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Kombucha Tea: Microorganisms Communities' Composition, Active Metabolites, Health Functions and Fermentation

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Abstract. As a tea drinking product, Kombucha is made from sugar, water and tea as raw materials and fermented by a variety of probiotics and contains diverse metabolites derived from active microorganisms. As kombucha contains a variety of microorganisms, active ingredients, and uses a fermentation process, it has a wide range of health care functions such as antioxidant, liver protection, cardiovascular and cerebrovascular, and anti-cancer, and thus has become the focus of people all over the world. As a result, in order to better exert the health as well as commercial value of kombucha tea product, it is necessary to carry out research on the diversity of microorganisms in kombucha, active substance's types and functions, and their relationship with the fermentation process. In order to further improve the flavor and health functions of this tea product and lay a solid foundation for the industrial production of Kombucha from the theoretical level, this thesis will provide a comprehensive analysis and summary of the latest research on microbial community's composition, active metabolites, health efficacy and fermentation process factors of kombucha tea.

Keywords: fermented foods, kombucha tea, fermentation microorganism, active metabolites

1. Introduction

Recently, as a beverage product, kombucha products originated in the Bohai region of China have become popular around the world [1]. Kombucha made from sugar, water and tea as raw materials and fermented by a variety of probiotics. During the fermentation process, the communities is easily affected by factors such as temperature, and the mechanism of this effect is complex. The fermentation of kombucha consists of fermentation broth and biofilm. The main fermentation products include a large number of biologically active substances such as lactic acid and amino acids [2]. These active substances have health effects such as anti-cancer, improving human digestive function, delaying oxidation, improving human immunity, and inhibiting intestinal pathogens [3], which have attracted the attention of people from all over the world. This article will make a brief introduction about microorganisms' communities and functional active substances in this tea product with its health care function as well as fermentation process microbial community composition, active metabolites, health benefits and fermentation process factors.

2. Kombucha tea's microbial composition

Recently, as the key to explore the health characteristics of Kombucha, the microbial composition of this tea product has been widely studied. This tea product is made from sugar, water and tea as raw

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materials and fermented by a variety of probiotics and contains diverse metabolites derived from active microorganisms. Fermentation broth is fermented by a variety of microorganisms derived from tea leaves and sugar solutions. With the continuous fermentation of microorganisms, the liquid gradually becomes clear and bright. Bacterial membrane attached to the liquid air interface is a kind of milky white membrane, which is formed by symbiotic bacteria [4]. The fermentation stage mainly includes the symbiotic fermentation of a variety of probiotics. As shown in Figure 1, it is the specific fermentation process of black tea fungus. There are some differences between fermentation broth and bacterial membrane, whether in microbial species or dominant microorganisms [3]. There is no doubt that acetic acid bacteria and yeast are present in kombucha; Nevertheless, they are not always detected. However, research results indicate that adding lactic acid bacteria to kombucha during the fermentation process can increase the production of lactic acid and glucuronic acid, making kombucha with stronger antioxidant and antibacterial properties [4, 5]. What's more, microbial flora has a complex and changeable structure, which will be affected by fermentation temperature and time, tea type, as well as sugar concentration [6]. Table 1 summarizes the latest findings on microbial bustards in this tea. Gluconobacter, etc. is the most common genera of acetate bacteria. Lactoba cillus nagelli, etc. is the most common form of lactic acid bacteria. There are many kinds of fungi, and Candida are very common to see [3]. In addition, these findings may also help people better understand the succession process of kombucha microbes. Ethanol is produced by Yeast through the glycolytic pathway. With the continuous accumulation of ethanol, it will prohibit the growth of lactic acid bacteria and accelerate the growth of acetic acid bacteria after reaching a certain concentration. Under aerobic conditions, acetic acid bacteria use ethanol to produce acetic acid. When the acetic acid accumulates continuously and reaches a certain concentration, it will inhibit the growth of yeast. The mutual inhibition and acceleration of each flora will determine the quality of kombucha [7].

It should be noted that the research methods can be divided into two types when studying the microbial structure in kombucha: that is culturable analysis methods and uncultivable analysis methods. The first method refers to a traditional method using plates for culture, purification as well as further analysis. Nevertheless, considering that some microorganisms are difficult to cultivate or not suitable for this method, this method cannot safely identify the complex microbial community structure in this tea. The second method has a certain dependence on DNA, which can improve the defects of the first method. It adopts a number of technologies such as high-throughput sequencing technology. The first method generally mixes bacterial membrane and fermentation broth for selection, and the second method analyzes them separately, which can establish a clearer understanding of the fermentation process, and can understand the functions of various microorganisms in the fermentation stage and changes in fermentation broth. The first method was mainly used to study the structure of the microbiota when scholars conducted preliminary research on kombucha [3]. In recent years, a growing number of teams have adopted the second method, or combined these two methods, conducting in-depth studies of the diversity of kombucha' microbial communities.

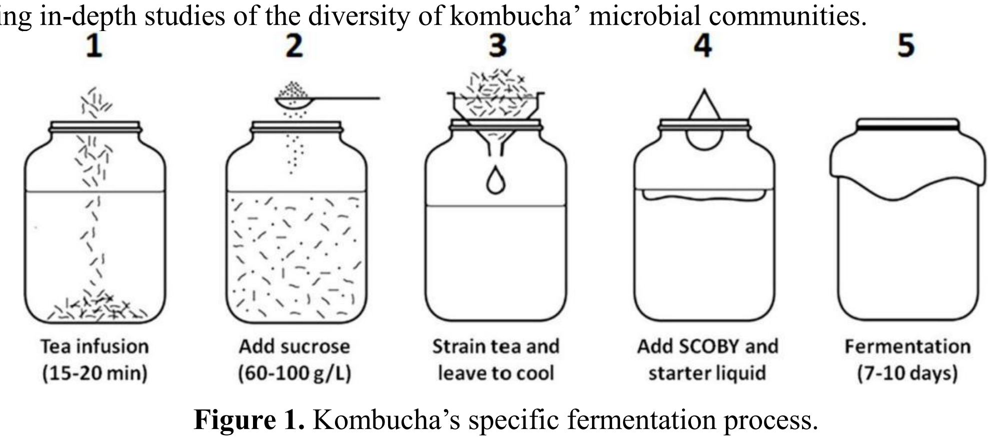

Figure 1. Kombucha's specific fermentation process.

Table 1. Summary of latest research results on microbial communities found in Kombucha after fermentation (continue).

<table>
<tr><th colspan="2">Method</th><th>Bacteria</th><th>Yeast</th><th>Source</th></tr>
<tr><td colspan="2" rowspan="9">Culturable analysis method</td><td>Komagataeibacter intermedius</td><td>Brettanomyces bruxellensis
Metschnikowia fructicola
Zygosaccharomyces bisporus</td><td>Liquid/biofilm</td></tr>
<tr><td>Komagataeibacter rhaeticus</td><td></td><td>Biofilm</td></tr>
<tr><td>Gluconacetobacter intermedius KN89</td><td>Dekkera bruxellensis KN89</td><td>Liquid/biofilm</td></tr>
<tr><td>Acetobacter tropicalis
Gluconacetobacter intermedius</td><td>Brettanomyces bruxellensis
Zygosaccharomyces bisporu</td><td>Liquid/biofilm</td></tr>
<tr><td>Bacillus cereus</td><td>Zygosaccharomyces rouxii ZSR2</td><td>Liquid</td></tr>
<tr><td>Komagataeibacter saccharivorans
Gluconacetobacter europaeus
Acetobacter aceti
Lactobacillus fermentum</td><td>Saccharomyces cerevisiae
Arxula adeninivorans</td><td>Liquid</td></tr>
<tr><td>Acetobacter xylinum
Acetobacter liquefaciens
Acidomonas methanolica
Lactobacillus</td><td>Saccharomyces cerevisiae
Schizosaccharomyces pombe</td><td>Liquid/biofilm</td></tr>
<tr><td>Gluconobacter entanii
Komagataeibacter rhaeticus
Komagataeibacter saccharivorans</td><td>Brettanomyces bruxellensis
Zygosaccharomyces parabailli</td><td>Liquid</td></tr>
<tr><td>Komagataeibacter intermedius</td><td>Brettanomyces bruxellensis
Zygosaccharomyces parabailli</td><td>Biofilm</td></tr>
<tr><td rowspan="2">Unculturable analysis</td><td rowspan="2">16S rRNA, high-throughput sequencing technology</td><td>Lactobacillus
Acetobacter
Gluconacetobacter sp.</td><td>Zygosaccharomyces lentus
Zygosaccharomyces bisporus
Dekkera bruxellensis</td><td>Liquid</td></tr>
<tr><td>Gluconacetobacter sp.</td><td>Zygosaccharomyces lentus</td><td>Biofilm</td></tr>
</table>

			Zygosaccharomyces bisporus Dekkera bruxellensis	
	16S rRNA 26S rRNA	Acetobacter indonesiensis Acetobacter papaya Komagataeibacter saccharivorans	Dekkera bruxellensis Hanseniaspora valbyensis Saccharomyces cerevisiae	Liquid
			Hanseniaspora opuntiae Pichia aff Galactomyces geotrichum	Biofilm
	Whole genome sequencing (WMS) and amplicons	Komagataeibacter sp.	Zygosaccharomyces sp.	Liquid/biofilm
	Metagenomics Sequencing	Gluconacetobacter sp. SXCC-1 Komagataeibacter medellinensis Komagataeibacter xylinus		Liquid/biofilm
	Oligonucleotide matching technology	Gluconacetobacter（Acetobacter）、G. xylinus、G. saccharivorans		Liquid/biofilm
	T-rflp analysis and high-throughput sequencing technology	Bifidobacterium Komagataeibacter sp. Gluconobacter sp.	Candida stellimalicola Candida tropicalis Lachancea fermentati	Liquid
		Komagataeibacter sp. Gluconobacter sp.	Candida stellimalicola Candida tropicalis Lachancea fermentati Meyerozyma guilliermondii	Biofilm

3. Active metabolites of kombucha tea

In the fermentation stage, kombucha will generate lots of metabolites, which are mainly divided into three fermentation stages of lactic acid, alcohol and acetic acid. In the preliminary fermentation stage, under the catalysis of invertase, the yeast converts sucrose into glucose and fructose, and in the glycolysis stage, the monosaccharide is converted into ethanol and carbon dioxide. Under the oxidation of ethanol, acetic acid bacteria will further generate acetic acid and acetaldehyde, and the glucose will undergo oxidation reaction to generate gluconic acid as well as glucuronic acid [4]. At the same time, fructose will be converted into glucose 6-phosphate through fructose and phosphoglucose isomerase by the acetic acid bacteria K. Xilinus, thereby producing cellulose [2]. What's more, sugar can be used to generate lactic acid by lactic acid bacteria which can significantly reduce the pH value of fermentation broth and play a role in inhibiting bacteria and promoting human digestion. During the fermentation stage, kombucha will not only generate organic acids, but also generate amino acids, DSL, and other active substances. Table 2 makes a summary about the main metabolically active substances detected in the latest study. In the fermentation process, there is a close relationship between the proportion of these metabolites, the diversity of microorganisms, tea varieties, sugar concentration as well as fermentation conditions, etc., and plays a significant part in the formation of the color, fragrance, flavor, and health benefits of kombucha. In kombucha, glucuronic acid with antioxidant and detoxification effect is regarded as the most significant bioactive substance. It has been proven that it not only protects the liver, but also prevents unsaturated fatty acids from oxidation. In unfermented tea leaves, this compound was not detected. Nevertheless, its concentration will increase accordingly with the metabolism of AAB during the fermentation process. DSL is regarded as a principal compound that prohibits glucuronidase activity and is involved in tumorigenesis in an indirect manner [8, 9]. Moreover, it has been confirmed that DSL has a certain protective effect on the liver [4].

Table 2. Summary of bioactive compounds and their beneficial effects.

Categories	**Sub-categories**						
Organic acid	Acetic acid	Gluconic acid	Glucuronic acid	D-saccharic acid-1,4-lactone (DSL)	lactic, citric	ascorbic	succinic acids
Vitamins	Vitamin B1	Vitamin B2	Vitamin B6	Vitamin B12	Vitamin C		
Minerals	Cu, Fe, Mn, Ni, Zn	Co, Cu, Fe, Mn, Ni, Pb, Zn					
Ethanol				-			
Total polyphenols				-			

As a natural antioxidant, tea polyphenols have multiple health benefits which can reduce blood lipids, prevent mutations, and scavenge free radicals from the body [10]. The content of tea polyphenols will increase during the fermentation process and total polyphenols as well as flavonoids will increase in this process. This may be related to the degradation of microbial enzymes. Nevertheless, there were certain differences in the content change trends of some polyphenols during the fermentation process. For instance, there were certain differences in the content change trends of theaflavins and theocyanins. According to the research, the concentration of theaflavin and theacyanin will increase and decrease respectively in the process of fermentation [9]. Some scholars have also explained that some of the theocyanins will be transformed during the fermentation process to generate theaflavins [11]. As to some polyphenols common to see in green tea, in the process of fermentation, EGC and EC are elevated but EGCG and ECG are not. Some scholars suppose that EGCG is transformed into EGC and ECG is transformed into EC [3, 6].

It is well known that kombucha have a certain aroma, which is mainly derived from the aromatic substances produced by yeast [12]. It has been confirmed that the brewing process of kombucha also increase the concentration of water-soluble vitamins such as vitamin B1. In addition, studies have found that essential minerals such as Fe are also increased during the fermentation process [13].

4. Health benefits of kombucha tea

Kombucha produces active metabolites such as tea polyphenols during the fermentation process, which also explains why it is beneficial to human health. Next, this article will discuss the specific effects of active substances contained in kombucha on human health from various perspectives such as anti-oxidation.

4.1. Anti-oxidation

Tea polyphenols can scavenge free radicals from the body, and it is the source of the antioxidant effect of kombucha [8]. As an unsaturated electron-deficient species, free radicals gain electrons from surrounding molecules and create a chain reaction that results in cell damage or death. Polyphenols containing various reducing chemical groups can take part in the reaction to eliminate free radicals from the body. The research results indicate that kombucha's antioxidant activity continued to increase during the dispensing process [14]. During the fermentation stage, polyphenols' content in kombucha will continue to increase, and after degradation, the macromolecular polyphenol complex will gradually transform into simple small molecules with stronger absorption rate and better physiological activity [6, 9, 15]. Moreover, kombucha has been shown to have certain protective functions on different types of cells in the human body, such as human monocyte ThP-1. What's more, kombucha has the properties of antioxidant activity, and also enables kombucha to lower blood pressure and prevent cardiovascular as well as cerebrovascular diseases [6, 13]

4.2. Liver protection activity

People are usually exposed to various drugs, use a variety of food additives and are exposed to air pollution. The water solubility and polarity of these toxic compounds change during continuous biotransformation, which in turn excretes these substances from the body [3]. The liver is the main region where this detoxification process takes place, and this process can be divided into three stages. Firstly, toxins are modified into a toxic compound that dissolves more easily in water. Secondly, glucuronic acid forms a complex that is completely water-soluble when combined with toxins. Eventually, the substance is excreted [16]. Actually, people regard glucuronic acid as one of the main detoxification compounds in the body due to its ability to bind toxic compounds. During the fermentation process, kombucha produces this organic acid, so this explains why kombucha is beneficial to protect the liver [4, 9]. Scholars have carried out a number of studies including cell lines and animal models in this regard, in which kombucha can play a protective role against liver toxicity derived from a variety of toxic agents, such as CCl4, CdCl, TBHP, TAA, APAP or aflatoxin B1. In existing studies, major enzyme markers such as alanine aminotransferase have been observed, and these markers can reflect the specific amelioration of kombucha on hepatotoxicity [3]. Moreover, it has been confirmed that kombucha can effectively prevent and alleviate liver tissue damage in poisoned mice [17].

4.3. Inhibition of intestinal pathogenic bacteria

The generation of organic acid lower the pH of the beverage during the fermentation stage, thereby inhibiting bacterial growth. Kombucha sustained antimicrobial properties by the cytoplasmatic acidification and accumulation of dissociated anions to toxic levels, that ultimately leading to irreversible cell damage as well as death [3, 18]. Moreover, the bacteriostatic ability was also related to other metabolites during fermentation. Red grape juice was used as a substrate to ferment kombucha tested the inhibitory level of kombucha against seven pathogens. The test results indicated that beverage acidity and other metabolite biosynthesis during fermentation stage can also enhance Kombucha's antibacterial activity [19].

4.4. Anticancer activity

Cancer is the second leading cause of death worldwide next to cardiovascular disease, the number of cancer patients will increase by 19.3 million, and the number of cancer deaths will reach 10 million in 2020 [20]. Despite great progress in survival and other areas, a lot of research still needs to be done to prevent cancer, provide specific therapeutic care or conduct early diagnosis. Kombucha's cytotoxic effect on cancer cells derive from 2-dimethyl malonate and vitexin [21]. In comparison with non-cancer cell lines, kombucha extract was more effective in cancer cell lines. Antioxidant capacity of polyphenols will have certain influence on the anti-proliferation and anti-tumor effects of kombucha [22]. These substances have a certain scavenging effect on human free radicals and have a certain regulatory effect on the expression of various signaling molecules in cell proliferation [3].

5. Fermentation process of kombucha tea and improvement

In kombucha, the type of raw materials and fermentation conditions will have a certain impact on the function of functional microorganisms.

5.1. Raw materials
Black tea, green tea and rob tea are used as substrates to ferment kombucha and discovered that in comparison with kombucha prepared from green tea or black tea, kombucha prepared from rob tea without catechins had lower antioxidant activity. Nevertheless, in terms of glucuronic acid content, kombucha made from locust tea is equivalent to kombucha made from black tea and contains other compounds. Under oxidative stress, these substances can improve the recovery of oxidative damage induced by H2O2 in fibroblasts [6]. An investigation was made on the antioxidant as well as starch hydrolase inhibitory activities of 4 types of teas inoculated with "tea fungus" after fermentation. It is indicated that Sri Lanka black tea was greatly increased in terms of antioxidant activity, and the 4 fermented teas have a more significant inhibitory effect on α-amylase thanα-glucosidase. As a result, among 4 types of back tea, the best for preparing kombucha is Sri Lankan black tea [23]. What's more, the antioxidant as well as starch hydrolysis enzyme inhibition activity of kombucha prepared with different sugars instead of white sugar were also studied, and it was discovered that the inhibition activity effect is more obvious for brown sugar as well as glucose, and it is observed that the inhibition activity effect for honey and palmyra raw sugar did not change significant [24].

5.2. Fermentation conditions
To determine the best fermentation condition, the fermentation conditions are mainly improved in terms of fermentation temperature, time, inoculum and inoculum ratio. In the product evaluation stage, sensory evaluation, pH value, etc. are mainly used for evaluation [25]. To determine which conditions can be used to increase gluconic and glucuronic acid production, an investigation was made on the effect of two fermentation temperatures. It is concluded that there is no relationship between fermentation temperature and dominant bacterial communities' structure at the genus level, but there is a positive correlation between the two at the subgenus level, which is due to the existence of 2 different Gluconacetobacter species at the subgenus level which can accelerate the growth of some subdominant bacterial communities. At 30 ℃, the contents of 2 types of gluconacetobacteria was higher in fermented Kombucha [26]. Under RSM, the influences of tea content and other factors on metal adsorption in Kombucha is optimized by means of CCD and it was discovered that it had the effect of removing heavy metals such as Hg^{2+} [27].

5.3. Fermentation time
Using traditional techniques, kombucha needs to be fermented for 7d~60d. In the process of fermentation, the microbial community structure will change continuously in kombucha, and the content of metabolites will also rise and fall [28]. Analysis was made about the change of microbial community structure at 4 types of fermentation times and discovered that at 0,2 and 4d, t acetic acid bacteria in tea biofilms had higher abundance; at 8d, the abundance of biofilm and bacterial fluid reached equilibrium; During the whole fermentation process, there was no significant difference in the richness of the two [29]. During fermentation process of Kombucha, The change trend of polyphenols content and antioxidant activity was explored, and what is discovered that they increased significantly after fermentation for 7 days which can be attributed to the diverse microorganisms in kombucha [9]. Scholars have conducted a series of studies on the fermentation time of kombucha, and in the results obtained, there are significant differences in fermentation time, which can be attributed to the features and development of microbes' communities in Kombucha in various studies. It can be concluded that it is extremely important to define pairs of quality indicators to further improve the fermentation process and establish quality models according to the differences in matrix and the different indicators.

6. Conclusion
The thesis mainly summarizes the research results of microbial structure, active metabolites health care function and the process of fermentation. As it is above-mentioned, scholars mainly studied kombucha from the aspects of microbial structure, active metabolites health care function and the process of fermentation. Despite the fact that scholars have conducted a lot of research on kombucha

and conducted a more comprehensive study, there are still some shortcomings, and further improvement is still needed. Although the fermentation technology of kombucha is constantly improving, it is still in the development stage and is not as mature as traditional fermented foods. Moreover, although scholars have confirmed that as a functional beverage, kombucha is beneficial to human health through studies, there is still a lack of clinical and experimental results. In addition, in order to explore the interaction between distinct microorganisms, we can regard the fermentation process of kombucha as a symbiotic fermentation module.

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