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BIOTRANSFORMATION OF HOPS-DERIVED COMPOUNDS IN BEER – A REVIEW

– Review –

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Abstract: Besides providing bitterness to beer, hops also impart a whole range of aromas, such as herbal, spice, floral, citrus, fruity and pine to this beverage. Although hops are usually added in relatively small amounts, they have a significant impact on the sensory characteristics of the product. Raw hop aroma significantly differs from the aroma resulting from its addition to the beer. The final aroma of the beer arises from substances in the malt, hops, other additives, and yeast metabolism. The biochemical transformation of hop compounds by yeast has become more and more popular in recent years. Knowledge of this process may allow more precise control over the final sensory characteristics of the beverage. The article describes the chemical composition of hops and discusses the influence of the hopping regime on the concentration of volatile compounds in the finished product. Moreover, the article describes the biotransformation of hop-derived compounds by traditionally used *Saccharomyces cerevisiae* yeast, as well as less commonly used non-*Saccharomyces* yeast. The paper outlines the current state of knowledge on biotransformation of hop-derived hydrocarbons, terpenoids, esters, sulfur compounds and glycosidically bound aroma precursors.

Keywords: yeast, beer, hop, biotransformation, terpenoids, esters

1. INTRODUCTION

Beer has continued to enjoy undiminishing consumer appreciation over the years. According to the WHO (WHO, 2019) it is the most popular alcoholic beverage in Europe, and its average annual per capita consumption for 2018, depending on the country, is 33-141 L. Traditionally, for many years the highest consumption has been observed in the Czech Republic, where the amount for 2018 was 141 L. Subsequent countries in terms of consumption are Austria, Germany and Poland, respectively 107, 102 and 100 L per capita. The size of this market can be exhibited by production volume, which only for European countries reached 406 050 hL in 2018 (WHO, 2019; The Brewers of Europe, 2019). It is a market that is dynamically developing and looking for new ideas, as evidenced by the emergence of the phenomenon called the craft beer revolution. This phenomenon occurred in different countries at different times, but it can be assumed that it took place at the turn of the last few decades. It has resulted in breaking the market, hitherto homogenized by large breweries and the emergence of many new beer styles, as well as the evolution of the existing ones, including those in which hops plays a key role (Garavaglia & Swinnen, 2017). Examples of styles particularly focused on hops include beers from India Pale Ale family, especially styles such as West Coast IPA or New England IPA. In recent years, hop-heavy styles gained substantial attention. In the finished product, hops no longer provides just bitterness, but imparts a whole range of aromas to the beer, including herbal, spice, floral, citrus, fruity, pine and grassy. Those fragrances are largely obtained by the addition of hops at various stages of the beer production process. However, the aroma of a given hop variety is usually far different from the aroma of beer made with its contribution. The final aroma of a beverage is acquired from complex combination of substances derived from malt, hops, other additives, and yeast metabolism. This intricate matrix of compounds undergoes dynamic changes during the beverage production process, due to physical, chemical as well as biochemical processes.

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The biochemical transformation of compounds by yeast cells, termed biotransformation, has attracted particular interest in recent years. The ability to oxidize and reduce compounds, as well as the enzymatic activities of β-glucosidases and hydrolases allow the transformation of aromatic and odorless compounds into other ones with different sensory properties (Praet et al., 2012). Various strains of *Saccharomyces cerevisiae*, as well as non-*Saccharomyces* yeast may exhibit different enzymatic activities towards hop-derived compounds, resulting in different sensory attributes of the beverage. In addition to the *S. cerevisiae* yeast biotransformation capacities, the article attempts to discuss these phenomena in yeast outside the genus *Saccharomyces*, due to their different enzymatic activities and their increasing use in the brewing industry. Knowledge of these activities can help brewers steer the sensory characteristics of the product they produce in the desired direction by choosing the adequate yeast strain.

The aim of this paper is to present the current knowledge on the biotransformation of hop-derived compounds by *S. cerevisiae* yeast, as well as non-*Saccharomyces* yeast.

2. COMPOSITION OF HOPS AND THEIR ROLE IN BREWING

Nowadays, hops (*Humulus lupulus* L.) are considered an essential ingredient, which imparts a specific taste and aroma to the beer. From the brewers' point of view, the main components of hops are the hop resins, also known as hard resins. They consist of α- and β-acids, which introduce the bitterness to the beer. Second component of hops are essential oils, also known as soft resins. They contain among others, compounds responsible for the aroma and flavor (Moir, 2000; Preedy, 2011). According to the literature, essential oils make up 0.5-3% of the hop cone dry weight, depending on the variety. Some sources state that their content can be as high as 4% (Preedy, 2011; Rutnik et al., 2021). Due to the multiplicity of compounds, their exact number in this fraction is difficult to determine. A paper published in 1992 by Nickerson & Van Engel (Nickerson & Van Engel, 1992) stated, that the number of compounds in this fraction could be over 250. However, more recent studies have suggested that it could be closer to 1000. Sharpe and Laws (Sharpe & Laws, 1981) separated constituents of this fraction into 3 groups: hydrocarbons, oxygen-containing compounds, and sulfur compounds. Chemically, 50-80% of the hop essential oil compounds are hydrocarbons, which can be divided into monoterpenes, sesquiterpenes and aliphatic compounds. The most quantitively important compound of monoterpenes is myrcene. It accounts for 10-72% of all hop essential oils by weight. Among sesquiterpenes, largest fractions are α-humulene and β-caryophyllene (15-42% and 2.8-18.2% of hop essential oils by weight, respectively). Aliphatic compounds are present only in trace amounts. Hydrocarbons are characterized by spicy, herbal, woody, and green aromas. Because they are poorly soluble in water and evaporate almost completely during boiling, in unchanged form they are mainly found in beers in small amounts, mostly in dry-hopped ones (Aberl & Coelhan, 2012; Holt et al., 2019; Janish, 2021; Rutnik et al., 2021).

The second group, accounting for about 30% of hop oils are oxygen-containing compounds. This group include a multitude of compounds belonging to alcohols, esters, acids, ketones, aldehydes, lactones, and epoxides. They mostly impart floral and fruity flavors. The alcohols found in hops can be divided into monoterpene, sesquiterpene and aliphatic alcohols. Among alcohols, linalool, β-citronellol, nerol, geraniol and α-terpineol are the most abundant, and are present both as free molecules and bound to carbohydrates (Schwab et al., 2009; Holt et al., 2019; Janish, 2021; Rutnik et al., 2021). Hop oil can contain up to 15% of esters. Those found in beer can be divided into two groups: those formed during the fermentation and lagering stages, and esters that pass from the hops in unchanged form. Compounds in the first group are produced by a number of transesterification reactions. They can also be formed from short-chain carboxylic acids derived from hops and malt. However, the exact processes are yet poorly understood. Examples of the second group include isobutyl isobutyrate, isoamyl butyrate, 2-methyl isobutyrate, and many forms of geranyl esters (Praet et al., 2012; Schubert et al., 2021). Saturated and unsaturated aldehydes occur only in small quantities in hops, but (Z)-3-hexanal is one of the most important determinants of fresh hop aroma. These are perishable compounds that undergo rapid transformations, including reduction to their corresponding alcohols. Those found in beer are mainly formed during wort boiling, as a result of yeast metabolism as well as by random decarboxylation of organic acids (Preedy, 2011).

Hop oil also contains a wide range of methylated, branched, and unsaturated ketones, among which 2-undecanone is the most abundant. However, ketones found in the finished beer are mainly those produced by yeast (Aberl & Coelhan, 2012; Preedy, 2011; Rutnik et al., 2021). Since oxygen-containing compounds are characterized by greater hydrophilicity than hydrocarbons, they are more soluble in beer, and are considered to affect its aroma to a higher degree (Rutnik et al., 2021).

The last group are the sulfur compounds, which include thioesters, thiophenes, methylsulfides, polysulfides and episulfides. The human olfactory system is highly sensitive to sulfur compounds. Although they occur only in minute quantities in hops, they have a profound influence on aroma of hops and beer. 4-mercapto-4-methylpentan-2-one (MMP) is an example of the compound commonly desired by brewers. In low concentration it bestows a blackcurrant aroma, however in higher quantities it starts to resemble a cat urine. In some cases, hops may contain sulfur compounds from crop protection products. Of the volatile compounds, sulfur dioxide, hydrogen sulfide, thiols and sulfides are usually found in hops in the highest amounts. Volatile sulfur compounds are believed to mostly evaporate during the wort boil (Preedy, 2011; Rutnik et al., 2021).

Aroma differences between hops may result from variability between the cultivars, growing region, weather conditions, harvest time, as well as and drying, handling and storage methods (Rutnik et al., 2021). Steinhaus et al. (Steinhaus et al., 2007) demonstrated distinct differences in the distribution of the content of selected compounds in Hellertau Perle, Hellertau Hersbrucket Spat, Slowenian Golding, Hellertau Smaragd and US Cascade hops. The study by Aberl & Coelhan (Aberl & Coelhan, 2012) on the chemical composition of 24 hop varieties, showed differences in total oil content between the cultivars. Different distributions of selected esters, ketones, epoxides, terpene alcohols, sesquiterpenes and monoterpenes were found (Aberl & Coelhan, 2012).

The concentration of volatile compounds as well as their precursors originating from hops is strictly dependent on the applied hopping methods. Thus, by using appropriate hopping regimes it is possible to direct the sensory characteristics of beer in a desired direction. Hops can be added at any of the beer production stages, but are mainly added during filtration, wort boiling, whirlpooling, fermentation and secondary fermentation. The method of hopping is important in the context of biotransformation. Actions that increase the concentration of substrates that undergo biotransformation reactions may exacerbate the degree to which this phenomenon affects the sensory attributes of the finished product.

The first of the above-mentioned methods, also known as first wort hopping, was widely used during the 20th century, however its usage faded over the next years. Only relatively recently it has started to be used again. This method involves adding hops to the wort during filtration and leaving them in the wort till the end of boiling. It is believed that adding hops in this manner provides a subtle, unobtrusive hop aroma and a more uniform bitterness. This technique has not been thoroughly researched, but it's safe to assume, that nearly all the volatiles evaporate when first wort hopping is used (Colicchio, 2012; Podeszwa & Harasym, 2016).

The classic approach to the hopping process, is to add hops during the boil. Hops designated to provide bitterness, usually those with high α-acid contents are added early in the boiling process. The main purpose of early hopping is to maximize the isomerization of insoluble, tasteless α-acids to bitter, water-soluble iso-α-acids, at the expense of evaporating a significant portion of the volatile compounds. Of the volatiles, hydrocarbons are among the most evaporative. They are usually found only in dry hopped beers. For example, 50% of myrcene evaporates within 10 minutes of boiling, and it evaporates completely within the typical 60 minutes' boil (Janish, 2021).

Hoping targeted for aroma is carried out in the last 15 minutes of the boil, but also during the whirlpool to reduce the evaporation of volatile compounds and to avoid excessive bitterness. In these types of hopping the isomerization of α-acids is limited, especially in whirlpool hopping. Thanks to shorter exposure to the high temperature, more of the volatile compounds are retained. Some of them can undergo thermal transformation and oxidation. Beer hopped in this way contain trace amounts of hop-derived monoterpene and sesquiterpene hydrocarbons, but do contain significant quantities of oxidized terpene derivatives, ethers, ketones, and esters (Podeszwa & Harasym, 2016; Rettberg et al., 2018).

Dry hopping is performed by adding hops to a beer which has finished fermentation or is in the process of it (for example, substantial amounts of hops are added during the fermentation to New England IPA beers). The sensory qualities of a beer hopped in this manner are significantly different from beers hopped in previously described ways. The degree to which individual compounds are extracted during this process

depends largely on their chemical structure. Polar compounds, such as linalool pass to the beer in large quantities, while non-polar compounds, such as myrcene are found only in trace amounts, mainly due to their low solubility. In a study conducted by Hauser et al. (Hauser et al., 2019), spent hops used for 24 h dry hopping contained 77% of the original essential oil content The concentration of terpene and sesquiterpene hydrocarbons decreased only slightly, while the levels of oxygenated compounds like terpene alcohols and aldehydes decreased up to 10% of the original amount. Factors such as beer temperature, contact time, use of mechanical agitation, fermenter size, alcohol size and IBU levels are known to have a significant impact on the extraction levels during dry hopping. When hops are added to beer containing live yeast cells, extraction may be accompanied by occurrence of biotransformation. Additionally, CO_2 volatilization during the fermentation can remove up to 80% of beer volatiles (Ferreira et al., 1996). Using late dry hopping could counteract this process. One of the undesirable effects of dry hopping is phenomenon called hop creep. If dextrins are present in the beer, they can be broken down into fermentable sugars due to the presence of hydrolyzing enzymes in the hops, which can result in refermentation. Additionally, prolonged dry-hopping time can increase extraction of undesirable vegetative materials, which is believed to cause appearance of 'grassy' flavors (Hauser et al., 2019; Janish, 2021; Oladokun et al., 2017; Podeszwa & Harasym, 2016; Rettberg et al., 2018). Sharp et al. (Sharp et al., 2017) studied the influence of 3 different regimes: 60-minute boil, 25-minute whirlpool rest and 3 day dry-hopping on the concentration of glycosidically bound terpenes and found no differences between studied hopping regimes. Levels of glycosides were determined by marking aglycone content resulting from the glycoside hydrolysis using β-glucosidase (Sharp et al., 2017).

In recent years, systems aimed to facilitate the process of dry hopping under industrial conditions have emerged, such as hop cannons, torpedoes, or rockets. They are referred to as advanced dry-hopping techniques. Examples of such system are: HopGun®, HOP-X-TRACT or HOPSTAR®. They are designed to maximize the extraction of essential oils during dry-hopping, as well as reduce dry-hopping time, hop consumption, and beer loss. At the same time, they minimize the introduction of oxygen, which is known to degrade aroma stability and increase the formation of staling aromas (Kemp et al., 2021; Podeszwa & Harasym, 2016).

3. BIOTRANSFORMATION OF SELECTED GROUPS OF AROMA COMPOUNDS IN BEER

Yeast of the genus *Saccharomyces* are known to exhibit a broad range of possible biotransformation reactions. However, many of the publications focuses on the biotransformation in the wine industry, especially in the case of non-*Saccharomyces* yeast. But wine-related results may be translatable for brewing. Researchers are uncovering possibilities for the use of yeasts recently considered as unrelated to brewing or bearing the label of harmful microflora. One such work is a paper by Krogerus et al. (Krogerus et al., 2022). It is widely believed that non-*Saccharomyces* yeasts produce more secondary metabolites (including fusel alcohols and esters) at the expense of lower ethanol and biomass production. An example of the commercial application of non-*Saccharomyces* yeasts can be found in the use of *Dekkera* and *Brettanomyces* for the production of Belgian lambic, which greatly facilitates a process that has hitherto relied on spontaneous fermentation (Krogerus et al., 2022; Toh et al., 2020).

3.1. Terpenoids

Terpenoids are group of compounds widely distributed in nature. They are synthesized from isopentyl pyrophosphate and can be divided into monoterpenoids (C_{10} consisting of two isopentyl units), sesquiterpenoids (C_{15} consisting of three isopentyl units), diterpenoids (C_{20} – four isopentyl units), carotenoids, sterols, phytols and quinones. Monoterpenoids and sesquiterpenoids are known for their intense sensory characteristics (Kovačevič & Kač, 2002; Preedy, 2011). Ascomycetous yeasts lack the ability to utilize monoterpenes as a sole carbon source (Satyanarayana & Kunze, 2009).

3.1.1. Terpene Hydrocarbons

Among the compounds in this group, biotransformation studies have focused most heavily on myrcene. Myrcene, despite making up a substantial portion of hop essential oils, above its sensory threshold (9.5 – 1000 μg/L) is found mainly in dry-hopped beers. It imparts herbaceous, resinous, green, balsamic, and fresh hops aroma.

Current literature indicates that yeast lacks the ability to transform compounds from this group found in hops (Haslbeck et al., 2017; Peltz & Shellhammer, 2017; Rutnik et al., 2021). Only few studies concerning biotransformation of aliphatic and aromatic hydrocarbons by yeast are available (Hashem et al., 2018). Myrcene can be transformed by strains of organisms such as *Rhodococcus erythropolis* and *Pseudomonas aeruginosa* (Esmaeili & Hashemi, 2011; Thompson et al., 2010).

Known transformations of myrcene occur at the boiling stage, such as its oxidation to myrcenol, as well as through autooxidation upon improper hops storage to compounds as α-pinene, β-pinene, linalool, geraniol, geranial, and neral. α-humulene, which is another hydrocarbon present in high quantities in hops is oxidized during wort boiling to monoepoxides, diepoxides, and triepoxides. Compounds from the last fore mentioned group are not present in finished beer. β-caryophyllene can undergo oxidation during boiling and lagering of beer, and currently 9 products of its oxidation have been identified (Rutnik et al., 2021). Haslbeck et al. (Haslbeck et al., 2017) traced the fate of myrcene during beer production using *S. cerevisiae* yeast. The authors determined its significant content in volatilized fermentation gas and on the surface of yeast cells. The loss of this compound in beer, due to the adsorption to yeast cells was up to 99%. This phenomenon is thought to be related to the non-polar structure of hydrocarbons molecules. They do not dissolve in the polar environment of beer, instead they adsorb on the non-polar surface of the cell wall. It is important to note, that myrcene does not exhibit anti-fungal activity (Peltz & Shellhammer, 2017). King and Dickinson (King & Dickinson, 2003) proposed, that hydrocarbons can dissolve in beers with higher alcohol levels, but this is contradicted by the results obtained by Haslbeck et al. (Haslbeck et al., 2017). The authors failed to dissolve noticable amounts of myrcene bound to yeast cells, even when using a 50% ethanol solution (Haslbeck et al., 2017; King & Dickinson, 2003; Preedy, 2011; Rutnik et al., 2021).

3.1.2. Terpene alcohols

Monoterpene alcohols are considered a very important group of volatiles in the case of brewing, due to their intense sensory characteristics. Interestingly, some of the compounds in this show strong anti-fungal activity. However, their concentrations in beers, even dry-hopped ones are few orders of magnitude less than the minimal inhibitory concentration (citral is a compound with one of the lowest MIC, at 96.7 mg/L) (Lafontaine & Shellhammer, 2018; Scariot et al., 2021).

Not all the monoterpene alcohols found in beer may originate from used raw materials. There are literature reports documenting the ability of yeast to synthesize trace amounts of monoterpenes *de novo*. Such capacities were found in strains of *Kluyveromyces lactis*, *Torulaspora delbrueckii*, *Kloeckera apiculata*, *Metschnikowia pulcherrima*, *Candida stellata* and *Ambrosiozyma monospora*. Carrau et al. (2005) showed that *S. cerevisiae* can synthesize linalool and α-terpineol, and in smaller amounts citronellol and geraniol. Previously, it was believed that synthesis of terpenes by yeast is related with sterol biosynthetic pathway. However, the authors suggest that in *S. cerevisiae* production of terpenes occurs probably in mitochondria and is affiliated with leucine metabolism (Carrau et al., 2005). Known biotransformation reactions catalyzed by *S. cerevisiae* are presented in Figure 1. In the results obtained by Haslbeck et al. (Haslbeck et al., 2018) regarding *de novo* production of terpenes, it was geraniol that was synthesized in the highest amounts. In one of the samples, it was present at its threshold concentration. These results suggest that the ability to synthetize terpenes is strongly strain dependent. What is important, the authors hypothesized that β-citronellol content usually increases mainly due to *de novo* synthesis, rather than geraniol reduction (Haslbeck et al., 2018). The possibility of the production of monoterpenes by yeast should be considered in studies of new yeast strains, to avoid the confusion that some compounds undergo biotransformation, when they are actually, at least partially biosynthesized. Further studies on the issue of terpene synthesis focus mainly on the production of these compounds by engineered yeasts. Sensory characteristics of described terpenes and their detection threshold (determined in beer, wine or model solutions) are presented in Table 1 (Peltz & Shellhammer, 2017; Sales et al., 2020; Sanekata et al., 2018).

King & Dickinson (2000) evaluated the biotransformation capacities of the *Saccharomyces cerevisiae* IWD78, *Torulaspora delbrueckii* NCYC 696 and *Kluyveromyces lactic* IFO1267 yeasts, on minimal medium supplemented with the respective monoterpenoids. In this study, none of the tested yeasts showed the ability to biosynthesize monoterpenes. During the experiment, the concentration of the tested compounds decreased. It was probably due to the evaporation, as well as their adsorption on the yeast cell walls. *S. cerevisiae* yeast expressed the

ability to reduce geraniol to citronellol, and translocate geraniol to linalool, but trace amounts of α-terpineol were also detected. α-terpineol probably resulted from the cyclization of linalool. No transformation products of citronellol were detected. *T. delbrueckii* showed the ability to produce linalool from geraniol, which was later converted into α-terpineol. However, none of the monoterpenes were transformed into citronellol. Nerol was converted mainly to α-terpineol, but trace amounts of linalool and geraniol were also detected. Again, no transformations of citronellol were detected. *K. lactis* showed the widest spectrum of reactions. It produced both citronellol and linalool from geraniol and converted linalool to α-terpineol. Nerol was transformed to α-terpineol, linalool, geraniol, and citronellol, the latter of which was probably formed directly from geraniol. *S. cerevisiae* and *T. delbruecki* produced (+) and (-) enantiomers of linalool and α-terpineol in equal amounts (King & Dickinson, 2000).

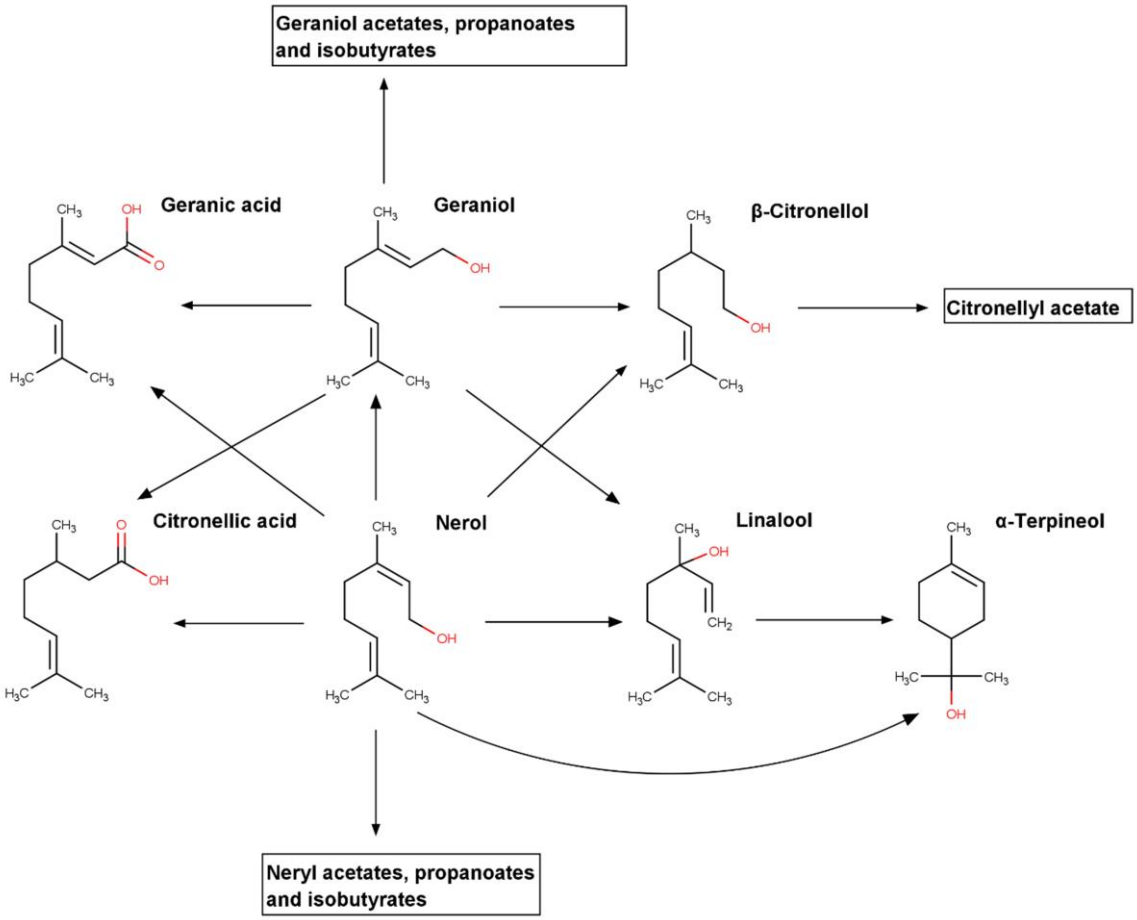


Figure 1. Known biotransformation reactions catalyzed by *S. cerevisiae*. Mentioned esters could undergo further hydrolysis (Ohashi et al., 2021; Steyer et al., 2013; Takoi et al., 2017; Wei et al., 2020)

Table 1. Sensory characteristics of described terpenes and their detection threshold (determined in beer, wine or model solutions) (Steinhaus & Schieberle, 2000; Inui et al., 2013; Peltz & Shellhammer, 2017; Sales et al., 2020; Sanekata et al., 2018).

Compound	**Sensory characteristics**	**Detection threshold (µg/L)**
α-Terpineol	Pine-like, woody	280-350
β-Citronellol	Fruity, green citrus	25
Citronellic acid	Fatty, waxy, heavy, floral	1% solution
Geranic acid	Oily, floral, plant-like	2200
Geraniol	Floral, rose, perfume	53
Linalool	Floral, citrus, coriander	9
Nerol	Fruity, green	500

In a study by Serra Colomer et al. (2020), 2 among the *Brettanomyces bruxellensis* strains tested showed a very intense capacity to transform geraniol into β-citronellol in beers after primary fermentation, where β-citronellol levels were similar to those typically detected in dry-hopped beers. In one sample, levels of this compound reached 31.5 μg/L, which is well above its sensory threshold (25 μg/L). Interestingly, the strains that produced high quantities of β-citronellol were also characterized by the lowest β-glucosidase activity. It should be mentioned that the authors did not indicate that they tested the ability of the studied strains to synthesize these compounds *de novo*. To a lesser degree, the strains showed the ability to convert linalool to α-terpineol. Similar biotransformation routes were detected in dry-hopped beers (Serra Colomer et al., 2020; Takoi et al., 2010).

The *Lachancea thermotolerans* strain MN477031 tested by Zdaniewicz et al. (2020) showed the ability to completely reduce limonene, perillene, nerol, and humulene present in the wort. Previously absent citronellol, β-damascenone, and nerolidol appeared in the beer. Compounds such as geraniol, perilla alcohol, β-farnesene, caryophyllene oxide, nerolidol, humulene epoxide II, aromadendrene epoxide, α-muurolol, and τ-muurolol were present in higher amounts than in the control sample with *S. cerevisiae*. However, authors did not investigate which processes lead to the higher content of those compounds (Zdaniewicz et al., 2020).

Terpene alcohols can also undergo esterification reactions. Esters of these compounds typically have a much higher detection threshold, compared to the original compounds. They are relatively rarely studied in publications, so it is not possible to determine how widespread these reactions are. For example, the detection threshold for citronellyl acetate is 250 mg/L, whereas detection threshold for citronellol is only 25 mg/L. According to the literature, *S. cerevisiae* can esterify geraniol and nerol to their corresponding acetates, propanoates and isobutyrates. Citronellol can be esterified to citronellyl acetate. Geranyl acetate has been detected in ciders fermented with *Pichia kluyveri* and *Hanseniaspora vinae* (Wei et al., 2020). Again, the occurrence of these reactions is probably strongly dependent on the strain, as well as the genus of yeast (Steyer et al., 2013; Van Opstaele et al., 2020).

Ohashi et al. (2021) showed that *S. cerevisiae* yeast having a single deletion of gene encoding alcohol dehydrogenase can biotransform geraniol and nerol to geranic acid, citronellic acid and citronellol. Geranic and citronellic acids have very high sensory thresholds, compared to the original compounds. It makes this enzymatic pathway potentially detrimental to beverage sensory attributes. However, these compounds possess unique aromatic enhancing properties for hop-derived terpenoids, even at subthreshold level, so the exact impact of this activity may require further study. To our knowledge, content of those compounds hasn't been tested in beers except in one paper by Takoi et al. (2017). There are no analogous studies for non-*Saccharomyces* yeast (Ohashi et al., 2021; Sanekata et al., 2018).

3.2. Esters

Esters are considered to be the most important group of fermentation derived flavor active compounds. They impart fruity, floral, and solvent-like fragrances, among other compound-specific flavors and aromas. Among the esters identified in hop oil, there are mainly a homologous series of methyl esters, from hexanoate to dodecanoate, as well as many branched-chain and unsaturated methyl esters (Bamforth, 2001). Ethyl isobutyrate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate and ethyl 4-methylpantanoate have a strong aroma and are believed to be responsible for the citrous flavor of beer. Geranyl esters (e.g., geranyl acetate, geranyl propionate and geranyl isobutyrate) are also a quantitatively important group. Although geranyl esters constitute a major faction of hop oil esters, they are found in only trace amounts in the finished beer. It is possible, that these trace contents are present thanks to the esterification by yeast, as mentioned earlier. It is believed that most of the geraniol found in beers is derived from the hydrolysis of its hop-derived esters (Forster et al., 2014; Rutnik et al., 2021). The results obtained by Forster et al. (Forster et al., 2014) are consistent with this thesis. Authors examined the beers brewed with Cascade and Comet hops, characterized by high levels of geranyl acetate. Finished beers obtained with these hops contained low amounts of this ester, while being characterized by a high level of geraniol (Forster et al., 2014). Takoi et al. (2017) divided hops into those that contain high geraniol content, and those containing mainly its precursors. Using varieties that contain mainly bound geraniol could allow

provide higher levels of this compounds in finished beer (Takoi et al., 2017).

Esters undergo significant chemical changes during fermentation and storage of beer. In addition to the chemical and physical factors that affect these perishable compounds, there are a number of factors that regulate their production by yeast. Literature lists that esters production can be altered by yeast strain, temperature, hydrostatic pressure, and wort composition (contents of sugars, free amino nitrogen, oxygen, and unsaturated fatty acids), among others (Pires et al., 2014). During beer aging, esters occurring above their chemical equilibrium levels become progressively hydrolyzed. Acetate esters of higher alcohols hydrolyze faster than their ethyl counterparts. Simultaneously also opposite reaction takes place, as the formation of esters of ethanol and organic acids occurs (Preedy, 2011).

It should be noted that the hopping method is of fundamental importance for the concentration of hop derived esters in beer/wort. Beer in which late hopping and/or dry hopping was used are characterized by a higher content of these compounds. For example, isobutyric esters are mainly found in late and dry hopped beers. No less important is the variety of hops. An example is the study by Takoi (Takoi, 2016), where the authors examined, among others, the concentrations of esters in late hopped beers. The levels of methyl isobutyrate in beer hopped with Bravo hops was 1.3 μg/L, while in that hopped with Polaris its amount was as high as 54 μg/L (Takoi, 2016). Sensory characteristics of described esters and their detection threshold (determined in beer, wine or model solutions) are presented in Table 2 (Burdock, 2016; Meng et al., 2017; Peltz & Shellhammer, 2017).

The esters mainly found in beer can be divided into two main groups: the acetate esters, and medium chain fatty acid (MCFA) ethyl esters (Menoncin & Bonatto, 2019). Esters of the first group are formed by the condensation reaction carried out by acetyltransferases, between higher alcohols and acetyl-CoA. Those belonging to the second group are formed by condensation of ethanol, and acyl-CoA, catalyzed by acyltransferases (Piškur i Compagno 2014). Esters can be degraded by enzymes from the esterases group. These enzymes catalyze the hydrolysis of the ester bond, but importantly, in some cases they can also form it. The balance between hydrolyzing and synthetizing activities of the enzymes from this group is important for the final concentration of esters in the product (Saerens et al., 2010). Kiyoshi et al. (1998) showed, that by changing balance between alcohol acetyltransferase and esterase active ties in *S. cerevisiae*, it is possible to manipulate the rate of isoamyl acetate production. Many enzymes from esterase group have a characteristic catalytic triad, which is also found in enzymes from other groups. It is possible that enzymes belonging to other groups, such as serine proteases can, to some degree, hydrolyze ester bonds (Kiyoshi et al., 1998; Saerens et al., 2010).

Takoi et al. (2017) investigated the fate of branched chain esters during fermentation of late hopped wort with *S. pastorianus*, at a hop dose equivalent to 0.8 g/L, analyzing 42 hop varieties. Hop-derived isobutyric esters consisting of isobutyric acid and three branched-chain alcohols (isobutanol, isoamyl alcohols and 2-methylbutanol), and ethyl esters of branched-chain fatty acids (isobutyrate, isovalerate and 2-methyl butyrate acids) were analyzed. During the time from wort production to obtaining finished product, the concentration of all isobutyric esters decreased, in some cases by as much as half. On the other hand, examined ethyl esters were present in the wort in only trace amounts, and their concentration increased during fermentation. The authors propose that 2-methylbutyl isobutyrate can be transesterified to ethyl isobutyrate (Takoi et al., 2017). In continuation of these studies, Takoi (Takoi, 2019) proposed a biotransformation pathway, in which isobutyric, isovaleric and 2-methylbutyl acids undergo esterification reactions to their respective ethyl esters and isobutyl isobutyrate, isoamyl isobutyrate and 2-methylbutyl isobutyrate are transesterified to ethyl isobutyrate (Takoi, 2019). One solution to ensure the higher concentration of these hop-derived esters is to use dry hopping. Fate of hop-derived branched-chain fatty acids and their esters during the fermentation with *S. cerevisiae* is presented in Figure 2 (Takoi, 2019).

Brendel et al. (2020) reports the formation of ethyl esters of 2- and 3-methylbutanoic acid, and methylpropanoic acid induced by the dry-hopping process. The amounts of esters in beer subjected to this process were higher than their content in hops, hence the authors indicate that their formation was initiated by the hopping process. However, the authors did not find a factor responsible for their formation. It was independent of the fermentation process as well as yeast activity. These esters were probably not formed from monocarboxylic acids derived from hop humulones and lupolones, as increased concentration of monocarboxylic acids did not translate into higher concentration of esters. Their formation is probably due to transesterification reactions (Brendel et al., 2020).

Table 2. Sensory characteristics of described esters and their detection threshold (determined in beer, wine or model solutions) (Verstrepen et al., 2003; Kishimoto et al., 2006; Burdock, 2016; Meng et al., 2017; Peltz & Shellhammer, 2017).

Compound	Sensory characteristics	Detection threshold (µg/L)
Ethyl 2-methylbutanoate	Powerful green-fruity, apple-like	18
Ethyl 3-methylbutanoate	Green, orange-like	6.89
Ethyl 4-methylpentanoate	Green, melon-like	0.75
Ethyl isobutyrate	Fruity	0.22
Ethyl isovalerate	Fruity, vinous, apple-like	n/a
Geranyl acetate	Floral, soapy	371
Geranyl isobutyrate	Light rose	13
Geranyl propionate	Fruit, flowery	n/a
Isoamyl acetate	Sweet, fruity, banana, fragrant	93.93
Isobutyl isobutyrate	Sweet, fruity, winey	37-4370
Methyl isobutyrate	Fruity (apple-pineapple)	19
Methyl geranate	Waxy, green, fruity, flower	n/a

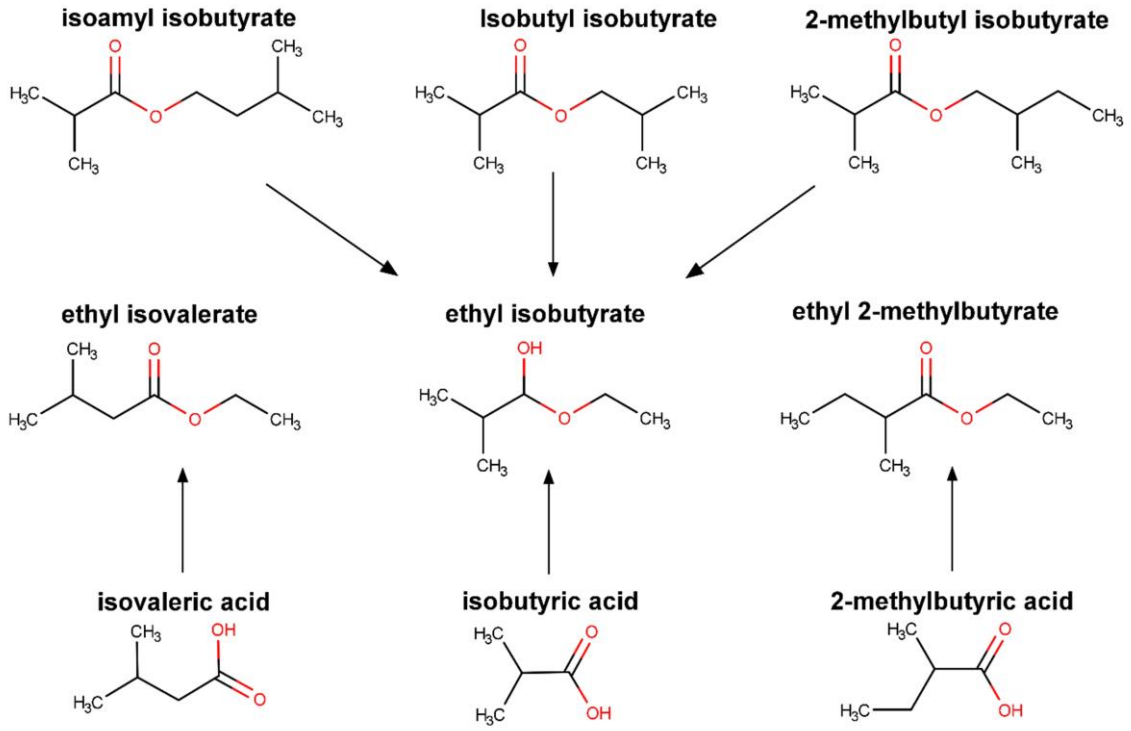


Figure 2. Fate of hop-derived branched-chain fatty acids and their esters during the fermentation with *S. cerevisiae* (Takoi, 2019)

Methyl esters derived from hops are hydrolyzed or converted to ethyl esters. The methyl esters found in beer are mainly due to the esterification of methanol resulting from the fermentation process (Haslbeck et al., 2018). Methyl esters of some conjugated acids, such as methyl geranate, are examples of compounds that do not undergo hydrolysis and can be determined in the finished product (Bamforth, 2001).

3.3. Sulfur Compounds

Sulfur compounds are perceptible by humans in very low concentrations, and compounds from this group found in the highest contractions cause serious defects in beer. Compounds such as SO_2 (sulfidic, burnt match), H_2S (sulfidic, rotten eggs), DMS (sweetcorn) and 3-methyl-2-butene1-thiol (skunk) are responsible for major beer defects (Preedy 2009). However, despite these negative consequences, sulfur compounds can impart desirable fruity flavors. Among the major desirable compounds in this group are: 3-

mercaptohexanol (3MH), 4-mercapo-4-methylpentan-2-one (4MMP) and 3-mercaptohexyl acetate (3MHA) (Michel et al., 2019). These compounds can occur in hops as flavor-active and flavorless precursors. Roland et al. (2016) studied in which forms 4MMP and 3MH occurs in 10 hop varieties. More than 99% of the 3MH present in hops was in the form of its precursors. Interestingly, the amount of bound 3MH in Cascade hops far exceeded its content found in grapes. In the case of 4MMP, the unbound fraction predominated. The authors demonstrated the presence of glutathionylated conjugates of 4MMP and 3MH, and cysteinated conjugates of 4MMP (Roland et al., 2016). These results explain the data obtained by Kishimoto et al. (Kishimoto, 2008). In the authors' study, during the boiling of hopped wort, the 4MMP content decreased, while the levels of 3MH increased. Additionally, the increase was faster at 100°C than at 90°C. The results suggest that 3MH was hydrolyzed during boiling. 3MH was also detected in unhoped beers, indicating that malt may also be a source of precursors of this compound (Kishimoto, 2008). Sensory characteristics of described sulfur compounds and their detection threshold (determined in beer, wine or model solutions) are described in Table 3 (Copper et al., 2021; Mafata et al., 2018; Michel et al., 2019; Peltz & Shellhammer, 2017; Zott et al., 2011).

β-lyases have been shown to be partially responsible for the conversion of odorless, bound thiols from grapes into their flavor-active forms. In a study by Michel et al. (Michel et al., 2019) the authors examined the activity of this enzyme among 158 strains belonging to *S. cerevisiae*, *S. pastorianus* and *T. delbruecki*. Selective medium containing S-methyl-L-cysteine as a sole nitrogen source was used to determine β-lyase activity. Additionally, IRC7 genetopes, which were thought to determine the capacities of yeast to release conjugated thiols were examined. The authors do not recommend screening with this substrate or analyzing IRC7 genotypes as it was found during the study that this method presents low specificity. They give as an example a strain that did not show growth on the medium but provided biotransformation levels similar to strains that did grow on the medium. The IRC7 gene was distributed very evenly among the strains tested. The authors also performed dry-hopped wort fermentation, using 2 strains from each species tested. All of them showed the ability to release bound thiols. Interestingly, higher amounts of 4MMP were detected in beers fermented with *S. cerevisiae* and *S. pastorianus*, than in those fermented with *T. delbrueckii*.

Zott et al. (2011) studied the release capacity of 4-methyl-4-sulfanylpentan-2-one (4MSP) and 3-sulfanylhexan-1-ol (3SH) from their S-cysteine conjugates in wines, by 15 strains belonging to 7 species. While all of the strains tested showed a lower capacity to release 4MSP than *S. cerevisiae*, the strains of *M. pulcherrima*, *T. delbrueckii* and *K. thermotolerans* showed a significantly higher capacity to release 3SH than the *S. cerevisiae* strains tested (Zott et al., 2011). Although aforementioned paper refers to wines, these compounds are also found in hops. 4MMP it thought to be one of the main compounds responsible for Cascade hop aroma, while 3SH is found in beers, hopped with Nelson Sauvin among others (Takoi et al., 2010). These results suggest that yeast from the genus *Saccharomyces* can release high amounts of 4MSP and 4MMP from their precursors.

In a study by Chenot et al. (2021) on the potential for release of cysteinated and glutathionylated sulfanylalkyl alcohols by *S. cerevisiae* in beer, these compounds were only detected after the maturation stage. Authors used unhopped wort, enriched with conjugated forms of thiols. These results indicate that maturation is a key step during which free thiols are released from yeast cells (Chenot et al., 2021). The results of this study can be extended with findings in paper published by Nizet et al. (2014) on the appearance of thiols during the refermentation process. Bottle beer refermentation is a frequently used process in home brewing. It is known to influence the sensory characteristics of beer. When this process is carried out correctly, its impact is judged to be positive. Nizet et al. (Nizet et al., 2013) showed that this process significantly increases the content of volatiles belonging to sulfanylalkylalcohols, sulfanylalkylacetates, and sulfanylalkylcarbonyles. The previously absent 1-Sulfanylpentan-3-ol, 3-sulfanylhexan-1-ol, and others appeared in beer. The concentration of other compounds, such as 2-Sulfanylethyl acetate (2SEA) significantly increased. The authors suggest that these compounds may be formed, among others, by transformation of α, β-unsaturated carbonyles with H_2S, substitution of H_2S with alcohols, and hydrolysis of hop cysteine adducts by yeast-derived lyases. These studies show that the content of volatile sulfur compounds increases further after the completion of the fermentation process (Nizet et al., 2013, 2014).

Table 3. Sensory characteristics of described sulfur compounds and their detection threshold (determined in beer, wine or model solutions) (Copper et al., 2021; Mafata et al., 2018; Michel et al., 2019; Nizet et al., 2013, 2014; Peltz & Shellhammer, 2017; Zott et al., 2011).

Compound	Sensory characteristics	Detection threshold (ng/l)
1-Sulfanylpentan-3-ol	Green odor	n/a
2SEA	Onion	n/a
3MH	Rhubarb, grapefruit flavor	60
3MHA	Passion fruit, grapefruit flavor	4.2
3SH	Grapefruit like flavor, rhubarb	0,8
4MMP	Black currant, passion-fruit-like flavor, tomcat urine	0.8
4MSP	Tree-like flavor	1.5

3.4. Glycosidically Bound Aroma Precursors

During the processes aimed to obtain the fermentation-ready wort, as well as during dry-hopping, odorless glycosides can pass from hops into the beer. As mentioned previously, the hopping method used does not seem to have a significant effect on the transfer rate of glycosides into beer. β-glucosidase (EC 3.2.1.21) is considered to be the most important enzyme that hydrolyzes glycosidically bound compounds. Methods of the detection of β-glucosidase activity are mainly based on the hydrolysis of a glucosidic substrate, which leads to an increase in biomass or the release of a detectable aglycone (Daenen et al., 2008). A study presented by Lafontaine et al. (2021) indicates the glycoside content of hops, depending on the cultivar, to be in the range of 0.46 – 1.81 1 μg/g hops, among which bound α-terpineol and linalool are the largest fraction. The authors project a new view on glycosidically bound volatile compounds, showing that glycosides in hops are mainly found in the form of pentose-hexose monoterpenols, and not hexose-monoterpenols as previously believed. Since β-Glucosidases lack endoglucanase activity, pentose-hexose monoterpenols cannot be released with their participation alone. Therefore, it is suspected that α-L-arabinofuranosidase and/or α-L-rhamnosidase or β-xylosidase, may also have an important function in the hydrolysis of hop-derived glycosides. However, these activities are currently poorly studied in yeast (Lafontaine et al., 2021; López et al., 2015; Rodríguez et al., 2007; Zietsman et al., 2011). Yanai and Sato (Yanai & Sato, 2000) studied 386 yeast strains belonging to 11 genera, examining their ability to hydrolyze p-nitrophenyl α-L-rhaminopyranoside, of which α-L-rhamnosidase activity was detected only in *S. cerevisiae, Cryptococcus terreus, Pichia capsulata,* and *Pichia angusta. P. angusta* expressed the highest enzymatic activity (Yanai & Sato, 2000). Belda et al. (Belda et al., 2016) studying α-L-arabinofuranosidase activity among 770 strains detected it only in *Meyerozyma guilliermondii, Wickerhamomyces anomalus, Aureobasidium pullulans, Rhodosporium toruloides* and *Cryptococcus amylolentus* (Belda et al., 2016). Manzanares et al. (Manzanares et al., 1999) studied the presence of β-D-xylosidase activity among 54 strains belonging to the genera: *Candidia, Dekkera, Hanseniaspora, Metschnikowia, Pichia, Rhodotorula, Schizosaccharomyces* and *Zygosaccharomyces*. The authors investigated the ability to hydrolyze methylumbelliferyl-β-xyloside to 4-methylumbelliferone, which gives a fluorescent halo surrounding yeast growth, under UV light. Investigated activity was detected in only four out of seven *H. uvarum* strains, in two *H. osmophilia* strains tested and in two *P. anomala* strains (Manzanares et al., 1999). The current state of knowledge indicates that these enzymes are present in very few yeast species, hence further studies may be required to investigate the exact mechanism of hop-derived glycosides hydrolysis.

Studies by many authors have shown that *S. cerevisiae* have low β-glucosidase activity. In a study by Daenen et al. (2008), this activity was not found in any of the 9 *S. pastorianus* and 32 of *S. cerevisiae* strains tested. The authors indicate that the gene encoding β-1,4-glucosidase is present in very few *S. cerevisiae* strains. However, all yeasts in this genus synthesize β-1,3-glucanases EXG1, which, as reported by the authors, can hydrolyze some glycosidically bound volatile compounds. The intensity of this ability depends on the expression levels of this enzyme in the strain. A great advantage of this enzyme is substrate non specificity, which allows it to attack, among others, laminarin, pustulan, laminaribose, p-nitrophenyl-β-glucoside and 4-methylumbelliferyl (Daenen et al., 2008). In many publications on *S. cerevisiae,* authors use the term β-glucosidase, while the discussed activities may be due to β-glucanases. Sharp et al. (Sharp et al., 2017) suggests that 4-methylumbelliferyl-β-D-

cellobioside should be used in β-1,4-glucosidase activity assays, to avoid the action of exo-β-1,3-glucanases. The authors tested 80 commercial and research yeast strains. An important finding of the study is that yeast affiliation (ale, lager, *Brettanomyces*) is not a clear determinant of their glucoside hydrolyzing activity. The authors also tested the quantitative ability to degrade these compounds, by monitoring the octyl glucoside and 1-octanol content using HS-SPME GC-MS in 12°P wort. In this study, two yeast strains with low enzyme activity, and two with high activity were used against pure β-glucosidase as a control. Interestingly, pure β-glucosidase managed to hydrolyze 96% of octyl-glucoside, while the yeast, regardless of the activity only about 11% of the substrate within 10 days. The low degree of hydrolysis could be due to aerobic conditions and high glucose concentration at the beginning of the fermentation (Sharp et al., 2017). Haslbeck et al. (Haslbeck et al., 2018) studied 6 commercially available *S. cerevisiae*/*pastorianus* strains. Authors determined the exo-β-1,3-glucanases activity during the fermentation process to be in range of 0.067–0.168 U/L (Haslbeck et al., 2018).

Literature sources attribute higher β-glycosidic bond hydrolysis capacity to non-*Saccharomyces* yeast, compared to *S. cerevisiae* (Porter et al., 2019). Escribano et al. (Escribano et al., 2017) performed a screening of non-*Saccharomyces* yeast for extracellular β-glucosidase activity in wines. This study was performed using an API-ZYM test system (BioMe´rieux, France). Of the strains tested, 6% *L. thermotolerans*, 63% *M. pulcherrima*, and 14% *Z. balii* showed enzymatic activity. No activity was found in the tested strains of *D. hanseii*, *P. kluyveri*, *S. salmonicor*, *T. delbrueckii*, and *W. pratensis* (Escribano et al., 2017).

Despite the exclusion of β-glucosidase activity in *T. delbrueckii* by Escribano et al. (Escribano et al., 2017), Azzolini et al. (Azzolini et al., 2012) investigated this activity in wine fermentations in strain TD291 using 4-nitrophenol-β-D-glucopyranoside (pNPG) as a substrate. Authors determined that its activity is higher than that of the tested *S. cerevisiae* strains, at 0.9 and 0.43 mM/min/G (Azzolini et al., 2012). In a similar study by Maturano et al. (Maturano et al., 2012) using pNPG as substrate, this yeast achieved β-glucosidase activity equal to that of *S. cerevisiae* (Maturano et al., 2012).

Yeasts of the *Brettanomyces* genus are widely considered to exhibit high β-glucosidase activity. In a study by Serra Colomer et al. (2020) using the MAK129 β-glucosidase assay kit (Sigma Aldrich), the *Brettanomyces bruxellensis* strains tested showed no significant ability to release glycosidically bound monoterpenes. The authors explain this fact mainly by intracellular enzyme activity. Strains containing BbBgl2 and BbBgl1 MAK129 β-glucosidase ORFs had the highest β-glucosidase activity. In turn, strains which lacks or have a single gene showed negligible activity. A significantly higher intracellular activity of this enzyme than among the *B. bruxellensis* strains tested, was detected in *B. anomalus* (Serra Colomer et al., 2020). Daenen et al. (Daenen et al., 2008) also detected significant activity in *B. custerii* CMBSLD72 isolated from fermenting lambic beer, but it should be noted that this is a slow fermenting strain. The β-glucosidase of this strain shows a broad spectrum against alkyl- and aryl-β-d-glucosides. On YPD medium enriched with hop glycoside extract, the concentration of linalool reached 13 mg/L, whereas the concentration in the sample with the *S. cerevisiae* was only 2 mg/L (Daenen et al., 2008).

In a study by Rodríguez et. al (2007) in wines, *Candida guilliermondii* V2 and V5, *Candida pulcherrima* V6 and *Kloeckera apiculata* V9 strains showed the ability to produce β-glucosidase, and all except *K. apiculata* V9 produced β-xylosidase. The β-glucosidase activity in these strains was related to their cell growth. The maximum activity of the enzyme was observed at the end of the exponential growth phase and decreased gradually during stationary phase in *C. guilliermondii* V_5 and *C. pulcherrima* V_6. It remained at the same high levels in the other two tested strains during stationary phase (Rodriguez et al., 2007).

Pando Bedrinaña et al. (2012) studied 420 cider yeast strains for β-glucosidase activity against arbutin as a substrate, and for β-xylosidase activity against 4-methylumbelliferyl-β-D-xyloside. The strains were classified into species *Hanseniaspora valbyensis, H. uvarum, H. osmophila, Metschnikowia pulcherrima, Candidia parapsilosis* and *Pichia guilliermondii*. In each of the studied species there were strains possessing β-glucosidase activity. In total, it was found in 33% of the examined microorganisms. It was most frequent among *M. pulcherrima* (73% of examined strains). β-xylosidase activity was detected in only 17 strains, include none in *C. parapsilosis*. It was the most common among *M. pulcherrima* and *H. uvarum* (Pando Bedrinana et al., 2012).

3.5. Other Compounds

There are about 50 different ketones in hop oil, consisting of a homologous series from heptan-2-

one to heptadeca-2-one, along with number of branched and unsaturated ketones (Bamforth, 2001). The ketone found in the greatest amounts in hop oil is 2-undecanone (Rutnik et al., 2021). In a study by Guan et al. (Guan et al., 2019), a nearly 6-fold decrease in its content was observed in beer after fermentation, relative to wort (Guan et al., 2019).

Terpenoid ketones β-ionone, and β-damascenone are regarded as important compounds from this group (Preedy, 2011). β-damascenone is removed during fermentation by reduction and/or adsorption, so its content in finished beer is usually relatively low. Chevance et al. (Chevance et al., 2002) fermented a wort containing 450 ng/g of β-damascenone, along with variants additionally fortified with 500 and 1000 ng/g of β-damascenone using *S. cerevisiae* yeast. After the completion of the fermentation process, the levels of this compound in beers were in range of 0-176 ng/g. However, it should be noted that the level of β-damascenone increases during beer aging. This is probably due to acidic hydrolysis of its potential precursors, allene triols and acylene diols. Since these compounds occur in glycosidically bound forms, yeast exhibiting β-glucosidase activity may increase its content. In this study, the addition of pure β-glycosidase to Dark Ale beer resulted in a significant increase in the levels of this compound (54-79 ng/g, compared to 8 ng/g in beers without the enzyme added). In White Ale beer, this effect was much less pronounced (7 and 11 ng/g, respectively, after enzyme addition) (Chevance et al., 2002). In a study by Zhang et al. (Zhang et al., 2020), the authors fermented grape must using *S. cerevisiae* yeast, and added β-glucosidases extracted from *Issatchenka terricola* SLY-4, *P. kudriavzevii* F2-24, and *M. pulcherrima* HX-13. β-damascenone content in wines with *I. terricola* and *P. kudriavzevii* β-glycosidases was 0.037 mg/L and 0.026 mg/L, respectively, while the compound was not detected in the sample with *M. pulcherrima* glucosidase, and in control without enzyme addition (Zhang et al., 2020). Sensory characteristics of described ketones and their detection threshold (determined in beer, wine, or model solutions) are presented in Table 4 (Peltz & Shellhammer, 2017; Van Opstaele et al., 2020).

Hop oil also contains about 20 compounds from the aldehyde group, which include saturated and unsaturated compounds. Monoterpene dervied aldehydes, such as neral and geranial has also been identified in freshly distilled hop essential oil. As mentioned earlier they can also be created by the degradation of hop derived hydrocarbons. Some of the unsaturated aldehydes are strongly flavor-active. These compounds are found in small amounts in hops. Additionally processes such as wedge drying reduce their content. They are reduced to their corresponding alcohols during fermentation (Bamforth, 2001; Preedy, 2011).

Table 4. Sensory characteristics of described ketones and their detection threshold (determined in beer, wine, or model solutions) (Langos et al., 2013; Peltz & Shellhammer, 2017; Van Opstaele et al., 2020).

Compound	**Sensory characteristics**	**Detection threshold (µg/L)**
2-undecanone	Floral, citrus	7-82
β-damascenone	Woody, fruity, pipe tobacco	74-195
β-ionone	Violet-like, fruity, woody	0.007 – 205

4. CONCLUSION

Although the phenomenon of biotransformation of hop-derived compounds is only one of the components that determine the sensory characteristics of a finished beer, these reactions can have profound effects on beverage properties. Literature data indicates that many compounds which are considered to be important determinants of raw hop aroma, are almost non-existent in finished beer. For years brewers have been searching for new ways to impart unique sensory attributes to their products. In recent years, great progress has been made in understanding the transformations of hydrocarbons, terpenoids, esters, glycosidically bound aroma precursors, and especially sulfur compounds. However, as of today, biotransformation still remains a vague concept. Although hop oil can contain up to 1000 distinct substances, the current state of knowledge on biotransformation concerns only a small fraction of them. Literature suggests that yeasts exhibiting a wide β-glucosidase and β-lyase activity may significantly enhance the aroma of beer. Yeast known for these abilities should be strongly considered, when floral and fruity aromas are desired. Hop-derived hydrocarbons, esters, aldehydes, and ketones probably do not directly influence the beer's aroma, as most of them is mostly removed or transformed during the course of the fermentation.

Most publications on brewing focus mainly on *Saccharomyces* yeasts, while sparse indicate, that non-*Saccharomyces* yeasts could transform some hop-derived compounds to a higher degree. Although strains from the genus *Brettanomyces*, or *L. thermotolerans* or *T. delbrueckii* among others are commercially available, still little is known about their biotransformation capacities. It can be concluded that further research in field of biotransformation, especially by non-*Saccharomyces* yeast is still necessary.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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