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REVIEW

Wine flavor and aroma

Gustav Styger · Bernard Prior · Florian F. Bauer

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Abstract The perception of wine flavor and aroma is the result of a multitude of interactions between a large number of chemical compounds and sensory receptors. Compounds interact and combine and show synergistic (i.e., the presence of one compound enhances the perception of another) and antagonistic (a compound suppresses the perception of another) interactions. The chemical profile of a wine is derived from the grape, the fermentation microflora (in particular the yeast *Saccharomyces cerevisiae*), secondary microbial fermentations that may occur, and the aging and storage conditions. Grape composition depends on the varietal and clonal genotype of the vine and on the interaction of the genotype and its phenotype with many environmental factors which, in wine terms, are usually grouped under the concept of "terroir" (macro, meso and microclimate, soil, topography). The microflora, and in particular the yeast responsible for fermentation, contributes to wine aroma by several mechanisms: firstly by utilizing grape juice constituents and biotransforming them into aroma- or flavor-impacting components, secondly by producing enzymes that transform neutral grape compounds into flavor-active compounds, and lastly by the de novo synthesis of many flavor-active primary (e.g., ethanol, glycerol, acetic acid, and acetaldehyde) and secondary metabolites (e.g., esters, higher alcohols, fatty acids). This review aims to present an overview of the formation of wine flavor and aroma-active components, including the varietal precursor molecules present in grapes and the chemical compounds produced during alcoholic fermentation by yeast, including compounds directly related to ethanol production or secondary metabolites. The contribution of malolactic fermentation, ageing, and maturation on the aroma and flavor of wine is also discussed.

Keywords Wine aroma and flavor · Primary and secondary yeast metabolism · Malolactic fermentation

Introduction

The final sensorial quality of a wine is the result of a multitude of interactions between all the chemical components within the wine and specific environmental factors such as the temperature of the wine. The chemical composition of the wine is dependent on the type and quality of the grapes. Viticultural practices aim primarily at producing quality grapes that would reflect varietal flavors and aromas and/or characters typical for a specific region or terroir. This involves harvesting grapes at specific stages of ripeness depending on the style of wine to be made. Once harvested, specific processing techniques and fermentation strategies that are implemented will further determine the aroma and flavor development of the wine. Beside some pre-fermentative treatments such as maceration, the composition of the microflora present in the grape must and in particular the wine yeast strains and the selective application of malolactic fermentation are of significant relevance [3, 31, 41, 126]. The final aroma and flavor profile is furthermore strongly dependent on all aspects of post-fermentation treatments such as filtration and maturation strategies, including aging in wooden containers.

Once the product has been finalized, the appreciation of wine requires various senses: firstly to observe the color and appearance, secondly to judge the wine bouquet, thirdly to taste the wine itself, and fourthly to enjoy the mouthfeel and aftertaste [121]. This sensory perception of

G. Styger · B. Prior (✉) · F. F. Bauer
Institute for Wine Biotechnology, Stellenbosch University, Stellenbosch 7600, South Africa
e-mail: bap@sun.ac.za

wine is highly complex. In the first place, wine contains a very large number of flavor- and aroma-active compounds. Terpenes, esters, methoxypyrazines, and aldehydes impart distinct aromas and flavors such as floral, fruity, pepper, and woody to wine [11, 12, 64, 112, 114, 126, 140]. The taste of wine can be described as sweet, sour, salty, and bitter, and generally these properties are the result of the presence of sugar, polyol, salt, polyphenol, and flavonoid compounds [44, 102, 103, 112]. Compounds such glycerol, polysaccharides, and mannoproteins contribute to the viscosity and mouthfeel of wine [101, 103, 108], anthocyanins to the color [16], and ethanol to a mouth-warming effect [52]. The unique and non-linear interaction between these numerous chemical compounds determines the final flavor, taste, aroma, and perception of a wine [54, 79]. Aroma release and perception is furthermore strongly dependent on physical and environmental aspects such as the temperature of the wine or even the shape of the wine glass which can greatly change the perception of aroma and flavor [107]. Wine tasting and perception is therefore largely a subjective experience, and simple factors such as the absence or presence of saliva greatly influence the release of aroma compounds from both red and white wines [49].

From a scientific perspective, sensory perception needs to be analyzed by isolating specific impact factors. In an initial analysis of a mixture of odorants, the assessment focuses usually on which odorants present the highest impact and will depend on the perception threshold of each odorant and its concentration [81]. Such studies will determine the odor activity value (OAV; also known as the flavor activity), which is equal to the concentration of an aroma component divided by its detection threshold level [54]. However, it has also been shown that low impact odorants may act to change the perception of other odorants in a mixture, may interact synergistically or antagonistically [131], and therefore may impact significantly on perception without being recognized for their OAV.

This review will provide an overview of wine aroma and flavor starting with a section on the aromas associated with varietal character of grapes and wines. The main focus of the review will describe how metabolites associated with primary yeast metabolism, as well as those formed from secondary metabolic pathways, can influence and establish wine aroma. More detail will be given on aroma compounds associated with amino acid catabolism. The effect of malolactic fermentation and storage on wine flavor and aroma will also be discussed.

Varietal flavors and aromas

Although the overall composition of most grape varieties is very similar, there are clear and distinct aroma and flavor differences between most cultivars. These differences can mostly be attributed to relatively minor variations in the ratios of the compounds that constitute the aroma profile of a grape. Only a few aroma compounds have been directly linked to specific varietal flavors and aromas [121]. Some of these compounds and their characteristics are listed in Table 1. Although most of these compounds are present at low concentrations in both grapes and in the fermented wine, they normally have a large OAV and thus can have a huge impact on the overall aroma profile [75].

Varietal wine aroma from muscat-related grapes, for example, is mainly due to the presence of various isoprenoid monoterpenes in the grapes, with the most important being linalool, geraniol, nerol, and citronellol [66]. These compounds are formed from the precursor mevalonate, a metabolite derived from acetyl-CoA (Fig. 1). The monoterpenes can be found in free and odorless glycosidically bound forms in grape berries. The ratio of free and bound forms changes during berry ripening, with mature berries showing more bound forms than free forms of these compounds [42, 133].

Table 1 Impact odorants contributing to varietal aromas of selected wines

Odor characteristic	Impact compounds	Cultivar	Refs
Floral	Linalool	Muscat	[41]
Citrus, floral	Geraniol	Muscat	[41]
Citrus, floral	Nerol	Muscat	[41]
Geranium oil	Tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2,5-*cis*-2*H*-pyran (*cis*-Rose oxide)	Gewurztraminer	[112]
Kerosene	1,1,6-Trimethyl-1,2-dihydronaphthalene	Riesling	[116]
Bell pepper	3-Isobutyl-2-methoxypyrazines	Sauvignon blanc	[112]
Coconut, woody, sweet	3,6-Dimethyl-3a,4,5,7a-tetrahydro-3*H*-1-benzofuran-2-one	Gewurztraminer	[5]
Black currant	4-Methyl-4-mercaptopentan-2-one	Sauvignon blanc	[130]
Grapefruit, citrus peel	3-Mercapto-1-hexanol (*R* isomer)	Sauvignon blanc	[128]
Passion fruit	3-Mercapto-1-hexanol (*S* isomer)	Semillon	[128]
Black pepper	Rotundone	Shiraz	[126, 140]

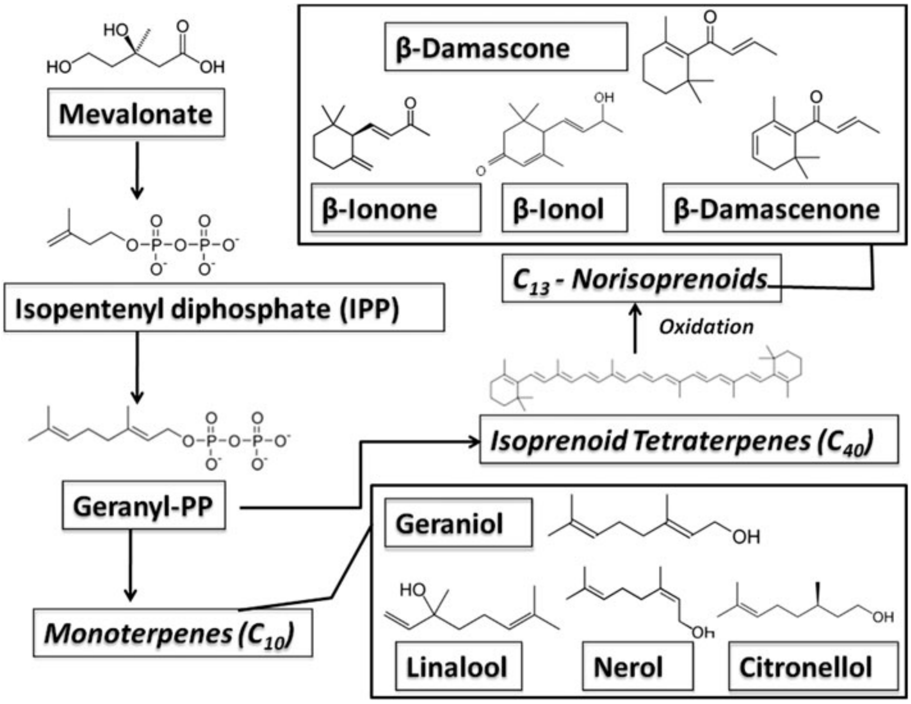

Fig. 1 Formation of the most important chemical compounds responsible for varietal aroma in wine. Both monoterpenes and C13-norisoprenoids are formed from the precursor mevalonate, itself a metabolite of acetyl-CoA. Figure adapted from Iriti and Faoro [66]

During fermentation yeast can release glucosidases and these enzymes can hydrolyze the glycosidic bonds of the odorless bound forms of monoterpenes, releasing more odor-contributing compounds to the wine [60, 100, 101]. Studies have also shown that skin contact treatment can significantly increase the concentrations of both free and bound odor compounds [134]. It has been found, however, that the formation of some aromas associated with varietal character can be an integral part of yeast metabolism and not a simple hydrolytic process, as previously thought [89]. Some studies, for example, show that yeast can synthesize some monoterpenes in the absence of grape-derived precursors [20]. Furthermore, the yeast strain has been shown to have an important influence on the levels of most varietal aroma compounds, affecting all families formed from precursor molecules, including C13-norisoprenoids, and monoterpenes [60].

Another set of varietal aroma compounds released from odorless bound precursors are volatile thiols that give Sauvignon blanc wines their characteristic bouquet, i.e., 4-methyl-4-mercaptopentan-2-one and 3-mercapto-1-hexanol (Table 1). These compounds are not present in grape juice in their active form, but occur in grape must as odorless, nonvolatile, cysteine-bound conjugates. The wine yeast is responsible for the cleaving of the thiol from the precursor during alcoholic fermentation [138]. It is interesting to note that some varietal aromas occur completely independent of each other. It is thought that the 'green' characters in Sauvignon blanc wines imparted by 3-isobutyl-2-methoxypyrazines (Table 1) can be manipulated through vineyard management. However, the 'tropical fruity' characters imparted by 4-methyl-4-mercaptopentan-2-one and 3-mercapto-1-hexanol appear to be largely dependent on the wine yeast strain used during fermentation and its ability to cleave the cysteinated precursors with a carbon–sulfur lyase enzyme [137].

The carotenoids also play a role in varietal aroma. These isoprenoid tetraterpenes originate from the precursor compound mevalonate, where five carbon units are condensed (Fig. 1). Oxidation of these carotenoids produces volatile and strong odor-contributing fragments known as C13-norisoprenoids including *β*-ionone (viola aroma), *β*-damascenone (exotic fruits), *β*-damascone (rose), and *β*-ionol (fruits and flowers) [66].

Another compound recently discovered that imparts a distinctive varietal pepper aroma to Shiraz wine (Table 1) is the sesquiterpene rotundone. Researchers identified the unknown peppery compound in white and black pepper and found that the same compound was responsible for the associated aroma and flavor in Shiraz grapes and wines. This disproved the previous hypothesis that this varietal pepper aroma was due to the complex interactions of many odorants, or to piperine and related alkaloids, which impart 'heat' in the mouth [135, 148]. However, many of the aroma and flavor compounds found in the finished wine come not from the grape, but rather from compounds formed during primary (essential) or secondary metabolism of the wine yeast during alcoholic fermentation. Some of the important compounds thus formed will be discussed below.

Flavors and aromas formed by the yeast during fermentation

According to Fleet [46], yeast influence wine aroma by the following mechanisms: (1) the biocontrol of molds by yeasts before harvest—mainly by apiculate yeast species competing for nutrients, (2) the alcoholic fermentation of the grape juice into wine, (3) the de novo biosynthesis of flavor and aroma compounds during alcoholic fermentation, (4) the metabolism of flavor-neutral grape compounds into active aroma and flavor compounds, (5) post-fermentation impact on wine via autolysis, and (6) the influence of the growth of malolactic and spoilage bacteria. Of these, the de novo biosynthesis of flavor and aroma compounds is probably the most important, because, in general, fermentation-derived volatiles make up the largest percentage of total aroma composition of wine in terms of numbers, if not always in terms of OAV [121] (Fig. 2). The formation of these compounds is variable and yeast strain specific [77]. This review will only discuss generalized aspects of metabolism of yeast aroma compounds.

Flavor and aroma compounds directly related to alcoholic fermentation

The aroma bouquet of a wine is a complex interaction between numerous volatile chemical compounds and these compounds interact with each other in various ways to achieve the final aroma and flavor palette. Quantitatively, metabolites that are direct products and by-products of glycolysis are found in the highest concentration. These compounds include ethanol, glycerol, and acetic acid. Although usually presenting low OAVs, their high concentration makes them important impact compounds. Studies have shown that a reduction of the ethanol concentration in a model wine from 10 to 9% had no effect on the flavor or aroma profile. When the ethanol concentration was further lowered to 7%, a marked increase in the intensities of the fruity, flowery, and acid flavors and aromas was seen. However, when the ethanol concentration was dropped to 3%, the model wine did not resemble wine anymore [54]. Another study showed that by reducing the alcohol levels in wine, the aromatic bouquet is affected not only by strengthening the perceived interactions between woody and fruity wine odorants, but also by modifying their chemical proportions [79].

Worldwide climatic changes are influencing vine and grape composition and ultimately the wines that are produced. One of the most important effects seen in many modern wines is an increased ethanol concentration due to increased grape sugar concentrations [29]. These high sugar concentrations not only increase the osmotic stress that the yeast has to tolerate during the initial stages of the fermentation, but also the subsequent levels of ethanol as well as by-products, glycerol, and acetic acid [9, 29]. These high levels of ethanol not only adversely affect the perception of flavor and aroma, e.g., higher ethanol levels were found to change the perception of a wine from fruity to herbaceous [51] and can also increase the perceived

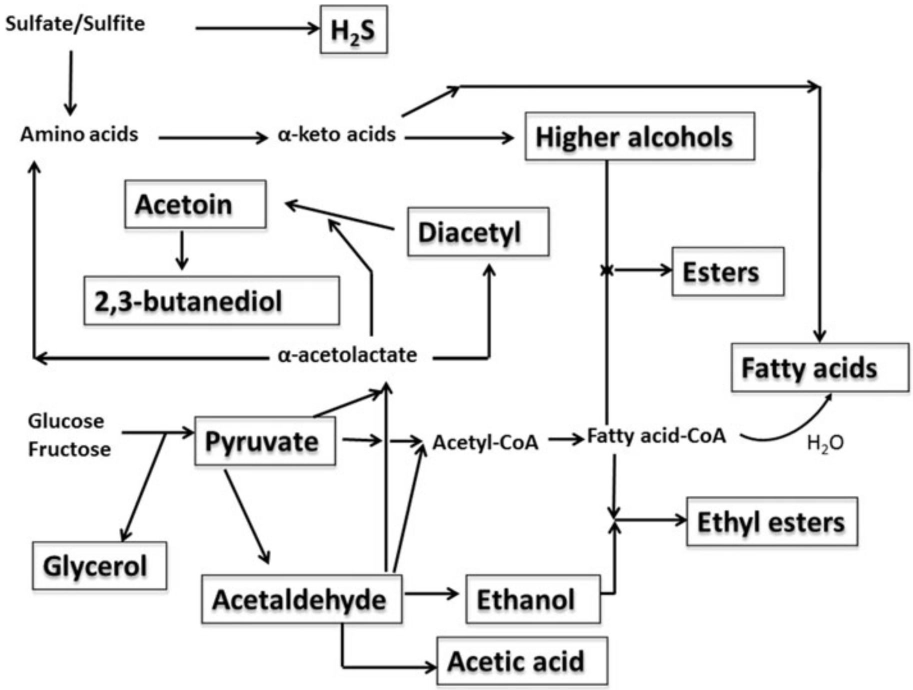

Fig. 2 Some of the major classes of aroma compounds (shown in *blocks*) produced by yeast during alcoholic fermentation as adapted from Bartowsky and Henschke [8] and Lambrechts and Pretorius [77]

astringency of the tannins and the bitterness, roughness, and hotness of wine [73, 112], but can also influence the metabolism of the yeast cell by inducing various stress responses, influencing global gene expression, and changing the structure of the cell membrane [1, 56, 70].

Glycerol is the most common liquid fermentation product after ethanol and this compound was historically thought to be a major contributor to the overall mouthfeel of wine. Higher glycerol concentrations were considered to enhance the desirable complexity of the wine. Normally dry wines contain about 5 g/l of glycerol [127]. However, little attention has been given to the interaction of glycerol and various flavor compounds and the role that glycerol plays in the formation of the aroma profile. Earlier studies with sensory analysis showed that the perceived overall flavor profile of a model wine and a white wine was not changed by the addition of glycerol indicating that glycerol does not play a part in establishing the aroma bouquet of wine [90]. However, recent data suggest that whereas no statistical association exists between glycerol concentration and quality of red wine, the relationship between the concentration of glycerol and the perceived quality of all styles of white wine is statistically significant [110].

Acetaldehyde is also an important aroma compound formed from pyruvate (Fig. 2) during vinification and constitutes more than 90% of the total aldehyde content of wine [111]. Acetaldehyde is also a precursor metabolite for acetate, acetoin, and ethanol synthesis. It has been found that acetaldehyde levels reach a maximum when the rate of fermentation is at its fastest, then decreases towards the end of fermentation, only to slowly increase again thereafter [77]. At low levels this compound imparts a pleasant fruity aroma to wine and other beverages, but at higher concentrations this turns into a pungent irritating odor reminiscent of green grass or apples [87]. Acetaldehyde is also extremely reactive and readily binds to proteins or individual amino acids to generate a wide range of flavor and odor compounds [76].

An important odorant formed from acetaldehyde is diacetyl (Fig. 2). Diacetyl is mainly formed by lactic acid bacteria during malolactic fermentation, but yeasts are also able to synthesize this compound during the alcoholic fermentation. However, the majority of diacetyl is further metabolized to acetoin and 2,3-butanediol [8]. Diacetyl at low concentrations (threshold value, 8 mg/l) adds yeasty, nutty, toasty aromas to wine, but at high concentrations, it has a characteristic buttery aroma that is associated with a lactic character [8, 130]. Once again this compound is highly reactive and has been found to react with cysteine, forming sulfur compounds that can influence wine aroma [2]. Neither acetoin nor 2,3-butanediol has a strong odor, with their detection threshold values of about 150 mg/l in wine [130].

However, many important aroma compounds are not directly related to the central carbon metabolism pathway. These so-called secondary metabolites can be synthesized during the metabolism of amino acids or fatty acids. The production of the most important compounds and their effect on wine aroma are described below.

Flavors and aroma compounds related to amino acid metabolism

During alcoholic fermentation yeast can use amino acids in several ways, particularly for protein synthesis, or for other metabolic processes [9]. Worldwide studies have shown that most grape musts contain insufficient amounts of yeast nutrients, especially assimilable nitrogen. Such deficiencies are seen as some of the main causes for sluggish and stuck fermentations, and nitrogen supplementation of grape musts has become common practice [145].

The nitrogen composition of the grape must affects not only the kinetics of alcoholic fermentation, but also the production of aromatic compounds, ethanol, and glycerol [61]. It has even been shown that the varietal aroma character of certain cultivars could be partially explained by the amino acid composition of the grape must [59]. The two main sources of yeast-assimilable nitrogen are primary amino acids and ammonium [61]. Although yeast strains differ greatly in their ability to use nitrogen and amino acids [21], various studies have shown that nitrogen supplementation in the form of assimilable nitrogen and amino acids influences the volatile aroma profile of the wine [48, 62, 69, 145].

The most important flavor and aroma compounds formed from amino acids are higher alcohols and their associated esters and volatile acids (Table 2). The process by which amino acids are catabolized into higher alcohols is called the Ehrlich reaction, indicated by the reactions and compounds marked with an asterisk in Fig. 3 [40]. The Ehrlich reaction also impacts directly or indirectly on the synthesis of other aroma compounds [58, 82]. In addition to the three nonpolar branched-chain amino acids (valine, leucine, and isoleucine), other amino acids can also be broken down to other metabolites via this reaction (Table 3) [4]. However, as the most important odor-related products (higher alcohols and volatile fatty acids) are produced from valine, leucine, and isoleucine (Table 2) the discussion in this review will mainly focus on them. The first step in the pathway is a transamination reaction in which the amino group from the amino acid is transferred to α-ketoglutarate to form an α-keto acid and glutamate (Table 3) [27, 28, 139]. The transamination reaction for the branched-chain amino acids has been shown to be catalyzed by mitochondrial and cytosolic branched-chain

Table 2 Branched-chain amino acid metabolites and their odor characteristics

Compound	Amino acid	Concentration in wine (mg/l)	Odor
Isovaleraldehyde	Leucine	Traces	Fruity, nut-like
Isobutyraldehyde	Valine	Traces	Slightly apple-like
2-Methylbutyraldehyde	Isoleucine	NR	Green (herbaceous), malty
Isobutyric acid	Valine	Traces	Sweet, apple-like
Isovaleric acid	Leucine	<3	Rancid, cheese, rotten fruit
2-Methylbutanoic acid	Isoleucine	NR	Fruity, waxy, sweaty fatty acid
Isoamyl alcohol	Leucine	45–490	Alcohol
Isobutanol	Valine	40–140	Fruity, alcohol, solvent-like
Amyl alcohol (active)	Isoleucine	15–150	Marzipan (almond)
Isoamyl acetate	Leucine	0.03–8.1	Banana, pear
2-Phenyl acetate	Phenylalanine	0.01–4.5	Rose, honey, flowery
Ethyl isovalerate	Leucine	0–0.7	Apple, fruity
Isobutyl acetate	Valine	0.01–0.8	Banana
Ethyl 2-methylbutanoate	Isoleucine	0–0.9	Strawberry, pineapple

Concentrations from Lambrechts and Pretorius [77]

NR not reported

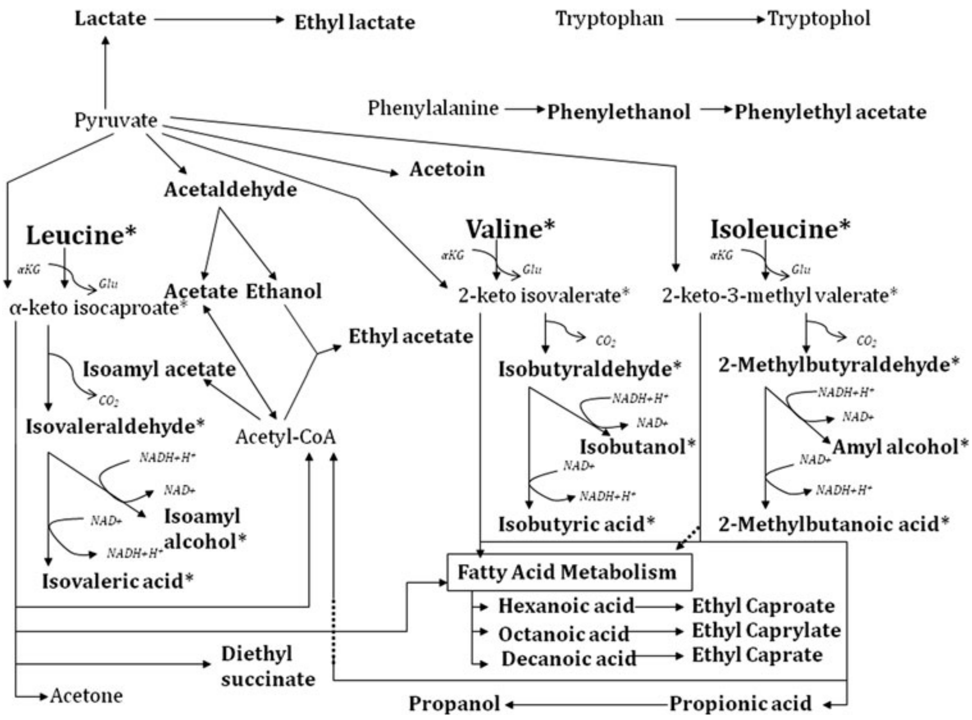

Fig. 3 A simplified metabolic map of yeast aroma compound production, indicating known metabolic linkages. *Bold type* indicates aroma compounds important to this study. Compounds marked with an *asterisk* constitute a diagrammatical representation of the Ehrlich pathway, responsible for the production of higher alcohols and volatile acids. Cofactors and transition metabolites are shown in *italics*. αKG is α-keto glutamate and Glu is glutamate [58, 82]

amino acid aminotransferases (BCAATases) encoded by the *BAT1* and *BAT2* genes [10, 38, 39, 74, 82, 122]. The Aro9p enzyme has been implicated in the transaminase reaction of the aromatic amino acids tryptophan, tyrosine, and phenylalanine [65]. Yeast, however, can also generate these α-keto acids through the so-called anabolic pathway, from glucose via pyruvate [23, 33, 34].

Further downstream in the Ehrlich pathway the α-keto acid is decarboxylated into an aldehyde (Table 3) [67]. Some researchers have suggested that the pyruvate decarboxylase genes (*PDC1*, *PDC5*, and *PDC6*) may play a part in this decarboxylation reaction, but are apparently not essential [140, 149]. Other genes that could possibly be involved in the decarboxylation of these α-keto acids are *KID1/THI3*, and *ARO10* [35, 146, 147].

The final fate of the branched-chain amino acid is thought to depend on the redox status of the yeast cell, as the Ehrlich pathway reaches a fork (Fig. 3) [147]. The aldehyde can either be reduced via a NADH-dependent reaction to its respective higher alcohol (Table 3) or it can

Table 3 Flavor-producing amino acid catabolism via the Ehrlich pathway

Amino acid	α-Keto acid	Aldehydes	Higher alcohols	Volatile fatty acids	Others	Refs
Leu	α-Ketoisocaproate	Isovaleraldehyde	Isoamyl alcohol	Isovaleric acid		[30]
Ile	α-Keto-β-methylvaleate	2-Methylbutyraldehyde	Amyl alcohol	2-Methylbutanoic acid		[31]
Val	α-Ketoisovalerate	Isobutyraldehyde	Isobutanol	Isobutyric acid		[32]
Phe	Phenylpyruvate	Phenylacetaldehyde	Phenylethanol	Phenylacetic acid		[139]
Tyr	*p*-OH-phenylpyruvate	*p*-OH-phenylacetaldehyde	*p*-OH-phenylethanol	*p*-OH-phenylacetic acid	*p*-Cresol	[3]
Trp	Indole pyruvate	Indole-3-acetaldehyde	Tryptophol	Indol-3-acetic acid	Skatole	[3]
Met	α-Ketobutyrate	3-Methylthiopropanal	3-Methylthiopropanol	3-Methylthiopropionic acid	Methanethiol	[3]
Asp	Oxaloacetate			Malate	Diacetyl, acetoin	[3]

Leu leucine, *Ile* isoleucine, *Val* valine, *Phe* phenylalanine, *Tyr* tyrosine, *Trp* tryptophan, *Met* methionine, *Asp* aspartic acid

be oxidized via a NAD$^+$-dependent reaction into a volatile carboxylic acid (Table 3). Various researchers have suggested that an alcohol dehydrogenase may catalyze this reductive reaction and an aldehyde dehydrogenase the oxidation reaction [35, 146].

The Ehrlich pathway is not the only way that amino acids can be metabolized into flavor and aroma compounds. In Fig. 4, alternative routes for the catabolism of threonine, methionine, and aspartic acid are shown. Aspartic acid (panel c) can be deaminated to form oxaloacetate. Some bacterial strains can further catabolize oxalacetate into acetoin, diacetyl, and 2,3-butanediol [4] but it is not known whether any yeast strains can complete this reaction. Threonine (panel a) can also be converted into acetaldehyde, an important odorant, and further into ethanol or acetic acid [4]. Methionine can be catabolized to release methanethiol following a demethiolation reaction. Methanethiol can be further converted to other sulfur compounds, and it could also react with carboxylic acids to produce thioesters [118].

These sulfur-containing compounds can have either a positive or negative impact on the aroma and flavor of wine, i.e., compounds such as 3-mercaptohexanol can impart fruity flavors to a wine. Of those that have a negative impact, hydrogen sulfide (H_2S)—with its rotten egg aroma—is the most important [138]. It has been found that the formation of hydrogen sulfide depends largely on the yeast strain and less on the composition of the grape must, as has been previously thought [16]. Therefore low hydrogen sulfide production is an important criterion in the selection of new yeasts for the industry. Various studies have identified the genes involved in the production of hydrogen sulfide and recently a yeast strain has been identified that produced little or no hydrogen sulfide [84, 85, 103].

Another amino acid, cysteine, can form various odor-impacting compounds through the so-called Maillard reaction, in which a chemical reaction between amino and carbonyl groups takes place to form new compounds [94].

Other flavor and aroma compounds

Volatile esters constitute one of the most important classes of aroma compounds and are largely responsible for the fruity aromas associated with wine and other fermented beverages [83]. The enzyme-free formation of esters is an equilibrium reaction between an alcohol and an acid. However, this manner of ester formation is apparently too slow to account for the large amounts of esters normally found in wine [77]. The enzymatic formation of esters was therefore identified as an initial activation of the acid by combining it with coenzyme A (CoA) before reacting with

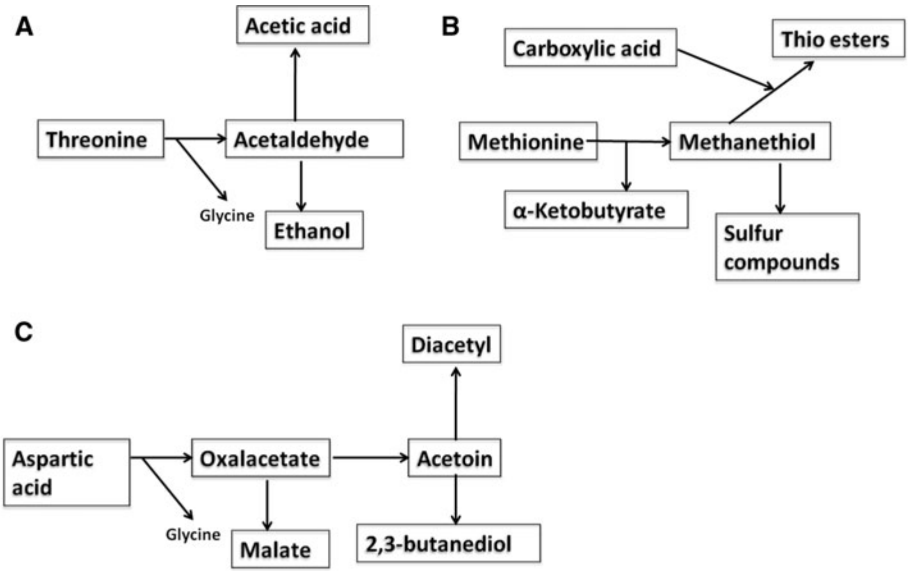

Fig. 4 Catabolism of three amino acids into compounds important for wine flavor and aroma. Panel **a** represents threonine, panel **b** represents methionine, and panel **c** represents aspartic acid. Figure adapted from Ardö [4]

the alcohol to form an ester [77]. The coenzyme donor can either be acetyl-CoA (formed from pyruvate) or any of a range of acyl-CoA compounds formed by the enzyme acyl-CoA synthetase [114]. Thus fatty acids, or ethyl esters (such as ethyl butanoate, ethyl hexanoate, ethyl octanoate), are formed from ethanolysis of acyl-CoA which is an intermediate metabolite of fatty acid metabolism. In this group of esters the ethanol group is derived from ethanol and the acid group from a medium-chain fatty acid [80, 132]. The other group of esters, the acetate esters (such as isoamyl acetate, propyl acetate, hexyl acetate, phenethyl acetate), are the result of the reaction of acetyl-CoA with alcohols that are formed from the degradation of amino acids, carbohydrates, and lipids [80, 132].

The synthesis of esters has been widely studied in *S. cerevisiae* during wine fermentation and various enzymes have been identified as playing a role in their formation. The formation of acetate esters is catalyzed by alcohol acetyltransferases (Atf1p and Atf2p), isoamyl alcohol acetyltransferase and ethanol acetyltransferase [83, 119, 142], whereas the formation of the ethyl esters has been attributed to two acyl-CoA:ethanol *O*-acyltransferase enzymes (Eeb1p and Eht1p) [132]. Formation of esters by oxidation of hemiacetal compounds (formed from alcohol and aldehyde mixtures) by alcohol dehydrogenases and the reaction of a ketone with molecular oxygen catalyzed by a Baeyer–Villiger monooxygenase are not important in wine fermentation [114]. However, the formation of esters differs widely between yeast strains and other external factors such as fermentation temperature, nutrient availability, pH, unsaturated fatty acid/sterol levels, and oxygen levels all play an important part in determining the end levels of esters in a wine [80, 124, 128, 150].

Volatile fatty acids also contribute to the flavor and aroma of wine. During yeast fermentation many medium- and long-chain fatty acids are also formed via the fatty acid synthesis pathway from acetyl-CoA [111]. Medium-chain fatty acids are thought to be toxic to the yeast cells and retard fermentation, but studies on stuck and sluggish fermentations have shown that high levels of these medium-chain fatty acids in these types of fermentations are symptomatic, rather than causative [7].

During wine fermentation *S. cerevisiae* is not the only microorganism that can contribute to the aroma and flavor of wine. Spontaneous fermentations involve many non-*Saccharomyces* species and some can impart novel aromas to the wine due to the production of enzymes that are either absent from *S. cerevisiae* or produced in very low amounts [104]. Some of the most important non-*Saccharomyces* species involved in wine fermentation include those from the following genera: *Candida*, *Kloeckera*, *Hanseniaspora*, *Zygosaccharomyces*, *Schizosaccharomyces*, *Torulaspora*, *Brettanomyces*, *Saccharomycodes*, *Pichia*, and *Williopsis* [72]. Studies have shown that small-scale fermentations carried out with single strains of *Kloeckera apiculata*, *Candida stellata*, *Candida pulcherrima*, and *Candida colliculosa* were not able to complete the fermentation. High residual sugar levels remained and these wines differed significantly from those produced by an industrial wine yeast strain [71]. These non-*Saccharomyces* yeasts can also produce compounds that negatively influence the aroma and flavor of wine. Tetrahydropyridines and 4-ethylphenol can be formed by *Brettanomyces* spp. and impart unpleasant "medicinal-like" or "mousy" characteristics to the wine [72]. However, currently there is a trend toward using so-called mixed starter cultures, containing one or more non-*Saccharomyces* yeasts as well as an established industrial *S. cerevisiae* wine yeast. This combined use of different species not only leads to the formation of novel aroma and flavor compounds, but yeasts can act synergistically upon one another, adding another level of complexity [24, 143].

Flavor and aroma compounds formed during malolactic fermentation

After alcoholic fermentation, some wines can undergo a secondary fermentation known as malolactic fermentation (MLF). This biological process is particularly desirable for high-acid wine produced in cool-climate regions, as MLF involves the deacidification of wine via the conversion of the dicarboxylic L-malic acid to the monocarboxylic L-lactic acid and carbon dioxide (Fig. 5a). This process is normally carried out by lactic acid bacteria isolated from wine, including *Oenococcus oeni*, *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp. [86]. MLF is also important in some wines from warmer regions because it changes the composition of the wine and improves its organoleptic quality. Moreover, it has been found that bacterial activity plays a role in the stabilization of wine and ensures an enrichment of its aromatic composition [108].

During MLF lactic acid bacteria can influence the aroma and flavor of wine by producing volatile metabolites and modifying aroma compounds derived from grapes and yeasts. Similarly to the role that yeast plays in aroma formation, the effect of these bacteria is strain specific and can vary greatly [14]. Generally it has been found that MLF can enhance the fruity aroma and buttery note but reduce the vegetative, green/grassy aroma of wine. Additionally, flavor characteristics ascribed to wines undergoing MLF include floral, nutty, yeasty, oaky, sweaty, spicy, roasted, toasty, vanilla, smoky, earthy, bitter, ropy, and honey. Besides aroma, MLF is also believed to increase the body and mouthfeel of wine and to give a longer aftertaste [86].

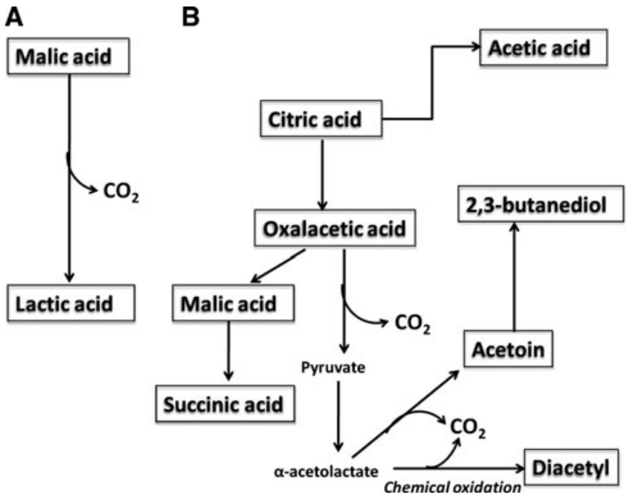

Fig. 5 The two most important biochemical reactions catalyzed by lactic acid bacteria during malolactic fermentation. Panel **a** represents the conversion of the dicarboxylic L-malic acid to the monocarboxylic L-lactic acid and carbon dioxide. Panel **b** represents the production of carbonyl or acetonic compounds, including diacetyl, acetoin, and 2,3-butanediol, from the metabolism of citric acid

Many lactic acid bacteria possess catalytic enzymes capable of liberating grape-derived aroma compounds from their natural non-aromatic glycosylated state [53]. Some of these enzymes include β-glucosidases, proteases, esterases, citrate lyases, and phenolic acid decarboxylases. All of these classes of enzymes can possibly hydrolyze flavor precursors and so influence wine aroma [13, 109, 141]. Many malolactic fermentations take place in oak barrels but recent studies suggest that the lactic acid bacteria can also influence wine flavor and aroma by producing additional oak-derived compounds [30]. It was observed that the concentration of vanillin, a powerful aroma compound, increased during malolactic fermentation in oak barrels. This finding suggests the existence of a vanillin precursor in the wood that is modified by lactic acid bacteria to release vanillin into the wine [11, 12].

Lactic acid bacteria can also produce or decrease aroma-impacting compounds via their own metabolism. It is thought that the enhanced fruitiness of wines that underwent MLF is due to the formation of esters by the lactic acid bacteria [86]. Much study is still needed on the effect of ester production by lactic acid bacteria during MLF, but evidence indicates that ethyl esters, such as ethyl acetate, ethyl lactate, ethyl hexanoate, and ethyl octanoate, are formed during MLF [30]. During storage of the wine, it has been observed that concentrations of certain esters increase, whilst those of others decrease. This is thought to be due to acid hydrolysis and chemical esterification [86]. The concentration of acetaldehyde, important in wine aroma, can be affected by the metabolism of lactic acid bacteria. It was shown that some species, especially *O. oeni*, can catabolize this compound, resulting in the formation of ethanol and acetate, with a subsequent reduction in the vegetative, green/grassy aroma of some wines [86]. However, the most important impact that malolactic fermentation has on wine aroma is the increased buttery flavor of wines. This is mainly the result of the production of carbonyl or acetonic compounds, including diacetyl, acetoin, and 2,3-butanediol from the metabolism of citric acid by lactic acid bacteria via several reactions in which citrate lyase plays a role (Fig. 5b) [108]. Another recent study also showed that *O. oeni* can metabolize the amino acid methionine, resulting in the production of aroma- and flavor-impacting sulfur-containing compounds such as methanthiol, methyl disulfide, and methionol 3-(methylsulfanyl) propionic acid [108].

Flavor and aroma compounds formed during ageing and maturation

As described above, wine aroma and flavor is generated through an immensely complex interaction of various classes of aroma compounds and various environmental and biological factors. However, wine is also a dynamic product that undergoes a period of ageing or maturation, be it in the bottle or in oak barrels. Generally, aging of wines leads to a loss of the characteristic aromas linked to the grape varietal and fermentation, and to the formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration [64, 78]. Specifically the concentrations of ethyl esters of branched-chain fatty acids change during ageing [32], and ageing of wine on the lees (mainly residue yeast cells) was found to decrease the concentrations of volatile compounds imparting a fruity aroma and increasing long-chain alcohols and volatile fatty acids [117]. Interestingly, it has also been shown that the lees can remove some of the unpleasant wine volatile phenols due to its biosorbent qualities [22, 102].

In order to form the lees, the yeast cells must first undergo the process of autolysis. This can be regarded as the hydrolysis of intracellular molecules into low molecular weight entities, resulting in cellular death [97]. Autolysis is a slow, complex process and can be induced by a variety of factors such as temperature and the activation of lytic cell enzymes. To a large extent this process also seems strain-dependent [96]. Autolysis is of enological importance because, as the cells lyse, they release various cellular components into the wine. Some of these components can contain nitrogen, amino acids, peptides, and proteins [95, 97]. An important aspect of this component is the mannoproteins. These are cell wall-associated proteins released into the wine that play a role in protection against haze formation, as well as color stabilization [26, 37]. Lipids are also released during autolysis and their liberated fatty acids can give rise to volatile components such as esters, aldehydes, and ketones—thus impacting on the aroma and flavor of the wine [123].

Additionally, the structural characteristics of the wood, i.e., the grain, porosity, and permeability, and its chemical composition, including polyphenols, tannins, and volatile compounds, can influence the complex biochemical processes that take place during the oxidative ageing of wine in barrels, changing the composition of the wine and adding to its stability. The simple extraction of aromatic compounds (volatiles and polyphenols) and tannins from wood can add a richness and complexity to the aroma and taste of wines [17, 44, 68].

Conclusion

It is clear from the above discussion that wine flavor and aroma is a complex interaction of chemical compounds and that differences between wines can be the result of certain varietal characters present in grapes such as odor compounds or precursors that are liberated during the fermentation process. Overall, about 1,000 chemical compounds make up the aromatic and flavor profile of a wine [6, 15, 18]. Some researchers have suggested that these profiles can be regarded as footprints or "aromagrams" and can in the future be used for identification and quality control purposes [120]. Not only are these aromagrams composed of various chemical classes of compounds (alcohols, esters, aldehydes, ketones, acids, and sulfur- and nitrogen-containing compounds), but these compounds have a very wide concentration range in the wine varying between the gram to the nanogram per liter level [15].

Further improvement of our understanding and knowledge of wine aroma and flavor and of an individual's perception thereof remains constricted by our limited ability to detect and quantify all relevant compounds in the complex matrix of wine. The detection of aroma compounds in wine is complex and no single detection method can satisfactorily detect and quantify all compounds. Thus a wide array of techniques have been developed to extract compounds from the wine matrix [18, 19, 25, 92, 106, 115, 151] and various detection methods have been developed. The most common chromatographic technique is gas chromatography, although various types of detection device may be coupled to it [25, 93, 105, 106]. More advanced systems—able to detect a vast range of compounds—comprise a gas chromatograph coupled to a mass spectrometer [45, 92, 100, 115].

A further scientific challenge is to improve our understanding of the perception of aroma, i.e., in particular of the sense of smell or olfaction [55]. Odorants are perceived via orthonasal and retronasal avenues and are transported across the olfactory mucus and presented to the olfactory receptors located on the olfactory receptor neurons, from where signal transduction occurs [55]. However, the perception of aroma and taste in humans are strongly dependent on a person's genetic make-up and cultural exposure [36]. In addition, other factors affect a person's taste and smell and include environmental factors such as organic and sulfur-containing air pollutants, as well as surfactants and heavy metals concentrated in the saliva [57]. Interestingly, taste and aroma were also found to be strongly influenced by the visual sense. Descriptions of the "nose" of a wine are dramatically influenced by its color and Fourier magnetic resonance imaging studies have shown a strong response in areas in the brain linked to visual stimuli when the olfactory system was stimulated [113].

During the fermentation process the winemaker only has a relatively limited set of tools available to enhance the production of favorable aroma and flavor compounds. Much depends on the quality of the grapes, as well as the fermentation potential—in this case measured as the potential to produce a sensorial complex wine—of the yeast used. At the moment wine makers are limited as to the range of yeasts that are able to impart some specific characteristic to a wine, and research focuses on issues such as enhancing color extraction from grape skins [43, 125, 144], adjusting the levels of aroma compounds, in particular esters and higher alcohols [82, 83], producing enzymes that will release additional volatile aroma compounds from the grapes [5, 50, 63, 116], and reducing the amount of alcohol to levels which allow a better perception and release of aroma and flavor compounds [91]. New yeast strains are continuously being developed by traditional breeding techniques leading to different flavor and aroma profiles in wine [98, 99, 129]. However, at present yeast strains developed by recombinant techniques are generally not accepted in the wine industry and by the public. Indeed, Fleet [47] stated that in searching for new wine yeast strains, the wine industry must look beyond *Saccharomyces* species. However, much recent research focuses on the use of hybrid yeasts in wine fermentations. Many hybrids between *S. cerevisiae*, *S. uvarum*, and *S. kudriavzevii* show promise in yielding wines with enhanced or different aroma and flavor profiles [52, 88, 136].

Lastly, it is also important to keep in mind that the appreciation of wine is entirely subjective. People will and should differ on the relative merits or attributes of a specific wine. After all, much enjoyment can be derived from discussing these differences in perception, and wine is certainly unique as a food product in creating passion and interest for detecting and debating the merits and demerits of individual products. There are certainly enough styles, cultivars, and wines on the market to satisfy all tastes.

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