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Research Progress on the effect of Bacillus on flavor substances of Maotai flavor Baijiu

Wu XUE[1], Liu JIAN[1], Wang QIAN[1], Pang XUELI[1], Zhu QIFA[2], Lin WEI[3], Huang DEWEN[4], Ning YANG[1]*

Abstract

The brewing of Chinese Baijiu is a microbial community fermentation system, in which there is a close and complex relationship between microorganisms and flavor quality, but the specific deep-seated mechanism still needs to be explored and interpreted continuously so far. In this review, Maotai flavor Baijiu was briefly introduced, and the microorganisms mainly affected by flavor substances, the main components of flavor substances, the factors affecting flavor formation and the application ways of functional microorganisms in Maotai flavor Baijiu were summarized. It provides a deeper understanding for the research of others flavor Baijiu.

Keywords: Maotai Flavor Baijiu; bacillus; functional components; bitterness; mechanism; influence factor.

Practical Application: This review will further reveal the flavor components of Maotai flavor Baijiu, provide inspiration for subsequent research, and provide more in-depth understanding for the study of bitter taste of other foods.

The six major distilled spirits in the world include whisky, brandy, vodka, rum and gin, as well as China's Baijiu (Shen, 1998). China's traditional fermented distilled spirits have a history of nearly one thousand years. Among the different flavor types of Baijiu in China, Maotai flavor Baijiu is the most mysterious, with the most complex and abundant chemical component sand flavors (Jin et al., 2017; Liu et al., 2018; Wang et al., 2019). The typical aroma characteristics of Maotai flavor Baijiu, such as Maotai flavor, caramel flavor, paste flavor, mellow flavor and fruit flavor, as well as the mellow, delicate and long aftertaste, are deeply loved by consumers all over the world (Shen, 1998; Xu et al., 2012b). In the process of tasting Maotai flavor Baijiu, it mainly includes two aspects: flavor and taste. The former is mainly soy sauce flavor, and the characteristic substances are mainly furan compounds, aromatic compounds and pyrazine compounds (Cang, 2013); The latter is mainly "aftertaste" bitter and astringent, that is, Baijiu remains on the root of the tongue or the back of the root of the tongue, which gives people an unpleasant feeling, affects the overall style of the liquor, and directly affects the sensory quality and grade of Baijiu (Xiao, 2014). Therefore, bitter and astringent taste is listed as the most common peculiar smell in Baijiu (Xiao et al., 2011). Baijiu in China has bitter and astringent taste to varying degrees (Cang, 2013; Xiao et al., 2014; Wu, 2005). The precursors of flavor substances are mostly macromolecular substances, and the degradation of macromolecular substances cannot be separated from the role of microorganisms. Most wine industry peers believe that high-temperature Daqu is the main source of soy sauce flavor substances. Under high temperature, only heat-resistant bacteria are left.

Bacillus is a strain with high biosafety. Because of its fast growth, strong resistance, inhibition of pathogens and other characteristics, it has become a dominant strain in soil and plant ecological environment (Chen et al., 2003, 2012, 2018b; Jing et al., 2018). Bacillus the Thermophilic bacteria, Bacillus subtilis and Bacillus licheniformis all have strong ability to produce amylase and proteinase (Pylypenko et al., 2021). Some amino acids and polypeptides can make the wine taste bitter and astringent, so they are important fungi for flavor generation and style formation of Maotai flavor Baijiu (Gao, 2010) Studies have confirmed that the characteristic components of Maotai flavor Baijiu are mainly furan compounds, aromatic compounds and pyrazine compounds (Zhuang, 2005), while thermophilic spores can produce acetoin (Xiao et al., 2009; Nicholson, 2008), tetramethylpyrazine (Adachi et al., 1964) and furanyl.

At present, Bacillus subtilis has been widely used in scientific research (Kang et al., 2021; Wang et al., 2019), food (Syahbanu et al., 2020; Alashbayeva et al., 2021), medicine and other fields. Among them, Bacillus subtilis is the most prominent strain, which is recognized as safe (GRAS) by the US Food and Drug Administration (FDA) because of its strong strain safety. Natto (Japan), tempeh, soy sauce (China), pickles, fermented bean curd and Baijiu are all used. gather γ- Glutamic acid(γ- PGA) is water-soluble, biodegradable and edible. Because of its strong viscosity, it can be used as thickener or flocculant. Research findings γ- PGA is made from soybeans fermented by Bacillus spp. and is also an ingredient of Japanese traditional food natto (Bang et al., 2011). Douchi is one of the most popular foods in China, because it has a strong Chifalavour (soybean flavor (Sun et al., 2022), a special ammonia odor different from natto). Bacillus subtilis is one of the most important microorganisms in

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[1] *Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao, Shandong, China*
[2] *Anhui Wannan Tobacco Co., Ltd, Xuancheng City, Anhui Province, China*
[3] *Nanping Branch of Fujian Tobacco Company, Nanping City, Fujian Province, China*
[4] *Hunan Tobacco Company Chenzhou Company, Chenzhou City, Hunan Province, China*
*e-mail: 3250955288@qq.com

the fermentation process of Douchi. Kosuge & Kamiya (1962) first proposed that Bacillus subtilis can synthesize tetramethylpyrazine TTMP more than half a century ago. Tetramethylpyrazine (TTMP) is a nitrogen-containing heterocyclic compound, which contributes to the formation of many aromatic and flavor compounds (Xiao et al., 2014; Liu et al., 2018; Gao et al., 2017; Wang et al., 2022; Fan et al., 2007a). TTMP belongs to pyrazine substances detected in fermented foods (Hou et al., 2016). A recent study proved that TTmp in Chinese Baijiu is mainly produced by Bacillus subtilis under micro oxygen conditions (Zhang et al., 2019). Bacillus subtilis used in Chinese sufu can improve the antioxidant activity, fibrinolytic function and sensory quality of sufu (Wang, 2022). In addition, Bacillus is often used as an excellent chassis cell and transformed into a microbial cell factory for the production of industrial enzymes, vitamins, functional sugars, health products, drug precursors and other target products, with strong industrial production and application capacity (Gu et al., 2018; Ÿztürk et al., 2016; Zweers et al., 2008).

Maotai flavor Baijiu is loved by consumers for its mellow Maotai flavor and long aftertaste. In this paper, the source of microorganisms, the main components and formation mechanism of flavor substances, and the factors affecting flavor formation in Maotai flavor Baijiu were reviewed. This review will provide inspiration for the follow-up study of Baijiu flavor, and provide a deeper understanding of the bitter and astringent taste of other foods.

1 Overview of Maotai flavor Baijiu

1.1 Research status of Chinese Baijiu

In order to sum up the traditional experience, Chinese Baijiu carried out pilot studies in Maotai, Fenjiu and Luzhou Laojiao in the 1950s and 1960s; Mechanization research in 1970s; Research on tick off technology from 1980s to 1990s; In 2007, universities and enterprises cooperated in the development (Shen, 1998; Shen & Tong, 2007; Yan, 2015). Now the research mainly focuses on the establishment of microorganisms, flavor substances, production technology, intelligent equipment system, etc. Baijiu is divided into 12 flavor types: thick, clear, soy sauce, rice, Phoenix, Jianhe, Dong, special, soy sauce, sesame, Lao baigan and Fuyu (Fan & Qian, 2010). As the representative of Maotai liquor in China, Maotai liquor attracts the attention of researchers at home and abroad due to its unique flavor and complex production technology. However, at present, most scholars study more Luzhou flavor Baijiu, while the typical Maotai flavor Baijiu is relatively less. The production process is the key to Maotai flavor. At present, the fermentation process of most Maotai flavor Baijiu is "four high and two long". In addition to the fermentation process, the fermentation metabolism mechanism of microorganisms is also the core technology. There is a saying about the fermentation mechanism that "fermentation depends on yeast, aroma production depends on bacteria, and saccharification depends on mold". In the process of bacterial metabolism, abundant and complex enzymes are produced, which participate in the formation of flavor substances, and thus determine the flavor and quality of Baijiu. Therefore, in recent years, the research on flavor producing functional bacteria using molecular biology technology and traditional microbial culture methods has gradually increased. Due to the open production environment of Maotai flavor Baijiu, the microorganisms in the fermentation process mainly include environmental entry and Daqu addition. The formation process of Maotai flavor in Maotai flavor Baijiu is precisely the reflection of mutual regulation and mutual growth of functional microorganisms in these bacteria. Now, the microorganisms in the fermentation process of Maotai flavor Baijiu (Wang et al., 2008a, 2014, 2015a, 2016a; Wu et al., 2012, 2013, 2015; Xiong, 2005; Xiu et al., 2012; Xu & Ji, 2012a; Xu et al., 2017; Yang et al., 2007b; Zhang et al., 2013; Zheng et al., 2016) are summarized in Table 1. At the present stage, the production of Baijiu still continues the traditional production process, which has strong experience and uncertainty. However, with the continuous deepening of the research on Baijiu, in order to improve the national liquor making level, we should innovate and develop on the basis of the traditional process. At present, the urgent research includes the analysis of flavor substances in Baijiu, the development of compound Baijiu, and the establishment of digital system. The healthy development of Baijiu industry is an important development direction of the brewing industry.

1.2 Traditional production process of Maotai flavor Baijiu

A sone of the six major distilled spirits in the world, Baijiu with Maotai flavor is loved by consumers for its unique flavor of "prominent Maotai flavor, elegant and delicate, soft and mellow, and long aftertaste". Maotai, the representative of Maotai flavor

Table 1. Microorganisms in the fermentation of Maotai flavor Baijiu.

	Fungi	Bacteria	Yeast
Da Qu	Aspergillus, Mucor, Rhizopus, Monascus, Trichoderma	Bacillus,Acetobacter, Lactobacillus, Clostridium,Weissella, Pediococcus, Leuconostoc, Saccharopolyspora,Erwinia,Planifilum, Brachybacteriumsp., Sphingobacterium, Acetobacter, Saccharomonospora, Halomonas, Desemzia	Saccharomyces, Hansenula, Candida, Pichia, Torulaspora
Stacking Frementation	Paecilomycs variotii, Rhizopus Microspores, Microascus cirrosus, Monascus purpureus, Penicillium, Chrysogenum, Aspergillus, Eurotium, Thermomyces, Byssochlamys	Bacillus, Acidithiobacillus, Kroppenstedtia, Lactobacillus, Acetobacter, Pediococcus,, Pantoea, Weissella, Thermoactinomyces, Enterbacter	Rhodotorula mucilaginosa,Kazachstania exigua, Debryomyces hansenii, Pichia kudriavzevii, Galactomyces geotric hum,Saccharomycopeis,Pichia,Zyg osaccharomyces,Saccharomyces,Sc hizosaccharomyces

Baijiu, is well-known at home and abroad (Shen, 1998; Li, 1999). Through research, it is believed that taste is closely related to brewing process. Maotai flavor Baijiu adopts the brewing process of "four highs and two longs, one big and one big". The "four highs" refer to high-temperature koji making, high-temperature stacking, high-temperature fermentation and high-temperature distillation, "two longs" refer to production cycle and storage time, "one big" refers to large koji consumption, and "many times" refers to multiple rounds (fermentation) of liquor taking. The production cycle is linked in a year (Wu et al., 2012; Xu & Ji, 2012a; Li, 1999; Cui, 2007a).

The quality of high-temperature Daqu determines the style and quality of Maotai flavor Baijiu. An abstract term is "Qu is the bone of liquor". The raw materials of Daqu are mostly sorghum or wheat, which are naturally fermented. During this period, a large number of microorganisms and flavor precursors will be gradually enriched, and then pressed into starter bricks, and then mature starter will be prepared. This process requires sufficient water, oxygen and high temperature. Under the condition of sufficient water and high temperature, high-temperature resistant microorganisms grow, reproduce and metabolize, decompose various enzymes to produce soy sauce flavor substances. On this basis, create an anoxic environment, so that high-temperature resistant, anaerobic and facultative anaerobic bacteria can multiply in large numbers to obtain the required flavor substances (Cui, 2007b; Ming et al., 2010; Hu et al., 2010).

The brewing stage of Maotai flavor Baijiu includes two parts: high-temperature stacking koji making and high-temperature stacking liquor making. During the preparation of cooked koji, a large number of yeasts died. In the process of stacking fermentation, open fermentation enable microorganism in the air environment to participate in the fermentation, transforming starch, protein and other macromolecules in raw materials into amino acids, oligosaccharides, fatty acids and other small molecules. This process is equivalent to "secondary koji making" (Peng et al., 2004; Xu, 2019; Sun et al., 2013). Bacteria, molds and their enzymes can decompose raw materials, which belongs to the role of high temperature accumulation in wine production, aroma generation and saccharification fermentation. In this process, there is a unique process that the fermented grains are piled into the pit, so that the ester producing yeast can ensure the need for subsequent fermentation and wine production. In the fermentation stage of the pit, the growth of some harmful microorganisms is inhibited because the temperature rises to generate acid. Acid can prevent the growth of certain rancidity bacteria, so that the koji is not sour and smelly, thus forming a favorable environment for the generation of soy sauce flavor.

Maotai flavor Baijiu needs to undergo eight rounds of fermentation, multiple rounds of fermentation and multiple rounds of liquor taking. The specific operation steps are shown in (Dai, 2019) Figure 1. However, the beneficial microorganisms in the fermentation process are more pure, the compound Maotai flavor in the fermented grains is more intense, the impurities of Maotai flavor precursors are less, and the wine style is better. In addition to multiple rounds, liquor recycling fermentation is also a major feature of Maotai flavor Baijiu fermentation. Liquor recycling fermentation process can nourish distillers' grains with liquor, increase aroma components with high boiling point, and then promote liquor body to be fragrant. The storage time of Baijiu determines the quality of liquor. The storage time of Maotai flavor Daqu liquor is not the same according to different koji. The storage time of Maotai flavor Daqu liquor is more than three

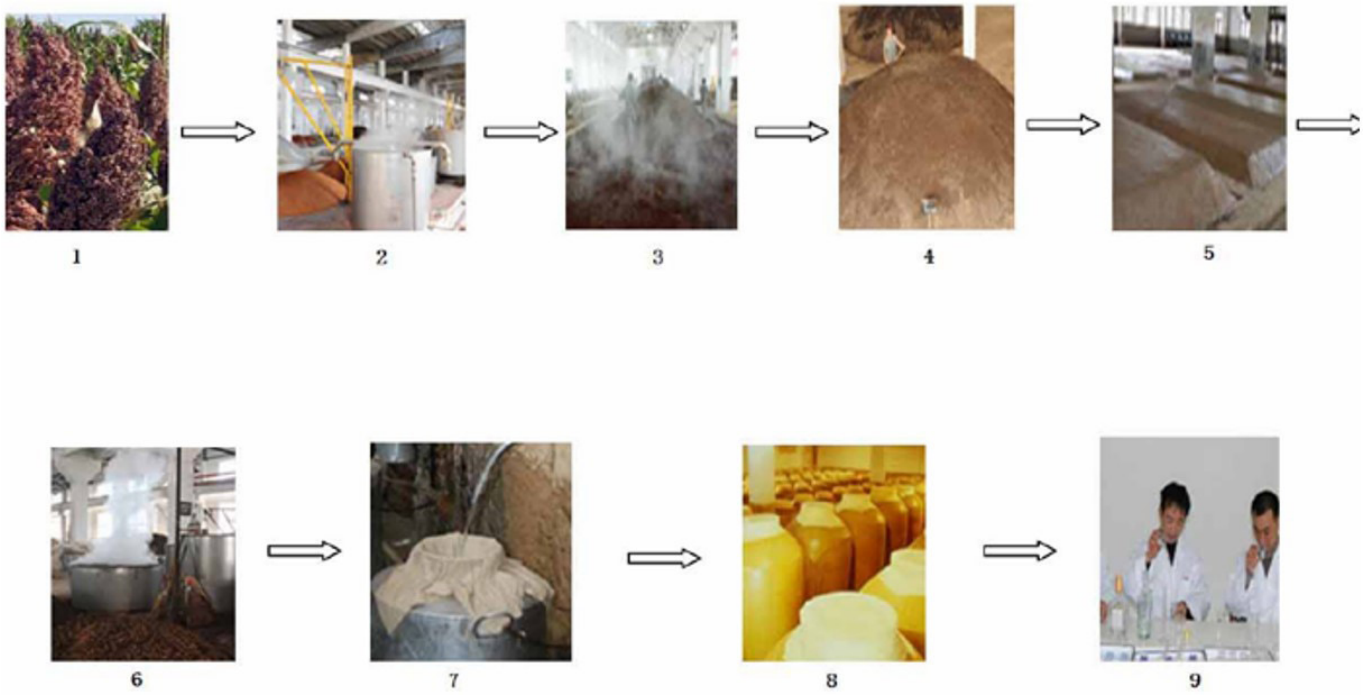

Figure 1. Main production process of Maotai flavor wine. 1. Sorghum 2. Steamed grain 3. Airing 4. Heap fermentation 5. Pit fermentation 6. Steamed wine 7. Take wine 8. Storage 9. Tick.

years, while the storage time of Maotai flavor bran koji is more than one and a half years. The complex production technology and storage means are the reasons why Maotai flavor Baijiu is different from others flavor Baijiu.

2. Research status of Bacillus in Maotai flavor Baijiu

The main power source of Maotai flavor Baijiu fermentation has always been considered to be the bacterial group (Fan & Qian, 2010), but because Maotai flavor Baijiu is an open stacking fermentation, the bacterial source should include two parts: environment and Daqu. High temperature accumulation is an important link commonly used in Maotai flavor Baijiu and thick sauce combined Baijiu. Its purpose is to enzymatically hydrolyze starch and protein in fermented grains into sugars and amino acids, and react to generate flavor substances (Zeng, 2012; Xiao et al., 2005). The environment of high-temperature brewing is very suitable for the growth of Bacillus groups, which leads to a large number of enrichments of Bacillus groups, making Bacillus the most abundant and abundant bacterial group in the brewing environment of Maotai flavor Baijiu (Wang et al., 2015a).

Flavor substances in Baijiu are mainly produced by the decomposition of raw materials by microorganisms (environment + koji) (Hai, 2013; Hu, 2015). Studies have found that molds decompose starch and protein to produce glucose and amino acids, and produce saccharifying enzymes and amylases through reproduction and metabolism; The bacteria metabolize glucose and amino acid to produce flavor components; Yeast metabolizes glucose to produce ethanol (Wang, 2014). As for environmental microorganisms playing a very important role in Baijiu brewing, it is often said in the industry that "if you leave Maotai Town, you can't brew Maotai liquor". However, at present, most of the studies on the micro ecology of Baijiu brewing are only focused on the succession of microbial communities during koji making or fermentation, and the studies on the microorganisms in the brewing environment are also limited to the study of air microorganisms in the environment. There is no in-depth study on other environmental microorganisms such as soil in the brewing environment. Moreover, due to the high diversity of wine making environment microecology, conventional culturable or PCR-DGGE technologies in the past could not have a good understanding of wine making environment microecology, and the role of environmental microorganisms in wine making lacked quantitative analysis. Therefore, it is of great significance to analyze the microbial structure of Baijiu fermentation environment, clarify the source of Baijiu brewing microorganisms, analyze the impact of environmental microorganisms on Baijiu fermentation, and analyze the formation mechanism of Daqu microecology to improve the controllability of Baijiu brewing and improve the quality of Baijiu.

2.1 research status of micro ecology of Baijiu fermentation

In recent years, some studies have shown that the formation of Maotai flavor of Baijiu is related to the role of bacteria, and functional microorganisms for Baijiu production have been isolated from Baijiu koji and fermented grains everywhere, including various functional bacteria for Maotai flavor (Peris & Escuder-Gilabert, 2009). Cheng Caiying (Cheng et al., 2014) and others conducted a detailed analysis of the diversity of bacterial flora in Maotai flavor Baijiu koji and fermented grains of different rounds. Through research, they found that there were common dominant bacterial flora in koji and fermented grains of different rounds: Bacillus licheniformis, the Actinomyces, B. subtilis-B. cereus-Lentibacillus. The unique brewing technology of Maotai flavor liquor forms a special microbial flora in its Daqu and fermented grains (Chen et al., 2013). Chang lu Wang et al. (2008b) used the culturable method to analyze the microbial population of Maotai Maotai Maotai Daqu at different maturity stages, and isolated more than 200 strains, mainly Bacillus. High temperature Daqu used in the production of Maotai flavor Baijiu is one of the main sources of Maotai flavor of Baijiu (Shen, 1998). Yang Daiyong (Yang et al., 2007a) and others preliminarily isolated 98 kinds of microorganisms from Maotai Daqu, including 41 kinds of bacteria, mostly Bacillus, 6 kinds of yeasts, 51 kinds of molds, including Aspergillus, Mucor, etc. Zhang Rong (Rong et al., 2010) and others screened three strains of Bacillus licheniformis with the function of producing Maotai flavor from Maotai flavor Baijiu. The results showed that acetoin, tetramethylpyrazine and furan twist may be related to the formation of Maotai flavor of Baijiu, but this result still needs further exploration and research. Yu Tingting (Yu et al., 2013) and others screened a functional Bacillus gzub-2 producing Maotai flavor from a Maotai flavor Baijiu and identified it as Bacillus licheniformis. Zhao Xingxiu (Zhao et al., 2016) and others analyzed the soy sauce ingredients in the soy sauce fermentation experiment through HS-SPME and GC-MS methods from three strains of soy sauce producing functional bacteria Bacillus subtilis in the high-temperature Daqu of a soy sauce distillery. The results showed that the contents of pyrazine and ketone in the soy sauce ingredients of the three strains were high, and the analysis of pyrazine and ketone may have a greater impact on the flavor of soy sauce.

2.2 Research status of microenvironment of Baijiu fermentation environment

The microenvironment of Baijiu fermentation environment is an important component of the microenvironment of Baijiu fermentation and an important factor affecting the quality, safety and flavor characteristics of Baijiu. Microecology of fermentation environment shall include all the environments directly or indirectly contacted by grain raw materials during operation, including but not limited to workshop floor, operating tools, air, workers and other factors.

Due to the close relationship between environmental microecology and Baijiu production, the research on brewing environmental microecology is also increasing. Wang Xingchu (Wang & Guo, 2011) used molecular biology and bioinformatics technology to study the air bacterial flora in the production environment of Maotai Liquor. The results showed that in the production environment of Maotai Distillery, bacteria in the air were selectively enriched, forming a bacterial flora with large cell density and complex structure. Wang Yanhua (Wang, 2016) used the second generation high-throughput sequencing technology to study the air of the new and old workshops of Fumao cellar liquor and the microbial flora structure in the accumulated and fermented fermented grains. The results showed that during

the process from accumulation fermentation to the end of fermentation in the cellar, the bacterial flora structure changed greatly, while the fungal flora structure changed slightly, and the fungal groups in the air of the brewing workshop were closely related to the brewing process of Fumao cellar liquor. Zhang Yali (Zhang, 2014) took the air microorganisms in Maotai ancient town as the research object, and carried out regular collection, culture, separation, purification, identification, total extraction, sequencing and microbial community analysis (divided by obvious seasons) four times a year. The research results showed that a large part of microorganisms in the brewing process of Maotai flavor Baijiu came from the air environment. Zhang et al. (2008) compared the composition of bacteria in the air inside and outside Luzhou Laojiao Workshop and believed that Bacillus is the main bacterial population in the air.

3 Research progress on flavor substances of Maotai flavor Baijiu

3.1 Research status of main aroma components

So far, it has been clear that the main aroma component of Luzhou flavor liquor is ethyl caproate; Fen flavor liquor is ethyl acetate; Rice flavored liquor may be ethyl lactate, ethyl acetate and p-phenylethanol. Some characteristic components were also found in other aromatic wines, but the main aroma and flavor components needed to be further analyzed. At present, there are many inferences about the characteristic flavor components of Maotai flavor liquor, but what its essential characteristics are still seems to be a mystery. The studied characteristic substances of Maotai flavor are summarized in Table 2.

4-Ethylguaiacol (4-EG)

In 1964, Maotai liquor pilot project (Li et al., 2021a), according to the conditions at that time, proposed for the first time that the main aroma component of Maotai flavor liquor was 4-ethyl guaiacol. 4-ethyl guaiacol is a typical representative of high boiling point compounds in soy sauce aroma, which has the characteristic aroma of soy sauce (Li et al., 1990a). In 1976 (Xiong, 2000, 2005a), a study was conducted by combining gas chromatography analysis with sensory taste, and it was found that 4-ethyl guaiacol had the smell of Beijing "fumigation". It was mellow, sweet, and had nothing to do with the soy sauce flavor, so it was not the main flavor of Maotai liquor.

Pyrazine

Some scholars believe that the main way of formation of soy sauce flavor substances is the thermal decomposition of amino acids and proteins; Sugar involved in the related reactions and some microbial metabolites. Cao Shushun also proposed that pyrazine compounds may be related to the formation of soy sauce flavor (Zhang et al., 2022b): high-temperature Daqu has a flavor similar to soy sauce flavor more than medium temperature Daqu, and its aromatic components may be related to the volatile compounds (such as aldehydes and pyrazines) generated by the reaction. In addition, factors such as large amount of koji, large concentration of matrix, high reaction temperature, etc. in the production process will inevitably produce or accelerate the Maillard reaction rate, thus increasing the production of pyrazine compounds. During distillation, pyrazine compounds volatilize with steam and condense into liquor due to the effect of vapor belt, creating Maotai flavor style. In the later study (Xiong, 1994), a researcher conducted sensory identification on tetramethylpyrazine, and found that its smell was not obvious, like the smell of soaking bean water, sweet and strong.

Pyrazine compounds have the aroma and baking smell of green pepper, which are found in large quantities in baked food and can make food produce flavors similar to popcorn, nuts, coffee, spinach and chocolate. However, compounds similar to soy sauce, soy sauce and wheat soy sauce have no aromatic effect except tetramethylpyrazine (Li et al., 1990b; Zhang et al., 2022c; Shen, 2010), so it is believed that pyrazine compounds have limited aromatic effect. Some of these compounds have very low thresholds, while others have very high thresholds. For example, the threshold of pyrazine can reach 300 mg/L (in water), the threshold of 2-methylpyrazine can reach 30 mg/L (in water) (Zhu et al., 2022), and the threshold of 2-methoxy-3-isobutylpyrazine is only 0.002 μ G/L (in water) (Ding et al., 2022), its threshold value is very different, and does not show soy sauce flavor. The addition test conducted by some researchers proved that (Fan & Yan, 2012), the addition of pyrazine, especially tetramethylpyrazine with high content, could not produce subtle soy sauce aroma. When pyrazine compounds were added to Luzhou flavor Baijiu, no Maotai flavor was found in Luzhou

Table 2. special flavor substances of Maotai flavor Baijiu.

Compound category	Flavoring substance	Threshold (μg/L)	Evaluation
Phenols	4-ethylguaiacol	33	Roasted and fried nuts
	4-Vinylguaiacol	300	Phenol, clove, smoke
	Guaiacol	/	Fruits, flowers, sweetness and caramel
	4-methylphenol	68	Pit mud odor, scorched skin odor, animal odor
Pyrazines	2,6-Dimethylpyrazine	400-1500	Roast barley and coffee
	trimethyl pyrazine	4000-9000	Roast aroma, roasted corn aroma
	TTMP	1000-10000	Baked incense, caramel
	2,5-Diethyl-3-methylpyrazine	15000	Caramelized
Furan	2-Acetylfuran	/	Nutty and caramel
	2-n-Butylfuran	5	Fruity, sweet and spicy
	Furfural	14100	Smell of burnt paste and nuts

flavor Baijiu; At the same time, when pyrazines were added to the atypical Maotai flavor Baijiu, it was not found that the Maotai flavor Baijiu became more typical. Therefore, pyrazine compounds are not the key aroma components or main aroma of Maotai flavor Baijiu, but they are the characteristic components of Maotai flavor Baijiu.

Furan and pyran derivatives

In 1983, some studies speculated that furans and pyranoids were important aroma substances and characteristic compounds of Maotai flavor Baijiu. The aroma of Maotai flavor Baijiu (Zhou, 1983) is mainly a compound aroma composed of Maotai flavor, burnt flavor, ester flavor and alcohol flavor. The important components of Maotai flavor Baijiu are Maotai flavor and burnt flavor. These two substances are derived from the fat and amino acid components in raw materials and the substances produced by the metabolism of yeast. In the production process, they are affected by raw materials, brewing process, koji, water quality and aging time. These furans and pyran compounds may include HEMF, HDMF, and HEMF has cake and biscuit aroma, which is obviously enhanced when added to soy sauce; When added to Baijiu, the style did not improve significantly. It indicates that the Maotai flavor of soy sauce or sauce is not the same as that of Maotai flavor Baijiu (Dong et al., 2016).

The furfural content of Maotai flavor Baijiu is higher than that of other flavor Baijiu, which has become a marker component of such flavor Baijiu. Its unique Maotai flavor may have some internal and inevitable relationship with the high content of furfural (Shen, 2010; Xu, 2002). Some studies reported that Wuliangye and Jiannanchun Liquor were studied by liquid-liquid extraction GC-MS, and seven furans were detected (Fan & Qian, 2006). In addition, hsspme technology was used to study the aroma substances in high-temperature Daqu, and nine furans were found (Fan et al., 2007b), which is the report with the highest content of furans found in the research on Chinese Baijiu Daqu (Zhao et al., 2008). Therefore, some researchers speculate that the main aroma components of Maotai flavor liquor may be furan and pyran derivatives.

3.2 Research status of auxiliary flavor

Sour, sweet, bitter, salty and fresh are the five basic tastes widely accepted by people (Zhang et al., 2017). In Baijiu, the taste of Baijiu mainly includes sour taste, sweet taste and bitter taste. Although an appropriate amount of bitter taste can enrich its taste (Zhang, 2011), in most Baijiu, bitter taste will be regarded as peculiar smell because of its high intensity. All kinds of Baijiu have some bitterness problems. Although there are a lot of studies on bitter substances in food (Wiener et al., 2012) internationally, the formation reason and chemical nature of bitter taste in Baijiu are still unclear. Recently, Wang Yinye et al. Took sesame flavor Baijiu as the research object, analyzed the volatile bitter substances in Baijiu, and identified volatile bitter compounds such as furfural, 2-methylpropanol, 3-methylbutanol, 1-butanol and n-propanol (Wang, 2018).

However, under normal circumstances, flavoring substances are often difficult to volatilize (Farmer, 1994). In China's Baijiu, the special solid-state distillation process makes Baijiu also contain some nonvolatile components (Zhang et al., 2014a), and studies show that compared with other flavor Baijiu, Maotai flavor Baijiu has the highest content of nonvolatile components and the most complex components (Wei, 2007). Therefore, we speculate that there are still some hard to volatile bitter substances in Maotai flavor Baijiu, which may contribute to the bitterness of Maotai flavor Baijiu to a certain extent, and the research on these taste substances needs to be in-depth.

Furfural

Furfural is the main component of furan system in Baijiu. It is produced by pentosan in rice husk and raw material skin under the action of microorganisms. Furfural is one of the important components of "grain aroma" or "burnt aroma" in wine, but excessive furfural will make wine scorched bitter, and acrolein bitter (Hu, 2006). However, at present, the research on furfural in Baijiu is only qualitative, quantitative and flavor analysis, and there is no report on its taste. The research on acrolein is only limited to the detection and quantification of acrolein in Baijiu for the first time (Zhu et al., 2016). Acrolein is easy to be produced in abnormal fermentation due to poor health management of Baijiu fermentation. It is produced by yeast and lactic acid bacteria acting on glycerol during Baijiu fermentation, so it is also known as glyceraldehyde. Acrolein not only has a pungent odor, but also has a great persistent bitter taste (Song et al., 2021).

Higher alcohols

Higher alcohols are alcohol soluble substances with high boiling points, which refer to univalent alcohols with more than three carbons. Liquor mainly containsn-butanol, isobutanol, n-propanol, isoamyl alcohol, etc. The mixture of these higher alcohols is what we usually called fusels. Fusels in Baijiu are not only the chromatographic skeleton components of Baijiu, but also an important component of Baijiu. It is generally believed that tyrosol in fusel is extremely bitter and persistent, isobutanol is extremely bitter, n-propanol is bitter, n-butanol is bitter, and isoamyl alcohol is slightly sweet and bitter (Wang, 2007). However, the reported studies on these bitter substances are only qualitative and quantitative (Wang et al., 2014), and there is no relevant literature report on the threshold measurement and taste analysis of their concentration in the wine body. The main way of producing higher alcohols in wine is that during fermentation, the protein in raw materials is hydrolyzed into amino acids, and the amino acids are deamination and carbon dioxide are used to generate higher alcohols one carbon less than amino acids. Tyrosol, also known as p-hydroxyphenylethanol, is one of the main diols in Baijiu. Although its aroma is very soft, its bitter taste is heavy and long. Generally, when Baijiu contains 0.5/10000 tyrosol, it will have a bitter taste in tasting. The formation of tyrosol is mainly caused by excessive yeast acting on tyrosine. Therefore, excessive use of koji will lead to excessive tyrosol, which makes the wine bitter. This is also the root cause of the bitter of Daqu (Song et al., 2021).

Other substances

There are many bitter components in Baijiu. The raw materials and auxiliary materials for liquor making contain more

tannins, which can be decomposed into catechol, pyrophenol, phloroglucinol and other monomers, all of which have bitter or astringent taste (Liu & Sun, 2018). It is generally believed that sulfide, disulfide (containing-S-S -) and sulfhydryl (- SH) compounds have bitter taste (Lai, 2009). At present, qualitative, quantitative and flavor studies have been carried out on sulfur-containing compounds in Baijiu (Sha et al., 2017). As for alkaloids, there are no reports about alkaloids in Baijiu. Most peptides are bitter peptides. There are peptides in Baijiu, so it is inevitable to have bitter taste (Wang et al., 2007). However, the current research on polypeptides in Baijiu is only limited to the discovery of polypeptides in Baijiu (Yan et al., 2014), and the quantification of polypeptides in Baijiu (Zhi et al., 2017). Amino acids exist in Baijiu (Zhang, 2011). The internationally reported bitter amino acids include L-histidine, L-valine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-tyrosine and L-arginine (Hufnagel & Hofmann, 2008). At present, 18 kinds of amino acids have been detected in Baijiu (Zhang et al., 2014b). Some soluble inorganic ions, such as magnesium ions and sulfate ions, will bring certain bitterness to the wine, such as MgSO4 and MgCl2, which will lead to bitter taste of Baijiu after blending (Wang, 2004).

4 Factors affecting the formation of flavor substances

4.1 Impact on brewing technology

Baijiu brewing is an alcoholic beverage made by cooking, saccharification, fermentation, distillation, storage, blending and other processes with sorghum, corn and wheat as raw materials and Daqu saccharifying starter (Zhao et al., 2020). Baijiu can be divided into 12 types according to flavor. The brewing process and style characteristics of Maotai flavor, rice flavor, Luzhou flavor and light flavor are shown in Table 3. The brewing process is closely related to the formation of Baijiu style. The most complicated brewing process is the Maotai flavor Baijiu process, which takes sorghum as raw material and adopts stone cellar fermentation. It has the characteristics of "four highs and two longs", that is, the "four highs" are high-temperature koji making, high-temperature fermentation, high-temperature cooking and high-temperature distillation; "Two long" means long production cycle and long storage time of base liquor.

High temperature is a crucial step in the process of Maotai flavor Baijiu (Yang et al., 2021), which can promote the decomposition of protein and amino acid, the reaction between sugar and protein, ammonia, browning reaction and microbial metabolism in Daqu, so as to form aroma components, such as ketones, aldehydes, alcohols, furans, Pyrans, thiophenols and pyrazines through Maillard reaction (Hai, 2016). The accumulation process can not only enrich many microorganisms, but also produce many biological and chemical reactions, which can form flavor substances, which is very important to enhance the Maotai flavor and improve the quality of Maotai flavor Baijiu. Liu Minwan et al. (Liu et al., 2019) found that the flavor compounds detected in the late stage of the accumulated fermented grains were more volatile than those in the early stage. The consistency between flavor substances and aroma components of Maotai flavor finished liquor was high. Yang ManJiang (Yang, 2011) studied the effect of stacking fermentation on flavor substances of Maotai flavor Baijiu. The research showed that Maillard reaction occurred during high-temperature stacking fermentation, and furans, pyrazines and aromatic compounds could be generated. The storage of base liquor also affects the style of Baijiu.

The base liquor has strong entrance irritation and poor taste, and the storage period of Maotai flavor Baijiu base liquor is generally not less than 3 years. Qin Dan et al. (Dan et al., 2021) showed that with the increase of storage time, the mass concentration of total acid and total alcohol in Maotai flavor

Table 3. Brewing and style characteristics of four kinds of Baijiu.

Flavor type classification	Representative liquor	Main raw materials	Fermentation equipment	Process characteristics	fermentation time/d	Style characteristics
soy sauce aroma style	Maotai	sorghum	Stone cellar	Four high and two long	365	Rich soy sauce flavor, full-bodied wine, long aftertaste, lasting fragrance
highly flavored type	Luzhou laojiao	sorghum	Mud cellar	Solid state fermentation, mixed steaming and burning, continuous tank ingredients	40-90	The cellar fragrance is rich, soft, sweet and clean, the fragrance is harmonious, and the tail is clean and long
Fragrant type	Fen wine	sorghum	Ground cylinder	Steaming	28	Pure fragrance, soft and sweet taste, harmonious fragrance, mellow and cool
Rice flavored type	Sanhua wine	rice	Pottery	Saccharification of Xiaoqu culture bacteria, semi-solid fermentation, kettle distillation	7	Rice fragrance is elegant, cotton is soft in the mouth, and the taste is pleasant

Baijiu decreased, while phenols, sulfur-containing and nitrogen-containing compounds increased. Jiang Jinjin et al. (Jiang et al., 2019) studied the volatile flavor substances of Maotai flavor Baijiu aged 3 months and 20 years, and found that besides the Maotai flavor compound furfural, there was also acetoin (the precursor of 4-methylpyrazine) in 20-year-old Baijiu. Zhang Jiaojiao et al. (Zhang et al., 2022a) analyzed the Maotai flavor liquor stored for 3 years, founding that its esters and acids accounted for a high proportion, while alcohols accounted for less.

4.2 Impact on brewing materials

Raw materials for making Baijiu include sorghum, corn, wheat and rice. Liquor making raw materials are not only required for the growth of liquor making microorganisms, but also can be decomposed into alcohol, acid, ester, aldehyde, ketone and other Baijiu flavor substances or precursor substances of flavor substances. The flavor substances produced by different Baijiu making raw materials are not the same. Therefore, liquor making raw materials are one of the important factors affecting the style, taste and quality of Baijiu (Zhong & Zhang, 2022). Glutinous sorghum and wheat are used to brew Maotai flavor Baijiu, among which sorghum contains starch, protein, tannin, fat and other components. The mass fraction of amylopectin in glutinous sorghum is not less than 80%, which is suitable for multiple rounds of fermentation of Maotai flavor Baijiu (Degree et al., 2022). Phenolic flavor substances can be formed by fermentation of proper amount of tannin and protein in waxy sorghum and ferulic acid in wheat. During fermentation, proteins in sorghum react with sugars to form furan, pyrazine, pyrrole and other flavor substances (Ding et al., 2019). Jiao Shaojie et al. (Jiao et al., 2015) showed that during the fermentation process, the fat in sorghum reacts to produce low molecular aldehyde or ketone, which causes the wine body to sour. Therefore, a small amount of fat in sorghum is beneficial to the wine body quality.

4.3 Microbial impact

In the brewing process of Maotai flavor Baijiu, a large number of microbial flavor, including bacteria, yeast and mold, are enriched through high-temperature koji making, high-temperature accumulation and fermented grains fermentation (Wei et al., 2020). When microorganisms grow and metabolize, macromolecular substances in raw materials are decomposed into small molecular substances by Secretase to provide rich flavor precursor substances; Some microorganisms produce flavor substances such as vitamins, auxin and amino acids through metabolism (Xu et al., 2016). Microorganism is an important source that affects the flavor of Maotai flavor Baijiu. In the high-temperature Daqu of Maotai flavor Baijiu brewing, bacteria are a very important group of microorganisms (Xin et al., 2021), abundant in quantity and the power source of flavor production. Flavor substances produced through fermentation and metabolism play an important role in the flavor formation of Maotai flavor Baijiu. Mo Zhenni et al. (Mo et al., 2022) analyzed the colony structure of Daqu of Maotai flavor Baijiu through high-throughput sequencing technology, and found that there were 55 bacterial genera, including lactobacillus, bacillus, polysaccharide polysaccharide, Cladosporium and Streptomyces. Li et al. (2014) screened three aroma producing strains, namely Bacillus subtilis, Bacillus licheniformis and Bacillus amyloliquefaciens. The fermentation products include propionic acid, 1,3-butanediol, acetic acid and methyl ester, which are similar to the flavor of soy sauce. Bacillus is a kind of soy sauce producing bacteria with strong ability to secrete enzymes (Min et al., 2017), which can metabolize into alcohols, pyrazine, esters and other flavor substances. Chen et al. (2018a) added Bacillus subtilis with ester producing aroma function to the fermented grains. Chromatographic analysis showed that the mass concentration of esters increased while that of alcohols, acids and ketones and aldehydes decreased during accumulation; During fermentation, the mass concentration of esters, alcohols, acids and ketones and aldehydes increased.

4.4 Environmental impact

China is a vast country with abundant resources. Different geographical environments will breed different kinds of microorganisms, which will produce wines with different qualities and styles. Maotai Town in Renhuai, Guizhou Province is the most famous Maotai Baijiu with Maotai flavor. Maotai town is located on the Bank of Chishui River. It is warm in winter, hot in summer, and has less wind and rain. This climate is conducive to the survival of microorganisms. Many microorganisms in Maotai flavor Baijiu come from the air environment. Zhang Yali (Zhang, 2014) isolated and cultured the air microorganisms in Maotai Town, and more than 300 kinds of microorganisms were isolated. Wang Li et al. (2021b) found that the types and quantities of mineral elements contained in Maotai flavor Baijiu in different regions (Chishui River Basin) were different.

5 Summary and perspectives

Maotai flavor Baijiu is the most complex Baijiu category among all mainstream flavor Baijiu in China. After years of research and exploration, researchers have obtained many results on the main flavor substance with sensory characteristics of "Maotai flavor", but they have never been verified. Therefore, in the future, we should further enrich the research methods and tools of flavor omics, but we should not unilaterally pursue the results of more detected substances and rely on limited instrument results; At present, the pre-treatment process of flavor substances detection is complex and time-consuming, and whether new physicochemical reactions are initiated remains to be verified. Therefore, the improvement of pretreatment means that the flavor substances in Baijiu are continuously mined through the improvement of the hardware or software of analytical instruments, the analytical data of instruments (flavor compounds) and sensory data (sensory characteristics) are well combined, and the correlation between flavor compounds and sensory characteristics is found by means of big data analysis. While paying attention to the volatile substances in Baijiu, we should also pay attention to the non-volatile substances in Baijiu, such as the contribution of lipopeptide substances to the flavor of Baijiu. In addition, the impact of nonvolatile substances on volatile substances, such as the size of aroma release and the difference in aroma perception, should be the focus of the future

research on flavor chemistry of Baijiu. Continue to explore for the realization of "quality clarity" of Maotai flavor Baijiu.

In addition, the sensory flavor characteristics of Baijiu are closely related to the environmental microecology, brewing microecology and flavor metabolites. A variety of different flavor substances exist in the liquor in a specific proportion, which determines the unique flavor characteristics of Baijiu. In the future, we should consider breaking through the research of flavor chemistry of Baijiu from different directions such as environmental microecology, brewing microecology and flavor metabolites. Metagenomics, meta transcriptomics and metabolomics can be used to deeply study the dynamic changes, interactions and metabolites of microorganisms in the brewing environment and fermentation process, so as to explore the metabolic pathways of various microorganisms in the fermentation process, reveal their specific functions and contributions to flavor, and gradually analyze the causes of complex flavor and quality of Baijiu.

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