A review of biotransformation of sulfur compounds in beer brewing

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Abstract: A large number of polyfunctional mercaptan precursors are found in hops and malt, which are not smelled by the nose, and these substances are not fully utilized in the beer production process, and much of the research on these sulfur-containing compounds has been to further explore how to improve the expression of multifunctional thiol in beer. Three effective thiols are known to contribute significantly to the tropical flavor of wine and beer, notably 3-mercaptohexyl alcohol (3MH), 3-mercaptohexyl acetate (3MHA), and 4-methyl-4-thiopentan-2-one (4MMP). With the increasing demand for floral and tropical fruit aromas in beer in recent years, a comprehensive understanding is needed to manipulate the mechanisms by which these aromatic merthiols are expressed in beer. In this paper, the recent understanding of the release of polyfunctional thiol in beer is summarized, which provides the direction for future experimental research and will contribute to further understanding of the biological transformation of hop aroma.

1. Introduction

Hops, the scientific name of hops, are dioecious. The male flower has no brewing value in the beer brewing process, and the female flower contains the hops gland, which is one of the important raw materials for beer brewing. It has antibacterial, anti-inflammatory, anti-tumor, anti-oxidation and other effects. Hop composition is complex, more than 400 volatile substances have been detected from the distilled hop essential oil alone. [1,2] Hop oil makes up about 3% of the total mass of hops,[3] consisting of three main compounds: hydrocarbons, oxygen and sulfur, of which only a small fraction (<1%) is sulfide, consisting of a low molecular sulfur bond organic compound called polyfunctional mercaptan.[4] The aroma threshold concentration of polyfunctional mercaptan is very low, but it has a great influence on the aroma of finished beer, and is the main contributor to the flavor and aroma of beer. In recent years, with the continuous rise in demand for craft beers with prominent hop styles, such as IPA, Seitao, and Belgian Wheat, [5] more and more brewers have begun to explore the use of different hop dry injection techniques to improve the impact of hops on beer aroma style.

Polyfunctional mercaptans are found in many plants, including hops, and are sulfur-containing compounds with a strong odor. They can provide tropical aroma properties such as grapefruit, passion flower, guava, raspberry, etc. [6] They have been the focus of research on beer flavor in recent years. Polyfunctional mercaptans commonly found in beer include, but are not limited to, 4-methyl-4-sulfopentane-2-one (4MSP), 3SH, 3SHA, 3-sulfopentane-1-ol (3SP), 3-sulfo-4-methylpentane-1-ol (3S4MP), and 3-sulfo-4-methylpentane-1-ol (3S4MP). [7] Polyfunctional mercaptan precursors are non-aromatic cysteine and glutathione complexes, which are present in hops in much higher concentrations than their free forms, especially glutathione complexes. In csacade hops, the combination of glutathione and cysteine-based 3SH precursors was measured at 19μg/g, It accounts for more than 95% of the total content of 3SH, and most of the 4MSP appears in the form of free.[8]Hop varieties such as Nelson Sauvin, Citra, and Amarillo are rich in citrus aroma because of the free form of 3SP precursors, which is why 3SH precursors are just as important as 3SP precursors. In addition, mercaptan precursors are also present in malt and hops, the main raw materials for beer production, and grapes, the main raw materials for red wine production. [9]

These plants are used as raw materials for wine products, some of which are produced by fermentation through yeast conversion.[10] This paper focuses on the main sulfur-containing compounds in beer and the factors that may affect their formation under actual production and experimental conditions, which will play a role in filling the gap in the study of mercaptan metabolism in the field of beer.

2. Sulfur compounds in beer brewing materials

2.1. Hops

The pleasant characteristic flavor and signature bitterness in beer are mainly provided by hops, so the comprehensive flavor characteristics of beer are actually directly influenced by hops, so it is also because different

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types, growth conditions and storage methods of beer hops will produce different aromas, so brewers will choose different types of hops to produce beer according to their own brand characteristics and consumer consumption habits, forming their own brand unique flavor.[11]The flavor component of hops is mainly derived from the hop oil contained in the lupin glands of the hop cone. [12] The global production of hops is characterized by year-to-year fluctuations, and production has increased slightly in the last three decades. However, the geographical distribution of hop production, as well as the area of hop cultivation, has changed significantly across the globe. Many European hop-growing countries have drastically reduced their acreage and production (e.g., Romania, Ukraine, Russia), or almost to zero (e.g., Serbia, Hungary). Germany's acreage decreased slightly, but total production increased significantly due to higher yields per unit area. The United States has a lot of development in this field, especially in the past decade, the increase in production is more pronounced. [13-14]

Growing hops in the United States is rich in fruit flavors, which can add rich tropical fruit smells to the finished beer, such as: blackcurrant, orange, passion fruit, raspberry scent. [10] Hop essential oils contain a large number of aromatic compounds, many of which have been found to consist mainly of monoterpenes and sesquiterpenes, less terpenoids, and a small amount of sulfur-containing mercaptan, the latter of which has a strong contribution to tropical aromas. [15]

According to brewing characteristics, hop varieties are divided into five categories,[16]Traditional flavor varieties known to the industry include long-bred Saaz, Spalt, Tettnang, and Hallertau Mittelfruh (Hallertau from Bavaria, Germany), which have low alpha acid content in their hops. Flavor varieties dominated by newer varieties (varieties with lower alpha-acid ratios as well as non-flavor varieties) have intense fruit and citrus flavors. High-formic acid varieties with high α-acid content are generally used to produce hop products and increase the bitterness value of beer in beer production. The acid content of the Facultal varieties is balanced by a balanced ratio of alpha and beta acids.[17]

In five classic hop varieties Tomahawk, Nelson Sauvay, Nugget, Cascade and Saz, 41 polyfunction merkiols are distributed[18]:Tomahawk has higher levels of 3-sulfoyl-2-ethylpropanal (skunk, plastic, sun odor) than other varieties. Tomahawk, Nelson Sauvin contains higher levels of 3-sulfo-2-ethyl propyl acetate (floral) and 3-sulfo-n-butanal (citrus, peach fruit) than the other three varieties, and Nelson Sauvin also has a higher beta-sulfoalkol content. The 3-sulfoheptane-1-ol content is higher in Cascade.

Compared to Lublin and Styrie, Saz contains higher levels of bergamocarotene (50 mg·−1 in fresh hops, stable after fermentation) and farnesene (800 mg·−1 in fresh hops). Compared to bitter varieties, Saz contains can cause the finished beer to exhibit a strong cheese and sulfur odor. [19]

The content of 3-mercapto-2-ethylpropanal in tomahawk is generally higher than that in most hop varieties.[20] Both Tomahawk and NS contain high levels of 3-mercapto-2-acetate and 3-mercapto-caprylic aldehyde. NS contains more β-sulfonyl alkyl alcohols. 3MH is found in almost all hop varieties, but the value is higher in Cascade. [7]

Figure 1. Biosynthesis of volatile mercaptan in hops

Note: 3MH/3MHA biosynthesis is in the upper pathway and 4MHA biosynthesis is in the lower pathway. The enzyme that catalyzes each reaction is indicated by an oblique line above the path. The red enzyme is only expressed in plants, and the blue enzyme is expressed by plants and saccharomyces cerevisiae.

Cys-3MH in hops has been shown to be the precursor of 3MH, the total amount of mercaptan precursors we focus on is determined by the specific hop variety, and the number of different mercaptan precursors in different varieties of hops varies greatly.[21]2MEA S-conjugate of cysteine (2-thioglycolate; Cys-2MEA) was identified as a potential precursor of 2MEA. [22] The main precursor of 2M3MB formed by yeast during beer fermentation has been identified as 2, 3-epoxy-3-methylbutyraldehyde (EMB), with a conversion rate of about 3%, and the sulfur source can react with EMB and then reduce the terminal aldehyde.[23]Several new mercaptan precursors have been shown to exist in hops: Amarillo-glutathione S-conjugate,3-mercapto-4-methylpentane-1-ol(G3M4MP);Citra-glutathione-S-conjugate-3-mercaptoamyl-alcohol(G-3MP);Hallertau,Blanc-cysteine-S-conjugate,3-mercapto-4-methylpentane-1-ol(Cys3M4MP);Polaris-cysteine-s-conjugate 3-thio-amyl alcohol (Cys-3MP). [24]

The main part of hop mercaptan potential is composed of cysteine-glutathione conjugated compounds (cysteine and G-conjugated compounds). G-3SHol and G-3SPol are ubiquitous in hops and are the source of most mercaptan pools in hops. The levels in Saz are the same as in cascade(95.7 and 2.5mg·kg-1−1G-3SHol and G3SPol, respectively, in Saz and 20.1−118.2 and 1.4−9.8 mg·kg−1, respectively, in cascade).Hops contain cysteine conjugations as high as 1.9mg.kg−1Cys-3SHol, 0.2mg.kg−1Cys-3SPol,and65mg.kgµ1Cys-3S4MPol (only 0.5mg.kg−1Cys-3SHol in Saz). [25-27]

2.2. Malt

In addition to hops, malt is another main raw material for brewing beer, which not only provides essential nutrients for yeast in the fermentation process of beer, but also gives beer rich color and taste like hops.[28] According to the different biochemical properties of the malt and the difference in the role played in the actual production, it can be divided into two types: pale malt and dark malt.[29] Pale malt can also be called base malt, because its flavor characteristics are easily masked by the characteristic aroma of hop esters and mercaptans in the brewing process, and its main function is to provide basic components such as enzymes, fermentable sugars, and amino nitrogen required for beer brewing. Dark malt is also known as "specialty malt" because of its strong caramel and baking aroma, depending on the amount added to the beer, it can provide a unique flavor and color to the finished beer.[30]Volatile flavor molecules in malt, including Maillard reaction products, thermal degradation products of oxidized fatty acids, and Strecker aldehydes and sugar coking products, are released during the malting process. [31]

Experiments showed that the mercaptan content of different types of beers (pale Ale, Belgian wheat, IPA, Pilsen) varied greatly in both type and content, and IPA beers with higher hop content contained relatively low 3mh(less than 180ng.L−1). This suggested that hops may not be the only source of mercaptan and mercaptan precursors in beer, and malt was hypothesized as another source.[32] However, further verification is still needed in combination with other precursors identified in grape (CysGly-3Mh, γGluCys-3Mh, G-3Mh-Al).

Although the levels of mercaptan precursor and free mercaptan were the same in the two fermentation conditions of hops in wort and YPD hop medium, the content of CG3SH was still significantly different, with a relatively high level in wort (109.1μg/L) and a low level in YPD hop medium (16.5μg/L). This study confirms that malt is a potentially important source of CG3SH. Reasons for the difference in increased 3SH and 3SHA content in post-fermented beer compared with post-fermented YPD hop medium in this experiment. [33] Another study showed that malt is also a source of cys-3MH precursors, albeit in small amounts.[34] Similar to wine, the abundant mercaptan precursors in wine come mainly from grapes, and given that malt is the other main raw material in the brewing process besides hops, wort has the necessary substrate for biosynthesis of mercaptan. [35] The concentration of free mercaptan and its precursors in malt also depends on the planting location (soil), harvest year (average annual precipitation, light intensity, temperature, humidity), and maturity at harvest. [36]

Therefore, the process of mercaptan precursors released by fermentation of different varieties of hops in wort and transformed by yeast into final aroma products can still be regarded as a research focus.

3. Beer contains mainly sulfur compounds

Table 1 Main flavor sulfur compounds in beer and their flavor descriptions

Beer contains mainly sulfur compounds	Odor attribute
4-mercapto-4-methylpentane-2-one (4MMPs)	Black currant, cat urine (in higher concentrations)
3-methyl-2-ene-1-mercaptan (3MBT)	skunk
3-mercaptohexan-1-ol (3MH)	Tropical fruits, grapefruit, blackcurrant, guava
3-mercaptohexyl acetate (3MHA)	Grapefruit, blackcurrant, mango
4-mercapto-4-methylpentane-2-one (4MMP)	blackcurrant
4-mercapto-4-methylpentane-2-ol (4MMPOH)	Grapefruit, flowers
3-mercapto-3-methylbutane-1-ol (3MMB)	
3-mercapto-3-methylbutyl acetate (3MMBA)	

The ability of yeast to release cysteine and glutathione bound mercaptan precursors has been extensively explored in wine industry studies, and the absence of volatile mercaptan in grapes indicates the importance of yeast fermentation in the winemaking process.[37] The precursors present in grapes have been shown to be thiols in the cysteine-bound form. The metabolic activity of yeast is necessary for the cleavage of cysteine-coupled precursors, different strains of wine yeast ferment result in the release of different amounts of volatile mercaptan. [38]Another volatile mercaptohexyl mercaptol (3Mh) present in wine also comes from the cysteine-bound precursor (Cys-3Mh).Studies on the formation of free mercaptan showed that lemon fungus extract or purified Escherichia coli tryptophanase lyzed Cys3MH precursors in vitro.[39] L. citri extract and purified tryptophanase have cysteine-binding lyase activity. Saccharomyces cerevisiae cleavage mercaptan precursors by a similar mechanism during the fermentation of grape juice.[40] The activity of β-lyase is a key factor in the formation of a free mercaptan and an intermediate that spontaneously degrades into pyruvate and ammonia. Therefore, the yeast carbon-sulfur lyase with elimination activity is involved in the process of releasing 4MMPs from Cys-4MMPs.This provides a theoretical basis and inspiration for studying the mechanism of yeast conversion of important mercaptan such as 3-mercaptohexyl alcohol in wort fermentation. The reason why IRC7, as a key gene, has become a research focus in the current mercaptan transformation process is that as a key node gene, it is responsible for encoding the enzyme that lyses the odorless cysteine mercaptan precursor found in grapes: carbon-sulfur lyase (CSL), through the metabolic transformation of this enzyme can release a considerable amount of free mercaptan molecules with odor.[41]

However, the specific role of this enzyme in beer production, especially in the important wort fermentation stage of beer production, has not been fully studied, which has caused a gap between beer and wine industry in the study of the role of B-lyase activity in the production of finished wine aroma. Therefore, based on the discovery of a large number of mercaptan precursors in the two main ingredients of beer production, similar to those found ingrapes, [42]Therefore, the hypothesis of cysteylglycines-conjugate (CysGly-4MMP) and γ-GluCys-4Mp (gamma-Glucys-4MP) in malt has been proposed, which has not yet been confirmed.

4. Factors affecting the formation of sulfur compounds in beer

4.1. Influence of fermentation medium on sulfide production

Wort fermentation showed a greater degradation rate of G3SH (the average degradation rate of G3SH in wort was 53% compared to 23% in YPD hop medium), highlighting the potential influence of fermentation media on precursor degradation. In each medium, there are also differences on the basis of strains, and this is most evident in wort fermentation. Among all yeasts, lager released the most 3SH (0.16%). The decomposition of G3SH to C3SH is not dependent on β lyase, but on transpeptidase activity.[43] The β lyase simultaneously breaks down C3SH to 3SH during the metabolism of G3SH transpeptidase, so it is difficult to determine the maximum concentration of C3SH formed during fermentation. However, it is worth noting that in the laboratory environment, the thiol content in the finished beer is higher because the continuous stirring fermentation goes faster.[44] Therefore, fermentation conditions (adequate agitation, suitable temperature, water quality) may also affect the release of mercaptan.

4.2. Nitrogen

Regulation of nitrogen catabolism is an important factor in inhibiting the release of mercaptan in wine. Therefore, assimilable nitrogen level is also a potentially important factor that may affect the release of mercaptan in beer. Assimilable nitrogen has been identified as a potential source of free mercaptan release. [45] Brewers typically detect nitrogen content by yeast assimilable nitrogen (Yen), which is derived from amino acids and peptides, and high levels of Yen inhibit the release of free mercaptans 4MSP, 3SH, and 3SHA.[46] However, the specific parameters about the effect of nitrogen level in wort on the ability of yeast to release mercaptan during brewing have not been confirmed under brewing conditions.

4.3. Influence of the process on sulfide production in the actual production process

The two major steps of beer production process are wort preparation and beer fermentation production: the wort preparation process refers to the process of pulverizing barley malt and other raw materials through high temperature saccharation and washing to prepare the main solution; beer fermentation refers to the process of adding hops and yeast to the wort to make the wort fully ferment, aging, cold storage and finally filtration. However, the production process of dry throwing hops in the boiling process of wort will also cause different degrees of loss of volatile flavor compounds due to the difference in the length of the boiling time after the wort is prepared and cooled, and the addition of hops after the fermentation process is completed. It has become a recognized method in the industry to enhance the aroma of finished beer. [47] After the hops are dried and before cold storage, the beer undergoes a "secondary fermentation" in which the active yeast reabsorbs compounds negatively associated with beer flavor, namely diacetyl (a compound associated with the aroma of buttery popcorn) into 2, 3-butanediol (a compound with a higher aroma threshold). Yeast mediated flavor changes may also occur during this period, such as hydrolysis of hop-derived odorless glycosides. [48]

Saccharomyces pastorianus strain can produce polyfunctional mercaptans from cysteated (Cys-) and glutathione (G-) complexes. [49] Studies have shown that reducing the nitrogen content and hair degree of wort (12℃) during wort fermentation can significantly improve the release rate of cysteine binding and the ester to alcohol ratio. However, this practice does not promote the release of G-conjugates and even prevents the yeast from producing acetate. Despite the lack of free precursors and their cysteine precursors, Saz hops and Pearson malt (classic components of fine beer) have been shown to be important sources of G-conjugates (3-sulfohexanol and 3-sulfo-4-methylpentyl alcohol). [50] Therefore, in the actual production, the differences of sulfur-containing compounds in different kinds of raw materials in the first part of beer brewing, the production process and the selection of raw materials will ultimately affect the characteristic aroma of the finished beer.

4.4. Yeast

According to the end step of the 3MH and 4MMP biosynthetic pathway in Figure 1, yeast is mediated by cysteine B-lyase to eventually produce 3MH and 4MMP, which is completed in yeast. Studies have shown that there are significant differences in the conversion ability of volatile mercaptan among different yeast strains. Under the same experimental conditions, beer fermentation was carried out with transgenic (GM) yeast that released multifunctional mercaptan (by inserting highly active CSL encoded by the gene from Citrobacter and ethanol acetyltransferase from apple and Escherichia coli) and unmodified original yeast strains at the same time, and the chemical properties of finished beer from transgenic yeast did not change. But 3MH was released at 73 times the concentration of the original yeast strain and more than 130 times the threshold for sensory detection in beer. This study demonstrated that yeast performance is one of the factors that effectively affect volatile chemical molecules such as polyfunctional mercaptan during beer brewing, and successfully validated the ability to promote mercaptan expression in beer by altering the genes of yeast strains.[50]

4.5. PH

Due to the different acidic properties of beer and wine, the typical pH range of wort is 5.2~5.4, and the pH range of finished beer is 4.0~5.0; The typical pH range for grapes is 3.2−4.0, and the pH range for finished wines is 2.8 to 3.8. [51] In particular, it has been concluded from the wine literature that PH is an important factor that may affect the biological conversion of aromatic mercaptan.

5. Summary and prospect

Sulfur-containing compounds have been extensively studied in the brewing industry, and it is a complex process to drive the biotransformation of aromatic mercaptan in the brewing process of complex substrates such as beer. However, in addition to grapes and wine, the beer industry has a relatively clear understanding of the basic composition, contribution and influence factors of sulfur-containing compounds in beer, but the mutual conversion of these glutathione S-conjugated derivatives is still lacking. In addition to mercaptan, many of the compounds produced by yeast during fermentation come from exogenous sources, such as malt and hops, which provide the desired characteristic aroma to the finished beer through a series of interactions during beer brewing. These compounds include, but are not limited to, esters, higher alcohols, C-13 norisoprene, pyrazine, and terpenyl alcohols. Based on the investigation and collection of this data, it is concluded that there are many factors affecting the metabolism of sulfur-containing compounds in the beer production process, and different yeast strains, fermentation liquid, ph, temperature, nitrogen content, etc. will affect the quality of finished beer. Therefore, subsequent molecular experiments can be carried out from these aspects to improve yeast strains or hops. However, too high concentration of sulfur compounds is harmful to the human body, which is also worth our attention.

With the increasing demand for craft beer with more characteristic aroma in China, more in-depth studies should be conducted to grasp the mechanism of their interaction, and on this basis, targeted methods should be found to study the interaction between specific mercaptan − ester and mercaptan −C13 isoprene, so as to improve the aroma of beer and finally further improve the quality of beer.

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