucha Determined Using Amplicon Sequencing and Shotgun Metagenomics. *J. Food Sci.* **2020**, *85*, 455–464. https://doi.org/10.1111/1750-3841.14992.
- 4. Dufresne, C.; Farnworth, E. Tea, Kombucha, and Health: A Review. Food Res. Int. 2000, 33, 409-421.
- 5. Sreeramulu, G.; Zhu, Y.; Knol, W. Kombucha Fermentation and Its Antimicrobial Activity. *J. Agric. Food Chem.* **2000**, 48, 2589–2594. https://doi.org/10.1021/jf991333m.
- 6. Jayabalan, R.; Malbaša, R.V.; Lončar, E.S.; Vitas, J.S.; Sathishkumar, M. A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 538–550. https://doi.org/10.1111/1541-4337.12073.
- 7. Roche, J.; Philosopher, T. The History and Spread of Kombucha. Available online: http://users.best-web.net/~om/~kombu/roche.html (accessed on 4 April 2023).
- 8. Jayabalan, R.; Malbaša, R.V.; Sathishkumar, M. Kombucha. *Ref. Modul. Food Sci.* **2016**, 1–8. https://doi.org/10.1016/b978-0-08-100596-5.03032-8.

9. Machado, G.; Leon, S.; Santos, F.; Lourega, R.; Dullius, J.; Mollmann, M.E.; Eichler, P. Literature Review on Furfural Production from Lignocellulosic Biomass. *Nat. Resour.* **2016**, *7*, 115–129. https://doi.org/10.4236/nr.2016.73012.

- 10. Kim, J.; Adhikari, K. Current Trends in Kombucha: Marketing Perspectives and the Need for Improved Sensory Research. *Beverages* **2020**, *6*, 15.
- 11. Bishop, P.; Pitts, E.R.; Budner, D.; Thompson-Witrick, K.A. Kombucha: Biochemical and Microbiological Impacts on the Chemical and Flavor Profile. *Food Chem. Adv.* **2022**, *1*, 1-9. https://doi.org/10.1016/j.focha.2022.100025.
- 12. Leal, J.M.; Suárez, L.V.; Jayabalan, R.; Oros, J.H.; Escalante-Aburto, A. A Review on Health Benefits of Kombucha Nutritional Compounds and Metabolites. *CYTA-J. Food* **2018**, *16*, 390–399. https://doi.org/10.1080/19476337.2017.1410499.
- 13. Jakubczyk, K.J.; Piotrowska, G.; Janda, K. Characteristics and Biochemical Composition of Kombucha—Fermented Tea. *Med. Ogólna Nauk. Zdrowiu* **2020**, *26*, 94–96. https://doi.org/10.26444/monz/118887.
- 14. May, A.; Narayanan, S.; Alcock, J.; Varsani, A.; Maley, C.; Aktipis, A. Kombucha: A Novel Model System for Cooperation and Conflict in a Complex Multi-Species Microbial Ecosystem. *PeerJ* 2019, 1, 1-22. https://doi.org/10.7717/peerj.7565.
- 15. Chakravorty, S.; Bhattacharya, S.; Chatzinotas, A.; Chakraborty, W.; Bhattacharya, D.; Gachhui, R. Kombucha Tea Fermentation: Microbial and Biochemical Dynamics. *Int. J. Food Microbiol.* **2016**, 220, 63–72. https://doi.org/10.1016/j.ijfoodmicro.2015.12.015.
- 16. Wang, B.; Rutherfurd-Markwick, K.; Zhang, X.X.; Mutukumira, A.N. Kombucha: Production and Microbiological Research. *Foods* **2022**, *11*, 1-18. https://doi.org/10.3390/foods11213456.
- 17. Antolak, H.; Piechota, D.; Kucharska, A. Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). *Antioxidants* **2021**, *10*, 1-20.
- 18. Villarreal-Soto, S.A.; Beaufort, S.; Bouajila, J.; Souchard, J.P.; Taillandier, P. Understanding Kombucha Tea Fermentation: A Review. *J. Food Sci.* **2018**, *83*, 580–588. https://doi.org/10.1111/1750-3841.14068.
- 19. Moreno, J.; Peinado, R. Sugars in Must. In *Enological Chemistry*; Elsevier. 2012; pp. 95–107. https://doi.org/10.1016/b978-0-12-388438-1.00007-8.
- Markov, S.; Jerinic, V.; Cvetkovic, D.; Loncar, E.; Malbasa, R. Kombucha Functional Beverage: Composition, Characteristics and Process of Biotransformation. Hem. Ind. 2003, 57, 456–462. https://doi.org/https://doi.org/10.2298/HEMIND0310456S.
- 21. Marsh, A.J.; O'Sullivan, O.; Hill, C.; Ross, R.P.; Cotter, P.D. Sequence-Based Analysis of the Bacterial and Fungal Compositions of Multiple Kombucha (Tea Fungus) Samples. *Food Microbiol.* **2014**, *38*, 171–178. https://doi.org/10.1016/j.fm.2013.09.003.
- 22. Gaggìa, F.; Baffoni, L.; Galiano, M.; Nielsen, D.S.; Jakobsen, R.R.; Castro-Mejía, J.L.; Bosi, S.; Truzzi, F.; Musumeci, F.; Dinelli, G.; et al. Kombucha Beverage from Green, Black and Rooibos Teas: A Comparative Study Looking at Microbiology, Chemistry and Antioxidant Activity. *Nutrients* **2019**, *11*, 1-22. https://doi.org/10.3390/nu11010001.
- Villarreal-Soto, S.A.; Bouajila, J.; Pace, M.; Leech, J.; Cotter, P.D.; Souchard, J.P.; Taillandier, P.; Beaufort, S. Metabolome-Microbiome Signatures in the Fermented Beverage, Kombucha. *Int. J. Food Microbiol.* 2020, 333, 1-13. https://doi.org/10.1016/j.ijfood-micro.2020.108778.
- 24. Coton, M.; Pawtowski, A.; Taminiau, B.; Burgaud, G.; Coulloume-Labarthe, L.; Daube, G.; Coton, E. Unravelling Microbial Ecology of Industrial-Scale Kombucha Fermentations by Metabarcoding and Culture Based Methods. *FEMS Microbiol. Ecol.* **2017**, 93, 1-16.
- 25. Teoh, A.L.; Heard, G.; Cox, J. Yeast Ecology of Kombucha Fermentation. *Int. J. Food Microbiol.* **2004**, *95*, 119–126. https://doi.org/10.1016/j.ijfoodmicro.2003.12.020.
- 26. Watawana, M.I.; Jayawardena, N.; Gunawardhana, C.B.; Waisundara, V.Y. Health, Wellness, and Safety Aspects of the Consumption of Kombucha. *J. Chem.* **2015**, 2015, 1-11. https://doi.org/10.1155/2015/591869.
- 27. Mayser, P.; Fromme, S.; Leitzmann, C.; Grunder, K. The Yeast Spectrum of the 'tea Fungus Kornbucha'. *Mycoses* **1995**, *38*, 289–295.
- 28. Herrera, T.; Calderon Villagomez, A. Species of Yeasts Isolated in Mexico from the Tea Fungus. Rev. Mex. Micol. 1989, 5, 205–210
- 29. Yamada, Y.; Yukphan, P.; Thi, H.; Vu, L.; Muramatsu, Y.; Ochaikul, D.; Tanasupawat, S.; Nakagawa, Y. Short Communication Description of *Komagataeibacter* Gen. Nov., with Proposals of New Combinations (*Acetobacteraceae*). J. Gen. Appl. Microbiol 2012, 58, 397–404.
- 30. Greenwalt, C.J.; Steinkraus, K.H.; Ledford, R.A. Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects. *J. Food Prot.* **2000**, *63*, 976–981. Available online: http://meridian.allenpress.com/jfp/article-pdf/63/7/976/1671742/0362-028x-63_7_976.pdf (accessed on 3 April 2023).
- 31. Dutta, D.; Gachhui, R. Novel Nitrogen-Fixing Acetobacter Nitrogenifigens Sp. Nov., Isolated from Kombucha Tea. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 1899–1903. https://doi.org/10.1099/ijs.0.64101-0.
- 32. Dutta, D.; Gachhui, R. Nitrogen-Fixing and Cellulose-Producing *Gluconacetobacter kombuchae* Sp. Nov., Isolated from Kombucha Tea. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 353–357. https://doi.org/10.1099/ijs.0.64638-0.
- 33. Watawana, M.I.; Jayawardena, N.; Gunawardhana, C.B.; Waisundara, V.Y. Enhancement of the Antioxidant and Starch Hydrolase Inhibitory Activities of King Coconut Water (Cocos Nucifera Var. Aurantiaca) by Fermentation with Kombucha "Tea Fungus". *Int. J. Food Sci. Technol.* **2016**, *51*, 490–498. https://doi.org/10.1111/ijfs.13006.

34. Kluz, M.I.; Pietrzyk, K.; Pastuszczak, M.; Kacaniova, M.; Kita, A.; Kapusta, I.; Zaguła, G.; Zagrobelna, E.; Struś, K.; Marciniak-Lukasiak, K.; et al. Microbiological and Physicochemical Composition of Various Types of Homemade Kombucha Beverages Using Alternative Kinds of Sugars. *Foods* **2022**, *11*, 1-15. https://doi.org/10.3390/FOODS11101523.

- 35. Yang, J.; Lagishetty, V.; Kurnia, P.; Henning, S.M.; Ahdoot, A.I.; Jacobs, J.P. Microbial and Chemical Profiles of Commercial Kombucha Products. *Nutrients* **2022**, *14*, 1-16. https://doi.org/10.3390/nu14030670.
- 36. Nyhan, L.M.; Lynch, K.M.; Sahin, A.W.; Arendt, E.K. Advances in Kombucha Tea Fermentation: A Review. *Appl. Microbiol.* **2022**, *2*, 73–101. https://doi.org/10.3390/applmicrobiol2010005.
- 37. Liu, C.; Hsu, W.; Lee, F.; Liao, C. The Isolation and Identification of Microbes from a Fermented Tea Beverage, Haipao, and Their Interactions during Haipao Fermentation. *Food Microbiol.* **1996**, *13*, 407–415.
- 38. Mohite, B.V.; Patil, S.V. A Novel Biomaterial: Bacterial Cellulose and Its New Era Applications. *Biotechnol. Appl. Biochem.* **2014**, *61*, 101–110. https://doi.org/10.1002/bab.1148.
- 39. Lahiri, D.; Nag, M.; Dutta, B.; Dey, A.; Sarkar, T.; Pati, S.; Edinur, H.A.; Kari, Z.A.; Noor, N.H.M.; Ray, R.R. Bacterial Cellulose: Production, Characterization and Application as Antimicrobial Agent. *Int. J. Mol. Sci.* **2021**, 22, 1-18. https://doi.org/10.3390/ijms222312984.
- 40. Shoda, M.; Sugano, Y. Recent Advances in Bacterial Cellulose Production. Biotechnol. Bioprocess Eng. 2005, 10, 1–8.
- 41. Tan, L.; Ren, L.; Cao, Y.; Chen, X.; Tang, X. Bacterial Cellulose Synthesis in Kombucha by *Gluconacetobacter* Sp and *Saccharomyces* Sp. *Adv. Mater. Res.* **2012**, *554*–*556*, 1000–1003. https://doi.org/10.4028/www.scientific.net/AMR.554-556.1000.
- 42. Chawla, P.R.; Bajaj, I.B.; Survase, S.A.; Singhal, R.S. Microbial Cellulose: Fermentative Production and Applications. *Food Technol. Biotechnol.* 2009, 42, 107–124.
- 43. Yu, X.; Atalla, R.H. Production of Cellulose II by Acetobacter Xylinum in the Presence of 2,6-Dichlorobenzonitrile. *Int. J. Biol. Macromol.* **1996**, *19*, 145–146.
- 44. Jones, D.; Ormondroyd, G.O.; Curling, S.F.; Popescu, C.-M.; Popescu, M.-C. Chemical Compositions of Natural Fibres. In *Advanced High Strength Natural Fibre Composites in Construction*; Fan, M., Fu, F., Eds.; Matthew Deans: Oxford, England, 2017; pp. 23–58. https://doi.org/10.1016/B978-0-08-100411-1.00002-9.
- 45. Overbeek, R.; Olson, R.; Pusch, G.D.; Olsen, G.J.; Davis, J.J.; Disz, T.; Edwards, R.A.; Gerdes, S.; Parrello, B.; Shukla, M.; et al. The SEED and the Rapid Annotation of Microbial Genomes Using Subsystems Technology (RAST). *Nucleic Acids Res.* **2014**, 42, 206–214. https://doi.org/10.1093/nar/gkt1226.
- 46. Ross, P.; Mayer, R.; Benziman, M. Cellulose Biosynthesis and Function in Bacteria. Microbiol. Rev. 1991, 55, 35-58.
- 47. Goh, W.; Rosma, A.; Kaur, B.; Fazilah, A.; Karim, A.A.; Bhat, R. Fermentation of Black Tea Broth (Kombucha): I. Effects of Sucrose Concentration and Fermentation Time on the Yield of Microbial Cellulose. *Int. Food Res. J.* **2012**, *19*, 109–117.
- 48. Yim, S.M.; Song, J.E.; Kim, H.R. Production and Characterization of Bacterial Cellulose Fabrics by Nitrogen Sources of Tea and Carbon Sources of Sugar. *Process Biochem.* **2017**, *59*, 26–36. https://doi.org/10.1016/j.procbio.2016.07.001.
- 49. Gargey, I.A.; Indira, D.; Jayabalan, R.; Balasubramanian, P. Optimization of Etherification Reactions for Recycling of Tea Fungal Biomass Waste into Carboxymethylcellulose. In *Green Buildings and Sustainable Engineering*; Springer Transactions in Civil and Environmental Engineering; Drück, H., Pillai, R., Tharian, M., Majeed, A., Eds.; Springer: Singapore, 2019; pp. 337–346. https://doi.org/10.1007/978-981-13-1202-1_29.
- 50. Kurtzman, C.P.; Robnett, C.J.; Basehoar-Powers, E. Zygosaccharomyces Kombuchaensis, a New Ascosporogenous Yeast from "Kombucha Tea". FEMS Yeast Res. **2001**, *1*, 133–138. https://doi.org/10.1016/S1567-1356(01)00021-6.
- 51. Amarasekara, A.S.; Wang, D.; Grady, T.L. A Comparison of Kombucha SCOBY Bacterial Cellulose Purification Methods. *SN Appl. Sci.* **2020**, *2*, 7. https://doi.org/10.1007/s42452-020-1982-2.
- 52. Dima, S.O.; Panaitescu, D.M.; Orban, C.; Ghiurea, M.; Doncea, S.M.; Fierascu, R.C.; Nistor, C.L.; Alexandrescu, E.; Nicolae, C.A.; Trica, B.; et al. Bacterial Nanocellulose from Side-Streams of Kombucha Beverages Production: Preparation and Physical-Chemical Properties. *Polymers* **2017**, *9*, 1-24. https://doi.org/10.3390/polym9080374.
- Ramírez Tapias, Y.A.; Peltzer, M.A.; Delgado, J.F.; Salvay, A.G. Kombucha Tea By-Product as Source of Novel Materials: Formulation and Characterization of Films. Food Bioprocess Technol. 2020, 13, 1166–1180. https://doi.org/10.1007/s11947-020-02471-4.
- 54. Muhialdin, B.J.; Voon, W.; Shobirin, A.; Hussin, M. Effects of Sugar Sources and Fermentation Time on the Properties of Tea Fungus (Kombucha) Beverage. *Artic. Int. Food Res. J.* **2019**, *26*, 481–487.
- 55. Mizzi, L.; Maniscalco, D.; Gaspari, S.; Chatzitzika, C.; Gatt, R.; Valdramidis, V.P. Assessing the Individual Microbial Inhibitory Capacity of Different Sugars against Pathogens Commonly Found in Food Systems. *Lett. Appl. Microbiol.* **2020**, *71*, 251–258. https://doi.org/10.1111/lam.13306.
- 56. Chen, C.; Liu, B.Y. Changes in Major Components of Tea Fungus Metabolites during Prolonged Fermentation. *J. Appl. Microbiol.* **2000**, *89*, 834–839.
- 57. Pou, K.R.J. Fermentation: The Key Step in the Processing of Black Tea. *J. Biosyst. Eng.* **2016**, 41, 85–92. https://doi.org/10.5307/JBE.2016.41.2.085.
- 58. Chacko, S.M.; Thambi, P.T.; Kuttan, R.; Nishigaki, I. Beneficial Effects of Green Tea: A Literature Review. *Chin. Med.* **2010**, *5*, 1-9. https://doi.org/10.1186/1749-8546-5-13.

59. Joubert, E.; Schulz, H. Production and Quality Aspects of Rooibos Tea and Related Products. A Review. *J. Appl. Bot. Food Qual.* **2006**, *80*, 138–144.

- 60. Butt, M.S.; Imran, A.; Sharif, M.K.; Ahmad, R.S.; Xiao, H.; Imran, M.; Rsool, H.A. Black Tea Polyphenols: A Mechanistic Treatise. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 1002–1011. https://doi.org/10.1080/10408398.2011.623198.
- 61. Wang, K.; Gan, X.; Tang, X.; Wang, S.; Tan, H. Determination of D-Saccharic Acid-1,4-Lactone from Brewed Kombucha Broth by High-Performance Capillary Electrophoresis. *J. Chromatogr. B* **2010**, *878*, 371–374. https://doi.org/10.1016/j.jchromb.2009.12.003.
- 62. Wei, W.; Bao-Chuan, G.; Bao-Ping, J. D-Glucaric Acid and Other Metabolites in Kombucha. Food Sci. 2004, 25, 147–151.
- 63. Bauer-Petrovska, B.; Petrushevska-Tozi, L. Mineral and Water Soluble Vitamin Content in the Kombucha Drink. *Int. J. Food Sci. Technol.* **2000**, *35*, 201–205.
- 64. Bhattacharya, S.; Gachhui, R.; Sil, P.C. The Prophylactic Role of D-Saccharic Acid-1,4-Lactone against Hyperglycemia-Induced Hepatic Apoptosis via Inhibition of Both Extrinsic and Intrinsic Pathways in Diabetic Rats. *Food Funct.* **2013**, *4*, 283–296. https://doi.org/10.1039/c2fo30145h.
- 65. Martínez-Leal, J.; Ponce-García, N.; Escalante-Aburto, A. Recent Evidence of the Beneficial Effects Associated with Glucuronic Acid Contained in Kombucha Beverages. *Curr. Nutr. Rep.* **2020**, *9*, 163–170. https://doi.org/10.1007/s13668-020-00312-6.
- 66. Vīna, I.; Linde, R.; Patetko, A.; Semjonovs, P. Glucuronic Acid from Fermented Beverages: Biochemical Functions in Humans and Its Role in Health Protection. *IJRRAS* **2013**, *14*, 217–230.
- 67. Murugesan, G.S.; Sathishkumar, M.; Jayabalan, R.; Binupriya, A.R.; Swaminathan, K.; Yun, S.E. Hepatoprotective and Curative Properties of Kombucha Tea against Carbon Tetrachloride-Induced Toxicity. *J. Microbiol. Biotechnol.* **2009**, *19*, 397–402. https://doi.org/10.4014/jmb.0806.374.
- 68. Abshenas, J.; Derakhshanfar, A.; Ferdosi, M.H.; Hasanzadeh, S. Protective Effect of Kombucha Tea against Acetaminophen-Induced Hepatotoxicity in Mice: A Biochemical and Histopathological Study. *Comp. Clin. Path.* **2012**, *21*, 1243–1248. https://doi.org/10.1007/s00580-011-1273-9.
- 69. Bhattacharya, S.; Gachhui, R.; Sil, P.C. Hepatoprotective Properties of Kombucha Tea against TBHP-Induced Oxidative Stress via Suppression of Mitochondria Dependent Apoptosis. *Pathophysiology* **2011**, *18*, 221–234. https://doi.org/10.1016/j.pathophys.2011.02.001.
- 70. Rasouli, L.; Aryaeian, N.; Gorjian, M.; Nourbakhsh, M.; Amiri, F. Evaluation of Cytotoxicity and Anticancer Activity of Kombucha and Doxorubicin Combination Therapy on Colorectal Cancer Cell Line HCT-116. *J. Educ. Health Promot.* **2021**, *10*, 1-8. https://doi.org/10.4103/jehp.jehp_1456_20.
- 71. Aloulou, A.; Hamden, K.; Elloumi, D.; Ali, M.B.; Hargafi, K.; Jaouadi, B.; Ayadi, F.; Elfeki, A.; Ammar, E. Hypoglycemic and Antilipidemic Properties of Kombucha Tea in Alloxan-Induced Diabetic Rats. *BMC Complement. Altern. Med.* **2012**, *12*, 1-9.
- 72. Kapp, J.M.; Sumner, W. Kombucha: A Systematic Review of the Empirical Evidence of Human Health Benefit. *Ann. Epidemiol.* **2019**, *30*, 66–70. https://doi.org/10.1016/j.annepidem.2018.11.001.
- 73. Murphy, T.E.; Walia, K.; Farber, J.M. Safety Aspects and Guidance for Consumers on the Safe Preparation, Handling and Storage of Kombucha-A Fermented Tea Beverage. *Food Prot. Trends* **2018**, *38*, 329–337.
- 74. Bintsis, T. Foodborne Pathogens. AIMS Microbiol. 2017, 3, 529–563. https://doi.org/10.3934/microbiol.2017.3.529.
- 75. Marroquín-Cardona, A.G.; Johnson, N.M.; Phillips, T.D.; Hayes, A.W. Mycotoxins in a Changing Global Environment—A Review. *Food Chem. Toxicol.* **2014**, *69*, 220–230. https://doi.org/10.1016/j.fct.2014.04.025.
- 76. Lokunarangodage, C.; Wickramasinghe, I.; Ranaweera, K.K.D.S. Impact of HACCP Based Food Safety Management Systems in Improving Food Safety of Sri Lankan Tea Industry. *J. Tea Sci. Res.* **2016**, *6*, 1–16. https://doi.org/10.5376/jtsr.2016.06.0006.
- 77. Phan, T.G.; Estell, J.; Duggin, G.; Beer, I.; Smith, D.; Ferson, M.J. Lead Poisoning from Drinking Kombucha Tea Brewed in a Ceramic Pot. *Med. J. Aust.* **1998**, *169*, 644–646. https://doi.org/10.5694/j.1326-5377.1998.tb123448.x.
- 78. Tejedor-Calvo, E.; Morales, D. Chemical and Aromatic Changes during Fermentation of Kombucha Beverages Produced Using Strawberry Tree (Arbutus Unedo) Fruits. *Fermentation* **2023**, *9*, 1-14. https://doi.org/10.3390/fermentation9040326.
- 79. Greenwalt, C.J.; Ledford, R.A.; Steinkraus, K.H. Determination and Characterization of the Antimicrobial Activity of the Fermented Tea Kombucha. *LWT-Food Sci. Technol.* **1998**, *31*, 291–296.
- 80. WEBMD. Webmd.Com_BLACK TEA: Overview, Uses, Side Effects, Precautions, Interactions, Dosing and Reviews. Available online: https://www.webmd.com>vitamins>ingredientmono-997 (accessed on 4 April 2023).
- 81. Morton, J.F. Rooibos Tea, *Aspalathus linearis*, a Caffeineless, Low-Tannin Beverage 1 The Plant Is of Very Limited Distribution, Occurring Naturally Only in the Western Districts of Cape Province, Particularly the Cedarberg Mountains and Higher Areas. *Econ. Bot.* **1983**, *37*, 164–173.
- 82. Joubert, E.; Gelderblom, W.C.A.; De Beer, D. Phenolic Contribution of South African Herbal Teas to a Healthy Diet. *Nat. Prod. Commun.* **2009**, *4*, 701-718.
- 83. Joubert, E.; de Beer, D. Rooibos (*Aspalathus linearis*) beyond the Farm Gate: From Herbal Tea to Potential Phytopharmaceutical. *S. Afr. J. Bot.* **2011**, 77, 869–886. https://doi.org/10.1016/j.sajb.2011.07.004.
- 84. Hillis, W.E.; Inoue, T. The Polyphenols of Nothofagus Species-II. The Heartwood of Nothofagus Fusca. *Phytochemistry* **1967**, *6*, 59–67. https://doi.org/10.1016/0031-9422(67)85008-8.

85. Huang, C.F.; Gan, X.W.; Bai, H.Y.; Ma, L.; Hu, L.H. Schoepfin A, B, C: Three New Chalcone C-Glycosides from Schoepfia Chinensis. *Nat. Prod. Res.* **2008**, 22, 623–627. https://doi.org/10.1080/14786410701614184.

- 86. Krafczyk, N.; Glomb, M.A. Characterization of Phenolic Compounds in Rooibos Tea. *J. Agric. Food Chem.* **2008**, *56*, 3368–3376. https://doi.org/10.1021/jf703701n.
- 87. Bramati, L.; Minoggio, M.; Gardana, C.; Simonetti, P.; Mauri, P.; Pietta, P. Quantitative Characterization of Flavonoid Compounds in Rooibos Tea (*Aspalathus linearis*) by LC-UV/DAD. *J. Agric. Food Chem.* **2002**, *50*, 5513–5519. https://doi.org/10.1021/jf025697h.
- 88. CARMIEN. Rooibos and Your Mental Health; CARMIEN: Citrusdal, South Africa, 2023; pp. 1–5. Available online: https://carmientea.co.za/rooibos-tea-and-your-mental-health-stress-anxiety (accessed on 4 April 2023).
- 89. Marnewick, J.L.; Rautenbach, F.; Venter, I.; Neethling, H.; Blackhurst, D.M.; Wolmarans, P.; MacHaria, M. Effects of Rooibos (*Aspalathus linearis*) on Oxidative Stress and Biochemical Parameters in Adults at Risk for Cardiovascular Disease. *J. Ethnopharmacol.* **2011**, 133, 46–52. https://doi.org/10.1016/j.jep.2010.08.061.
- 90. Marnewick, J.L.; Gelderblom, W.C.A.; Joubert, E. An Investigation on the Antimutagenic Properties of South African Herbal Teas. *Mutat. Res.-Genet. Toxicol. Environ. Mutagen.* **2000**, *471*, 157–166. https://doi.org/10.1016/S1383-5718(00)00128-5.
- 91. Ulicna, O.; Vancova, O.; Waczulikova, I.; Bozek, P.; Janega, P.; Babal, P.; Liskova, S.; Greksak, M. Does Rooibos Tea (*Aspalathus linearis*) Support Regeneration of Rat Liver after Intoxication by Carbon Tetrachloride? *Gen. Physiol. Biophys.* **2008**, 27, 179–186.
- 92. Ajuwon, O.R.; Katengua-Thamahane, E.; Van Rooyen, J.; Oguntibeju, O.O.; Marnewick, J.L. Protective Effects of Rooibos (*Aspalathus linearis*) and/or Red Palm Oil (*Elaeis guineensis*) Supplementation on Tert -Butyl Hydroperoxide-Induced Oxidative Hepatotoxicity in Wistar Rats. *Evid.-Based Complement. Altern. Med.* 2013, 2013, 19. https://doi.org/10.1155/2013/984273.
- 93. Uličná, O.; Vančová, O.; Božek, P.; Čársky, J.; Šebeková, K.; Boor, P.; Nakano, M.; Greksák, M. Rooibos Tea (*Aspalathus linearis*) Partially Prevents Oxidative Stress in Streptozotocin-Induced Diabetic Rats. *Physiol. Res.* **2006**, *55*, 157–164. https://doi.org/10.33549/physiolres.930778.
- 94. Sasaki, Y.F.; Yamada, H.; Shimoi, K.; Kator, K.; Kinae, N. The Clastogen-Suppressing Effects of Green Tea, Po-Lei Tea and Rooibos Tea in CHO Cells and Mice. *Mutat. Res.* 1993, 286, 221–232.
- 95. Ajuwon, O.R.; Oguntibeju, O.O.; Marnewick, J.L. Amelioration of Lipopolysaccharide-Induced Liver Injury by Aqueous Rooibos (*Aspalathus linearis*) Extract via Inhibition of pro-Inflammatory Cytokines and Oxidative Stress. *BMC Complement. Altern. Med.* 2014, 14, 392. https://doi.org/10.1186/1472-6882-14-392.
- 96. Hopfe, S.; Flemming, K.; Lehmann, F.; Möckel, R.; Kutschke, S.; Pollmann, K. Leaching of Rare Earth Elements from Fluorescent Powder Using the Tea Fungus Kombucha. *Waste Manag.* **2017**, *62*, 211–221. https://doi.org/10.1016/j.wasman.2017.02.005.
- 97. Aduri, P.; Ankita Rao, K.; Fatima, A.; Kaul, P.; Shalini, A. Study of Biodegradable Packaging Material Produced from SCOBY. *Life Sci. Inform. Publ.* **2019**, *5*, 389–404. https://doi.org/10.26479/2019.0503.32.
- 98. Kamiński, K.; Jarosz, M.; Grudzień, J.; Pawlik, J.; Zastawnik, F.; Pandyra, P.; Kołodziejczyk, A.M. Hydrogel Bacterial Cellulose: A Path to Improved Materials for New Eco-Friendly Textiles. *Cellulose* **2020**, *27*, 5353–5365. https://doi.org/10.1007/s10570-020-03128-3.
- 99. Najafpour, A.; Rajabi Khorrami, A.; Aberoomand Azar, P.; Saber Tehrani, M. Study of Heavy Metals Biosorption by Tea Fungus in Kombucha Drink Using Central Composite Design. *J. Food Compos. Anal.* **2020**, *86*, 1-9. https://doi.org/10.1016/j.jfca.2019.103359.
- 100. Murugesan, G.S.; Sathishkumar, M.; Swaminathan, K. Supplementation of Waste Tea Fungal Biomass as a Dietary Ingredient for Broiler Chicks. *Bioresour. Technol.* **2005**, *96*, 1743–1748. https://doi.org/10.1016/j.biortech.2005.01.006.
- 101. Kruk, M.; Trząskowska, M.; Ścibisz, I.; Pokorski, P. Application of the "Scoby" and Kombucha Tea for the Production of Fermented Milk Drinks. *Microorganisms* **2021**, *9*, 1-17. https://doi.org/10.3390/microorganisms9010123.
- 102. Zhu, C.; Li, F.; Zhou, X.; Lin, L.; Zhang, T. Kombucha-Synthesized Bacterial Cellulose: Preparation, Characterization, and Biocompatibility Evaluation. *J. Biomed. Mater. Res.* **2014**, *102*, 1548–1557. https://doi.org/10.1002/jbm.a.34796.
- 103. Shanmugavel, M.; Nandhini, N.; Supriya, B.; Vasantharaj, S.; Inbasekaran, S.; Gnanamani, A. Kombucha Fungus Mediated Silver Nanoparticles and Their Biological Activities. *Int. J. Appl. Bioeng.* **2017**, *11*, 22–26.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.