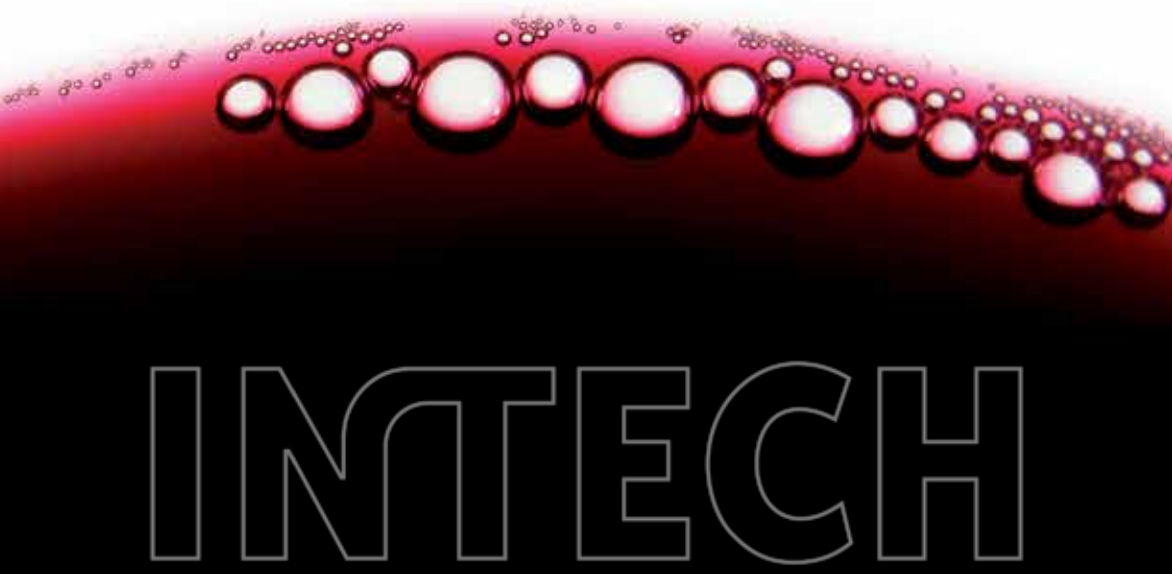
GRAPE AND WINE BIOTECHNOLOGY

Edited by **Antonio Morata** and **Iris Loira**



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Grape and Wine Biotechnology
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Contents

Preface

This book shows a multidisciplinary approach on many current and essential topics of vine and wine biotechnology in its 21 chapters. Authors are experts in their respective research fields at international level and work in research centers or universities from 13 wine-producing countries. The topics in the chapters describe the innovative technologies or processes in wine production, but these are presented at a technical/reader-understandable level. Grape production and wine industry are evolving to improve sensory quality, in a safe, sustainable, and eco-friendly way. Innovations in cultivar selection or vine plant biotechnology are directed to improve grape sensory quality and at the same time to obtain cultivars with improved resistance to diseases and pests and to adapt to environmental conditions as a way to reduce the use of chemical pesticides. Wine biotechnology is focused on the improvement of both alcoholic and malolactic fermentations, as well as the microorganisms respectively involved in them—yeasts and malolactic bacteria. The goal is on the improvement of sensory quality trying to adapt fermentative quality to the specific aromatic and flavor profile of each variety and region. Also it is essential to improve wine safety in order to provide consumers with healthy wines. Reduction of alcohol content in beverages is another major concern in the framework of making wines. Moreover, this can be essential in a scenario of increasing global climatic changes, which caused higher contents of sugar in grapes, resulting in the increase of alcohol content. The use of non-*Saccharomyces* yeasts is also a current trend to improve wine quality at sensory and technological level. Moreover aging of wine is the traditional way of polishing its structure to increase its complexity and roundness. Wine aging in barrel is an emerging technique to modulate wine sensory profile.

The book has been divided into three sections according to specific topics: grape and vine biotechnology, fermentation and wine biotechnology, and analysis and origin authentication.

Chapters 1–4 are devoted to the management of fungal diseases at molecular level and pathogenic processes. Dr. Arce-Johnson et al. focus on molecular biotechnology in vine and how to manage biotic and abiotic stress in vine plants to reduce economic losses and in turn to get a good quality. Particular attention is given to fungal diseases and UV radiation and the effect of these stresses in plant development. Dr. Suzuki et al. describe the pathogenic alteration related to proteins in plants explaining the main plant defense mechanisms, proteins involved, and their role in pathogen control. Dr. Nita explains the use of cultivar selection to control fungal diseases in grape and vine from an integrated management with the aim to minimize the use of chemical pesticides. In Chapter 4 by Dr. Lijavetzky et al., the main molecular tools to obtain transgenic vines with improved properties are studied. New technologies of gene edition are explained highlighting their application in functional study of grapevine.

In Chapter 5, Dr. Martínez-Ávila et al. describe the main available technologies to extract and recover phenolic compounds from grapes and by-products to obtain bioactive compounds to be used as pigments, organic acids, or antioxidants. In a similar way, Dr. Gómez et al. (Chapter 6) describe the extraction and potential therapeutic applications of vine leaves' polyphenols in human diseases. Grape drying is used to produce raisins, another expansive grapevine production at world level. In Chapter 7, Dr. Xiao et al. explain the cur-

rent status and future trends of grape drying technologies. In Chapter 8, Dr. Domínguez et al. explain the use of earthworms as a biological and eco-friendly process to produce a high-quality biofertilizer to be used in soil management.

Chapters 9–15 are focused on wine-grape microorganisms and the management of fermentations. Dr. Vilela et al. describe many metabolites that can be found in grapes but also those produced in alcoholic and malolactic fermentations. Their main compounds and average concentrations in grapes or wines and analytical techniques to elucidate them are exhaustively described. Dr. Tsaltas et al. pay attention to the natural grape microbiome and the distribution and ecology of microorganism species according to geographical origin, cultural practices, varieties, and climatic conditions. They also describe the potential applications of wine strains as starter cultures in wine fermentations. Dr. Arroyo et al. focus the reader's attention on the role of nonconventional yeasts or non-*Saccharomyces* yeasts in wine production, describing new applications to improve wine sensory quality with yeast species that were traditionally eliminated in wine fermentations. Dr. Zhu et al. describe the main volatile compounds in wines explaining the main factors affecting wine aromatic properties. Dr. Morata et al. explain the main application of the use of selected *Saccharomyces* and non-*Saccharomyces* yeast species to improve the formation of stable pigments and color stability in wines. Dr. Benito et al. describe the main applications of *S. pombe*, a non-*Saccharomyces* yeast that recently is gaining importance in wine fermentation for its specific properties and metabolism. Dr. del Monaco et al. explain the current application of the use of selected wild yeasts in Argentinian wines in Patagonian region.

Chapters 16–19 are focused on wine technology, aging, and stabilization. Dr. Fia explains the use of wine lees as a tool for biological aging of wines and describes a new method to apply it and to facilitate the process. Reduction of alcohol content in wines is a hot topic, and Dr. Olego et al. describe several viticultural and biotechnological techniques to achieve it. Bentonite is an additive used in enology for settling and clarification; Dr. Lambri et al. explain the main properties of this silicate and the interaction of wines with protein colloids, polyphenols, and aromatic compounds to understand better the applications in wine stabilization. Dr. Kunicka-Styczyńska explains the main technologies and processes used in wine industry in Poland.

Finally, Chapters 20–21 are dedicated to the use of instrumental analytical techniques in wine analysis and origin authentication. Dr. Ronkainen et al. focus the attention on trace elements analyzed by atomic spectroscopy and electroanalytical techniques, and Dr. Chantzi et al. highlight the application of NMR, stable isotopes, 14C radiocarbon, and isotopic techniques in wine authentication and the determination of geographical origin.

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Chapter 9

Grape and Wine Metabolites: Biotechnological Approaches to Improve Wine Quality

Fernanda Cosme, Berta Gonçalves, António Inês, António M. Jordão and Alice Vilela

Additional information is available at the end of the chapter

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Abstract

Grape metabolites can be affected by many extrinsic and intrinsic factors, such as grape variety, ripening stage, growing regions, vineyard management practices, and edaphoclimatic conditions. However, there is still much about the *in vivo* formation of grape metabolites that need to be investigated. The winemaking process also can create distinct wines. Nowadays, wine fermentations are driven mostly by single-strain inoculations, allowing greater control of fermentation. Pure cultures of selected yeast strains, mostly *Saccharomyces cerevisiae*, are added to grape must, leading to more predictable outcomes and decreasing the risk of spoilage. Besides yeasts, lactic acid bacteria also play an important role, in the final wine quality. Thus, this chapter attempts to present an overview of grape berry physiology and metabolome to provide a deep understanding of the primary and secondary metabolites accumulated in the grape berries and their potential impact in wine quality. In addition, biotechnological approaches for wine quality practiced during wine alcoholic and malolactic fermentation will also be discussed.

Keywords: grape physiology, grape metabolites, wine biotechnology, alcoholic fermentation, malolactic fermentation, microbial metabolites

1. Introduction

Grape berry chemical composition is complex, containing hundreds of compounds. Water (75–85%) is the main component followed by sugars and then organic acids. Other important compounds include amino acids, proteins, and phenolic compounds. Berry sugar composition

has a key role in wine quality, since it determines alcohol content in wines [1]. Grape sugar, acidity, pH, and color are considered to mark harvest. *Bouquet* and flavor are related to the winemaker's expertise, stabilization, and storage processes, but primarily they are related to grape varietal character and its particular expression in a given *terroir*.

Nowadays, wine fermentations are driven mostly by single-strain inoculations, allowing greater fermentation control, leading to more predictable outcomes and decreasing the risk of spoilage by other yeasts [2]. During must fermentation, *Saccharomyces cerevisiae* produces a plethora of active-aroma secondary metabolites and releases many aroma compounds from inactive precursors present in grape juice, which significantly affect the sensory quality of the final wine [3, 4]. Besides yeasts, lactic acid bacteria (LAB) are members of the normal microbiota that appears in all type of wines (white and red), and, therefore, they also play an important role in their final quality. Malolactic fermentation (MLF), a long-standing process of deacidification in winemaking carried by LAB, is a reaction of L-malic acid decarboxylation to L-lactic acid. Complex metabolic activities also occur, thus suggesting that MLF can positively or negatively affect the final wine quality [5, 6].

2. Grape berry physiology and metabolome

2.1. Morphology and anatomy of grape berries

After successful pollination and fertilization of ovules within a flower berry development initiates [7]. The formation and growth of grape (*Vitis vinifera*) berries follows a double sigmoid pattern with three distinct phases [8]: I, rapid cell division and expansion in green berries; II or lag phase, in which cell expansion ceases; and III, in which growth is reinitiated and the fruit matures. The berry fruit comprises up to four seeds surrounded by the inner endocarp, the middle mesocarp, pulp or flesh, and the outer exocarp or skin [8, 9] (**Figure 1**).

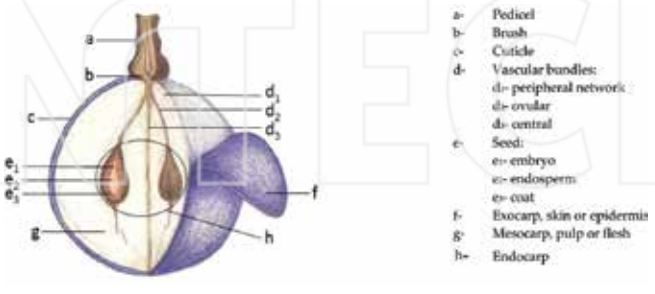


Figure 1. Structure of a ripe grape berry. Illustrated by Sílvia Afonso.

The exocarp consisting of a cuticle-covered epidermis, which represents 5–18% of the fresh weight of the fruit [10] and several layers of underlying thick-walled cells of hypodermis,

contains most of the skin flavonoids [11], notably anthocyanins in the outermost layers of the red grape varieties [8], interspersed with cells rich in needle-like crystals (raphides) [12]. Epidermis has non-photosynthetic cells with vacuoles containing large oil droplets [8]. Small berries have greater color, tannins, and flavor compounds than large berries because skin has a higher percentage of the total mass of small berries [7]. Scanning electron microscopy showed very few but functional stomata on young berries and wax-filled stomata on older berries [13], which accumulate polyphenolics and abnormally high concentrations of silicon and calcium in the peristomatal protuberances of up to 200 μm diameter [14].

At harvest, the cuticle of grape berry had an amorphous outer region and a mainly reticulate inner region [15]. During fruit development, the composition of the cuticular waxes changed, being oleanolic acid the main constituent, representing 50–80% of the total weight [16]. The soft wax was a mixture of long chain fatty acids (C_{16} and C_{18} fatty acid esters [17]), alcohols, aldehydes, esters, and hydrocarbons [18].

The mesocarp consists of thin-walled parenchyma [12]. The cells are round to ovoid and contain large vacuoles, which are the primary sites for the accumulation of sugars and phenolics [8], water, and organic acids [9] during grape berry ripening. According to Coombe [19], the translucent and hydrated mesocarp composes 85–87% of the berry's spherical volume. Altogether these make up 99.5% of the juice mass and hence are the major determinants of berry size and quality [9, 20]. The remaining 0.5% of berry components are phenolics, terpenoids, lipids, cellulose, and pectin [20]. The endocarp consists of crystal-containing cells (druses) and an inner epidermis [12].

Grape seeds are contained in locules (**Figure 1**), and are composed of an outer seed coat, the endosperm, and the embryo [9]. As with most seeds, the endosperm comprises the bulk of the grape seed and serves to nourish the embryo during early growth. The normal or perfect number of seeds in the grape is four [9], but lack of ovule fertilization or ovule abortion reduces the number of developing seeds, generally resulting in smaller berry size [7]. Based upon recent molecular evidence, auxin is synthesized in the ovule and transported to the pericarp upon fertilization, where it induces gibberellin (GA) biosynthesis. The GA then degrades DELLA proteins that repress ovary growth and fruit initiation [21]. The size of mature berries at harvest is also a function of the number of cells divisions before and after flowering, extent of growth of these cells [22], and the extent of preharvest shrinkage [23].

High level of tannins is observed in the seed coat [9, 11]. Similar to the tannins and phenols found in the flesh, these tannins also decline greatly on a per-berry basis after *véraison* [24].

Berry vascular tissue develops directly from that of the ovary. It consists primarily of a series of peripheral bundles that ramify throughout the outer circumference of the berry and axial bundles that extend directly up through the stem [8]. Grape berry is provided through the berry stem or pedicel by a vascular system composed of xylem and phloem vessels [25]. Water, minerals, hormones, and nutrients are transported from the root system throughout the vine by the xylem tissue [25]. Present evidence indicates that in the final stages of grape development, water movement through the xylem vessels decreases markedly [25]. But, it seems that the fruit is not hydraulically isolated from the parent grapevine by xylem occlusion then,

rather, is "hydraulically buffered" by water delivered via the phloem [9]. Berry is also supplied by the phloem, which is the vasculature involved in photosynthate (sucrose) transport from the canopy to the vine [25].

2.2. Grape primary and secondary metabolites

2.2.1. Sugars

One of the main features of the grape-ripening process is the accumulation of sugars in the form of glucose and fructose within the cellular medium, specific in vacuole. In addition, sugar content is an important indicator often used to assess ripeness and to mark grape harvest. But, it is also possible to quantify small traces of sucrose in *V. rotundifolia* and hybrids between *V. labrusca* and *V. vinifera* grapevines [26]. Liu et al. [27] analyzed sugar concentration of 98 different grape cultivars and concluded that glucose (45.86–122.89 mg/mL) and fructose (47.64–131.04 mg/mL) were the predominant sugars in grape berries. During grape berry maturation, sucrose is produced in leaves by photosynthetic carbon assimilation and is transported to the berry in the phloem [24]. Sucrose is loaded into the phloem by either a symplastic or apoplastic mechanism [28]. However, it is at *véraison* that begins the sugar accumulation and the imported sucrose is converted into hexoses as a result of the activity of invertases [29].

Grape berries accumulate glucose and fructose in equal amounts at a relatively constant rate during ripening [29]. In addition, after *véraison* there is a considerable accumulation of glucose and fructose in the vacuoles of mesocarp cells, while 20 days after this period, the hexose content of the grape berry is close to 1 M, with a glucose/fructose ratio of 1 [19, 30]. Grape sugar concentration and composition is mainly determined by several factors, such as genotype [26, 31], vineyard management [32, 33], and climatic conditions [34, 35]. Moreover, in last years, as a result of climate change, there is a tendency for a sugar increase in grapes [36]. But, according to Mira de Orduña [35], the extremely high sugar levels reached at harvest today, especially in warm climates, may be rather associated with the desire to optimize technical or polyphenolic and/or aromatic maturity.

2.2.2. Organic acids and nitrogenous compounds

L-Tartaric and L-malic acids contribute to around 90% of the organic acid content in mature grapes [37, 38]. Minor amounts of citric, succinic, lactic, and acetic acids are also present in ripened grapes [39]. Despite L-tartaric and L-malic acids having similar chemical structures, they are synthesized and degraded by evidently different metabolic pathways in the grape berries. L-Tartaric acid synthesis in grape berries occurs during the period of grape growth [19, 40]. Tartaric acid pathway using L-ascorbic acid (vitamin C) is considered to be responsible for >95% of grape L-tartaric acid production [41]. L-Malic acid synthesis indicates that-carboxylation of pyruvate or of phosphoenol pyruvate is the most important pathway [42]. Accumulation of acids usually occurs at the beginning of berry development. The organic acid content increases up to *véraison* and then declines. The content of organic acids is determined by a balance between their synthesis and degradation. L-Tartaric acid was the most prominent acid from *véraison* until the fruits were fully mature. L-Malic acid content increased gradually

until *véraison*, after which it decreased with fruit ripening [37]. Grape acid composition is influenced by many factors such as grape variety, environmental conditions, and cultural practices [43]. High malate-producing grape varieties have been identified, such as Carignane, Chardonnay, Grenache, Malbec, and Pinot Noir, as well as high tartrate-producing grape varieties such as Merlot, Semillon, Riesling, and Thompson Seedless [44]. Temperature is a key factor in the rate of L-malic acid degradation during the berries ripening; with low temperatures, higher concentration of L-malic acid was observed [43]. L-Tartaric acid is presumed to be more stable when exposed to higher temperature, being the slight decreases during ripening due to dilution from berry expansion [45, 46].

Grapes nitrogenous compounds include ammonium cations and organic nitrogenous compounds such as amino acids, hexose amines, peptides, nucleic acids, and proteins. As maturation happens, organic nitrogen progressively increases while ammonia slightly declines. The synthesis of amino acids, peptides, and protein occurs during the last 6–8 weeks of berry ripening [47]. In grapes, the main free amino acids include proline (up to 2 g/L), arginine (up to 1.6 g/L), and to a lesser extent, alanine, aspartic acid, and glutamic acid [48]. However, compositional differences in amino acids were observed by Stines et al. [49] among grape varieties, proline and arginine always being the major grape amino acids. In all grape varieties, most of the proline accumulation happened late in ripening, nearby 4 weeks of post-*véraison*. In opposite, arginine accumulation started before *véraison* and continued to maturity, excluding grape varieties in which a great level of proline accumulated [49]. The variation of amino acid profile and their concentration in grapes depends on grape variety, but also on viticultural management and environmental conditions [43, 50, 51].

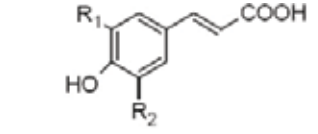
According to Hsu and Heatherbell [52], grapes contain naturally a wide range of different proteins, up to 41 protein fractions with molecular mass ranging from 11.2 to 190 kDa and isoelectric point from 2.5 to 8.7 [53, 54]. Soluble proteins in grape are globular proteins, mainly albumins [55, 56]. There is a significant increase in grape total protein content after *véraison* being a small content of proteins synthesized significantly during grape ripening [55, 57]. The most abundant grape proteins synthesized during ripening are pathogenesis-related proteins, including chitinases (32 kDa) and thaumatin-like proteins (24 kDa) [29, 57, 58].

2.2.3. *Aroma and flavor compounds*

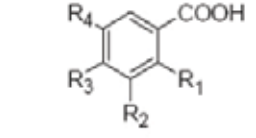
Free and bound terpene grape content has been used to measure berry flavorant development and potential. Numerous types of flavorants existed in the form of glycosidic precursors. Analysis of the total precursor content by assessment of the glycoside glucose (GG) content of the grapes may yield a more complete depiction of the grape flavorant potential [59]. During grape maturity, changes in the concentration and diversity of aroma precursors and volatile compounds occurred [60, 61]. Lacey et al. [60] observed that grapes grown under cool temperatures showed higher grape methoxypyrazine concentration than grapes grown under hot temperatures. Grape methoxypyrazine levels were relatively high at *véraison* but decreased markedly with grape ripening. However, since grape maturation is genetically controlled, it is considerably influenced by environmental conditions [60].

2.2.4. Phenolic compounds

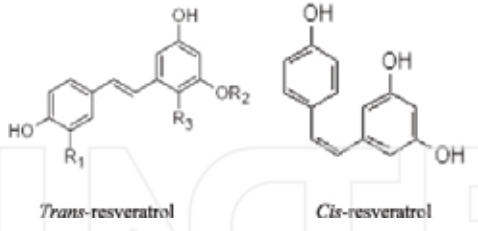
Phenolic compounds are very important for wine quality because they are responsible for most of the wine sensory characteristics, particularly color and astringency. These groups of compounds constitute a diverse group of secondary metabolites that exist in grapes, mainly in the grape berries' skins and seeds [62] and also in grape stems [63]. The phenolic compounds in *V. vinifera* grapes include two classes of phenolic compounds: non-flavonoids and flavonoids. The non-flavonoid compounds include phenolic acids divided into hydroxybenzoic acids and hydroxycinnamic acids, but also other phenol derivatives such as stilbenes (**Figure 2**). Non-flavonoids incorporate C_6-C_3 hydroxycinnmates acids, C_6-C_1 hydroxybenzoic acids, and C_6-C_3-C_6 stilbenes *trans*-resveratrol, *cis*-resveratrol, and *trans*-resveratrol glucoside.



Hydroxycinnamic acids	R_1	R_2
Caffeic acid	OH	H
Ferulic acid	OCH_3	H
p-Coumaric acid	H	H
Sinapic acid	OCH_3	OCH_3



Hydroxybenzoic acids	R_1	R_2	R_3	R_4
Gallic acid	H	OH	OH	OH
p- Hydroxybenzoic acid	H	H	OH	H
Vanillic acid	H	OCH_3	OH	H
Syringic acid	H	OCH_3	OH	OCH_3



Stilbenes	R_1	R_2	R_3
Trans-resveratrol	H	H	H
Piceid	H	OCH_3	OH

Figure 2. Main non-flavonoid compounds found in *V. vinifera* grapes.

For flavonoid compounds, there are a large number of subclasses, such as flavonols, flavanols, and anthocyanins [64]. Flavonols are the most abundant phenolic compounds in grape skins [65], while grape seeds are rich in flavan-3-ols [66]. Flavonoids are characterized by a basic structure of 15 carbon atoms comprising two aromatic rings bound through a three carbon chain (C_6-C_3-C_6). The major C_6-C_3-C_6 flavonoids in grapes include conjugates of flavonols quercetin, and myricetin; flavan-3-ols (+)-catechin and (−)-epicatechin; and malvidin-3-*O*-glucoside and other anthocyanins (**Figure 3a–c**).

According to Pastrana-Bonilla et al. [67], the average concentration of the total phenolic compounds in different grape fractions varied from 2178.8 mg/g gallic acid equivalent in seeds

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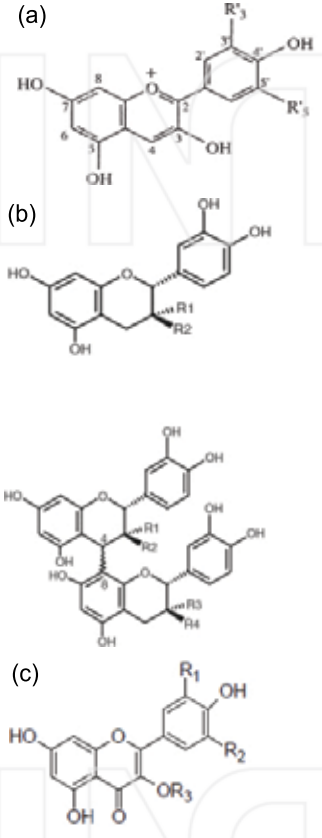
to 374.6 mg/g gallic acid equivalent in skins. In addition, it is also possible to found low concentrations of phenolic compounds in pulps (23.8 mg/g gallic acid equivalent).



Anthocyanidins	R'3	R'5
Delphinidin	OH	OH
Cyanidin	OH	H
Petunidin	OH	OCH_3
Peonidin	OH	OCH_3
Malvidin	OCH_3	OCH_3

Flavan-3-ols	R_1	R_2
(+) - catechin	H	OH
(+) - epicatechin	OH	H

Dimers (C_6-C_6)	R_1	R_2	R_3	R_4
Procyanidin B1	OH	H	H	OH
Procyanidin B2	OH	H	OH	H
Procyanidin B3	H	OH	H	OH
Procyanidin B4	H	OH	OH	H

Flavonols	R_1	R_2	R_3
Myricetin	OH	OH	H
Myricetin-3-O-glucoside	OH	OH	gluc
Quercetin	OH	H	H
Quercetin-3-O-glucoside	OH	H	gluc
Kaempferol	H	H	H
Kaempferol-3-O-glucoside	H	H	gluc

Figure 3. (a) Main flavonoid compounds (anthocyanidins) found in *V. vinifera* grape varieties. (b) Main flavonoid compounds (flavan-3-ols and procyanidins) found in *V. vinifera* grape varieties. (c) Main flavonoid compounds (flavonols) found in *V. vinifera* grape varieties.

In general, the phenolic composition of grapes is influenced by different factors, such as grape variety [68, 69], sunlight exposition [70], solar radiation [71] altitude [72], soil composition [73], climate [70, 74–76], cultivation practices [43, 74], exposure to diseases [77], and the degree of grape ripeness [63, 69].

The quantification of phenolic acids, stilbenes, monomeric anthocyanins, flavan-3-ols, and proanthocyanidins in red grape varieties is summarized in **Tables 1–3** and the quantification of phenolic acids, stilbenes, flavan-3-ols, and proanthocyanidins in white grape varieties is summarized in **Table 4**.

Phenolic compounds	Grape variety	Concentration	References
Phenolic acids	Negroamaro	7.3[a]	
Gallic acid	Susumaniello	45.0[a]	Nicoletti et al. [78]
	Malvasia Nera	77.3[a]	
	Aglianico	151.9[a]	
	Merlot	66.6[a]	
	Carménère	2.8[b]	Obreque-Slier et al. [79]
	Cabernet Sauvignon	3.5[b]	
	Merlot	9.8[c]	Montealegre et al. [80]
	Cencibel	7.3[c]	
	Cabernet Sauvignon	9.0[c]	
	Shiraz	6.8[c]	
Protocatechuic acid	Negroamaro	42.0[a]	Nicoletti et al. [78]
	Susumaniello	8.5[a]	
	Malvasia Nera	46.0[a]	
	Aglianico	37.4[a]	
	Cesanese	31.1[a]	
	Merlot	328.7[a]	
	Cencibel	1.5[b]	Montealegre et al. [80]
	Cabernet Sauvignon	2.4[b]	
	Merlot	1.7[b]	
	Shiraz	2.4[b]	
	Merlot	8.7[c]	Montealegre et al. [80]
	Cencibel	3.3[c]	
	Cabernet Sauvignon	7.1[c]	
	Shiraz	6.2[c]	
Caftaric acid	Primitivo	1.89[a]	Nicoletti et al. [78]
	Negroamaro	8.5[a]	
	Susumaniello	171.7[a]	
	Malvasia Nera	171.9[a]	
	Aglianico	320.4[a]	
	Cesanese	28.8[a]	
	Alphonse	645.0[a]	
	Merlot	746.3[a]	
	Carménère	0.6[b]	Obreque-Slier et al. [79]

Phenolic compounds	Grape variety	Concentration	References
	Cabernet Sauvignon	0.7[b]	
Stilbenes	Primitivo	30.7[a]	Nicoletti et al. [78]
Trans-piceid	Negroamaro	4.14[a]	
	Susumaniello	150.3[a]	
	Uva di Troia	15.3[a]	
	Malvasia Nera	98.0[a]	
	Aglianico	75.7[a]	
	Cesanese	12.0[a]	
	Merlot	26.3[a]	
	Alphonse Lavallée	24.1[a]	
	Castelão	67.24[c]	Sun et al. [81]
	Syrah	10.43[c]	
	Tinta Roriz	11.57[c]	
Trans-resveratrol	Primitivo	13.9[a]	Nicoletti et al. [78]
	Negroamaro	3.6[a]	
	Susumaniello	63.0[a]	
	Uva di Troia	4.6[a]	
	Malvasia Nera	48.5[a]	
	Aglianico	61.1[a]	
	Cesanese	8.1[a]	
	Merlot	9.2[a]	
	Alphonse Lavallée	40.0[a]	
	Blauer Burgunder	0.5[d]	Mikeš et al. [82]
	Lemberger	0.3[d]	
	Saint Laurent	1.0[d]	
	Saint Laurent	2.3[d]	Balík et al. [83]
	Blauer Portugieser	0.4[d]	
	Andre	0.4[d]	
	Castelão	6.8[d]	Sun et al. [81]

[a]mg/kg of berry dry weight.
[b]mg/kg of fresh grape skin.
[c]mg/kg of fresh grape seed.
[d]mg/kg dry skin.

Table 1. Quantification of phenolic acids and stilbenes in red grape varieties.

Monomeric anthocyanins	Grape variety	Concentration	References
Delphinidin 3-*O*-glucoside	Cabernet-Sauvignon	431.6[a]	Ortega-Regules et al. [84]
	Merlot	231.7[a]	
	Syrah	258.0[a]	
	Cabernet Sauvignon	4.67[b]	Revilla et al. [85]
	Garnacha	2.26[b]	
	Graciano	6.81[b]	
	Mencia	5.13[b]	
	Merlot	7.53[b]	
	Tempranillo	10.9[b]	
	Castelão Francês	6.2[c]	Jordão et al. [86]
	Touriga Francesa	0.9[c]	
Cyanidin 3-*O*-glucoside	Cabernet-Sauvignon	53.1[a]	Ortega-Regules et al. [84]
	Merlot	48.2[a]	
	Syrah	27.9[a]	
	Cabernet Sauvignon	0.90[b]	Revilla et al. [85]
	Garnacha	1.02[b]	
	Graciano	1.28[b]	
	Mencia	2.15[b]	
	Merlot	5.52[b]	
	Tempranillo	3.26[b]	
	Castelão Francês	2.6[c]	Jordão et al. [86]
	Touriga Francesa	0.1[c]	
Petunidin-3-*O*-glucoside	Cabernet-Sauvignon	337.4[c]	Ortega-Regules et al. [84]
	Merlot	270.9[a]	
	Syrah	385.2[a]	
	Cabernet Sauvignon	4.21[b]	Revilla et al. [85]
	Garnacha	3.73[b]	
	Graciano	7.21[b]	
	Mencia	6.68[b]	
	Merlot	7.0[b]	
	Tempranillo	11.11[b]	
	Castelão Francês	8.5[c]	Jordão et al. [86]
	Touriga Francesa	2.5[c]	
Peonidin 3-*O*-glucoside	Cabernet-Sauvignon	259.5[a]	Ortega-Regules et al. [84]

Monomeric anthocyanins	Grape variety	Concentration	References
	Merlot	381.9[a]	
	Syrah	299.2[a]	
	Cabernet Sauvignon	4.87[b]	Revilla et al. [85]
	Garnacha	12.69[b]	
	Graciano	12.79[b]	
	Mencia	14.85[b]	
	Merlot	14.27[b]	
	Tempranillo	7.81[b]	
	Castelão Francês	11.7[c]	Jordão et al. [86]
	Touriga Francesa	3.6[c]	
	Cabernet-Sauvignon	2506.3[a]	Ortega-Regules et al. [84]
Malvidin 3-*O*-glucoside	Merlot	1834.7[a]	
	Syrah	2889.7[a]	
	Cabernet Sauvignon	41.45[b]	Revilla et al. [85]
	Garnacha	64.69[b]	
	Graciano	53.69[b]	
	Mencia	47.40[b]	
	Merlot	35.54[b]	
	Tempranillo	46.35[b]	
	Castelão Francês	59.2[c]	Jordão et al. [86]
	Touriga Francesa	46.3[c]	

[a]μg/g grape skin.
[b]Relative amount of anthocyanidins (%).
[c]% weight of anthocyanins/weight grape.

Table 2. Quantification of monomeric anthocyanins in red grape varieties.

Phenolic compounds	Grape variety	Concentration	References
Flavan-3-ols	Baboso Negro	51.61[a]	
(+)-Catechin	Listán Negro	54.25[a]	Pérez-Trujillo et al. [87]
	Negramoll	51.31[a]	
	Tintilla	50.10[a]	
	Vijariego Negro	49.09[a]	
	Touriga Nacional	0.012–0.021[b]	Mateus et al. [88]
	Touriga Francesa	0.012[b]	

Phenolic compounds	Grape variety	Concentration	References
	Merlot	240.0[c]	Montealegre et al. [80]
	Cencibel	82.0[c]	
	Cabernet Sauvignon	270.0[c]	
	Shiraz	120.0[c]	
(−)-Epicatechin	Baboso Negro	16.50[a]	Pérez-Trujillo et al. [87]
	Listán Negro	13.77[a]	
	Negramoll	15.07[a]	
	Tintilla	20.55[a]	
	Vijariego Negro	16.13[a]	
	Touriga Francesa	0.010[b]	Mateus et al. [88]
	Merlot	210.0[c]	Montealegre et al. [80]
	Cencibel	60.0[c]	
	Cabernet Sauvignon	130.0[c]	
	Shiraz	130.0[c]	
Proanthocyanidins	Touriga Nacional	0.013[b]	Mateus et al. [88]
Procyanidin B3	Merlot	64.0[c]	Montealegre et al. [80]
	Cencibel	43.0[c]	
	Cabernet Sauvignon	50.0[c]	
	Shiraz	55.0[c]	
Procyanidin B1	Baboso Negro	15.95[a]	Pérez-Trujillo et al. [87]
	Listán Negro	15.00[a]	
	Negramoll	14.69[a]	
	Tintilla	13.64[a]	
	Vijariego Negro	13.39[a]	
	Touriga Nacional	0.184–0.260[b]	Mateus et al. [88]
	Touriga Francesa	0.090–0.138[b]	
	Merlot	170.0[c]	Montealegre et al. [80]
	Cencibel	74.0[c]	
	Cabernet Sauvignon	150.0[c]	
	Shiraz	100.0[c]	
Procyanidin B4	Merlot	80.0[c]	Montealegre et al. [80]
	Cencibel	39.0[c]	
	Cabernet Sauvignon	57.0[c]	
	Shiraz	33.0[c]	

Phenolic compounds	Grape variety	Concentration	References
Procyanidin B2	Baboso Negro	10.39[a]	Pérez-Trujillo et al. [87]
	Listán Negro	5.74[a]	
	Negramoll	7.55[a]	
	Tintilla	9.92[a]	
	Vijariego Negro	7.44[a]	
	Touriga Nacional	0.020[b]	Mateus et al. [88]
	Touriga Francesa	0.011–0.015[b]	
	Merlot	37[c]	Montealegre et al. [80]
	Cencibel	21.0[c]	
	Cabernet Sauvignon	41.0[c]	
	Shiraz	23.0[c]	

[a]Molar percentages.
[b]mg/g dry weight.
[c]mg/kg of fresh grape seed.

Table 3. Quantification of flavan-3-ols and proanthocyanidins in red grape varieties.

Phenolic compounds	Grape variety	Concentration	References
Phenolic acids	Grüner Veltliner	3.9[a]	
Gallic acid	Hibernal	4.0[a]	Mikeš et al. [82]
	Malverina	3.5[a]	
	Müller Thurgau	2.6[a]	
	Rheinriesling	2.1[a]	
	Welschriesling	1.8[a]	
	Neuburger	3.9[a]	
Protocatechuic acid	Chardonnay	4.8[b]	Montealegre et al. [80]
	Sauvignon Blanc	4.4[b]	
	Moscatel	3.6[b]	
	Gewürztraminer	6.0[b]	
Caftaric acid	Moscato	48.4[c]	Nicoletti et al. [78]
Stilbenes	Chardonnay	1.1[a]	Balík et al. [83]
Trans-piceid	Welschriesling	0.4[a]	
	Pinot Gris	0.6[a]	
Trans-resveratrol	Moscato	3.89[c]	Nicoletti et al. [78]
	Grüner Veltliner	0.1[a]	Mikeš et al. [82]
	Hibernal	0.3[a]	

Phenolic compounds	Grape variety	Concentration	References
	Malverina	0.3[a]	
	Müller Thurgau	0.3[a]	
	Rheinriesling	0.2[a]	
	Welschriesling	0.5[a]	
	Neuburger	1.5[a]	
	Chardonnay	0.3[b]	
	Welschriesling	1.6[b]	Balík et al. [83]
	Pinot Gris	1.1[b]	
Flavan-3-ols	Chardonnay	123[a]	
(+)-Catechin	Welschriesling	61.0[a]	Balík et al. [83]
	Pinot Gris	481[a]	
	Ugni blanc	2.6–222.0[d]	De Freitas and Glories [89]
	Sémillon	12–35.2[d]	
	Chardonnay	390.0[c]	Montealegre et al. [80]
	Sauvignon Blanc	200.1[c]	
	Moscatel	350.0[c]	
	Gewürztraminer	500.0[c]	
	Riesling	400.0[c]	
	Viogner	120.0[c]	
(−)-Epicatechin	Chardonnay	144[a]	Balík et al. [83]
	Welschriesling	84.3[a]	
	Pinot Gris	251[a]	
	Ugni blanc	0.04–3.0[d]	De Freitas and Glories [89]
	Sémillon	0.03–1.6[d]	
	Chardonnay	310.0[c]	Montealegre et al. [80]
	Sauvignon Blanc	130.0[c]	
	Moscatel	120.0[c]	
	Gewürztraminer	150.0[c]	
	Riesling	160.0[c]	
	Viogner	110.0[c]	
Proanthocyanidins	Ugni blanc	0.2–0.3[d]	De Freitas and Glories [89]
Procyanidin B3	Sémillon	0.01–0.2[d]	
	Chardonnay	52.0[c]	Montealegre et al. [80]
	Sauvignon Blanc	52.0[c]	
	Moscatel	39.0[c]	
	Gewürztraminer	56.0[c]	

Phenolic compounds	Grape variety	Concentration	References
	Riesling	43.0[c]	
	Viogner	51.0[c]	
Procyanidin B1	Ugni blanc	1.1–1.9[d]	De Freitas and Glories [89]
	Sémillon	0.02–0.4[d]	
	Chardonnay	380.0[c]	Montealegre et al. [80]
	Sauvignon Blanc	250.0[c]	
	Moscatel	330.1[c]	
	Gewürztraminer	460.0[c]	
	Riesling	620.0[c]	
	Viogner	200.0[c]	
Procyanidin B4	Ugni blanc	0.04[d]	De Freitas and Glories [89]
	Chardonnay	71.5[c]	Montealegre et al. [80]
	Sauvignon Blanc	54.0[c]	
	Moscatel	40.0[c]	
	Gewürztraminer	70.0[c]	
	Riesling	95.0[c]	
	Viogner	53.0[c]	
Procyanidin B2	Ugni blanc	0.06–0.2[d]	De Freitas and Glories [89]
	Chardonnay	33.0[c]	Montealegre et al. [80]
	Sauvignon Blanc	19.0[c]	
	Moscatel	15.0[c]	
	Gewürztraminer	22.0[c]	
	Riesling	33.0[c]	
	Viogner	19.0[c]	

[a]mg/kg fresh grape weight.
[b]mg/kg of fresh grape seed.
[c]mg/kg of berry dry weight.
[d]mg/g dry weight.

Table 4. Quantification of phenolic acids, stilbenes, flavan-3-ols, and proanthocyanidins in white grape varieties.

3. Biotechnological approaches for wine quality

More than 800 volatile compounds have been identified in wines, with a concentration range from hundreds of mg/L to the μg/L or ng/L [90]. The wine bouquet is formed by secondary metabolites synthesized by an extensive range of microbial species (yeasts and bacteria). Wine alcoholic fermentation (AF) is the key for innovation or creation of biotechnology that will change the expanding market [91] (**Figure 4**).

Figure 4. Grape juice is converted into wine by the action of wine yeast and bacteria during alcoholic and malolactic fermentations. Some wine components are wholly generated by these microorganisms as part of metabolism, while others are essentially synthesized by the grapevine. Wine quality and style is determined by the quality and quantity of compounds produced or modified by must/wine microflora.

In addition to yeasts, LAB also appears in all type of wines, being responsible for MLF that normally occurs after AF but may also occur simultaneously [92]. During the winemaking process, indigenous populations of LAB vary quantitatively and qualitatively [93], through a succession of species and strains before, during and after the AF [94]. After a phase of latency, the surviving cells begin to multiply and entering the exponential growth phase, reaching populations from 10^6 to 10^8 cfu/mL, almost exclusively, constituted by strains of *Oenococcus oeni*, species that dominate this stage and performs the MLF. Normally, a great diversity of strains of *Oenococcus oeni* at the beginning of the MLF is detected, while at the end only one or two predominate [95].

3.1. Yeasts metabolites: the imperceptible search of perfection

Wine yeasts contribute to wine aroma by a number of mechanisms: (i) they utilize grape juice constituents and transform them into flavor-impacting components, then (ii) they produce enzymes capable to transform neutral grape compounds into flavor-active compounds, and finally (iii) they can synthesize many flavor-active compounds such as primary and secondary metabolites [96].

Esters, in wine, are mainly originated from yeast metabolism during AF. But, some esters are also found in grape berry [97], where they occur in small amounts, contributing to the aroma of *V. vinifera* varieties [98]. Esters are formed via an intracellular process, catalyzed by an acyl transferase or ester synthase [99]. The concentration of esters usually found in wine is mostly well above their sensory threshold levels. Fruity and floral terms in Chardonnay wines were related to 2-phenylethyl acetate, as a rose-like/honey aroma [100] (**Table 5**). In red wines, ethyl butyrate (pineapple aroma), ethyl 2-methylbutyrate (sweet, floral, fruity, and apple), ethyl 3-methylbutyrate (strawberry, ethereal, buttery, and ripe), isoamyl acetate (banana-like aroma), ethyl hexanoate (anise seed, apple, or pineapple aroma), and ethyl octanoate (sweet, cognac, and apricot aroma) made a main contribution to the fruity character of wines [101] (**Table 5**).

These esters also appear in higher levels in wines after bio-reduction (deacidification) of wine's volatile acidity [102]. A study of overexpression *S. cerevisiae* alcohol acetyltransferases genes, ATF1p, ATF2p, and Lg-ATF1p, was performed by Verstrepen et al. [103]. Analysis of the fermentation products confirmed that the expression levels of ATF1 and ATF2 greatly affected the production of ethyl acetate and isoamyl acetate. But, factors such as oxygen and temperature that allow ester and higher alcohol synthesis must be monitored during AF [104].

Compounds	Odor description	Det. Threshold (μg/L)	References
Isoamyl acetate	Banana	30	Guth [115]
2-Phenylethylacetate	Roses, honey	250	Guth [115]
Ethylpropionate	Ethereal, fruity, rum-like	1800	Etievant [116]
Ethylisobutyrate	Strawberry, ethereal, buttery, ripe	15	Etievant [116]; Ong and Acree [117]
Ethyl butyrate	Pineapple	20	Guth [115]
Ethyl 2-methylbutyrate	Sweet, floral, fruity, apple	1–18	Guth [115]; Ferreira et al. [118]
Ethylisovalerate	Fruity	3	Ferreira et al. [118]
Ethyl hexanoate	Anise seed, apple, pineapple	5–14	Guth [115]; Ferreira et al. [118]
Ethyl octanoate	Sweet, cognac, apricot	2–5	Guth [115]; Ferreira et al. [118]
Diethylsuccinate	Fruity, melon	1200	Peinado et al. [119]
Acetaldehyde	Grass, green, apple, sherry	100,000	Carlton et al. [120]
Benzaldehyde	Almond	3500	Delfini et al. [121]
Linalool	Rose, lavender	25	Ferreira et al. [118]
α-Terpineol	Lily of the valley	300	Mateo and Jiménez [122]
Citronellol	Citronella	100	Guth [115]
Geraniol	Rose-like; geranium flowers	~75	Pardo et al. [109]
2-phenylethanol	Roses	10,000	Guth [115]
Isoamyl alcohol	Marzipan, burnt, whisky -like	30,000	Guth [115]
Butyric acid	Rancid, cheese	173	Ferreira et al. [118]
Isovaleric acid	Rancid, sweaty	33.4	Ferreira et al. [118]
Hexanoic acid	Sweaty, cheesenotes	420–3000	Guth [115]; Ferreira et al. [118]
Octanoic acid	Grass acid- like	500–8800	Etievant [116]; Ferreira et al. [118]
Decanoic acid	Soapy	1000–15,000	Guth [115]; Ferreira et al. [118]

Table 5. Major wine-yeast aromatic compounds, odor description, and detection thresholds in white and red wines.

Ethanol and glycerol are quantitatively the largest group of alcohols found in wine. Both contribute to the textural aspects of wines [1]. The search of yeast that can impart specific desirable characteristics to wines led to investigations such as the production of optimal levels of glycerol (the overexpression of GPD1, GPD2, and FPS1, together with the deletion of the ALD6 acetaldehyde dehydrogenase gene) [105].

Medium-chain fatty acids and their ethyl esters are natural components of alcoholic beverages. Fatty acids (butyric, isovaleric, hexanoic, octanoic, and decanoic acids, among others; **Table 5**) are produced by yeasts as intermediates in the biosynthesis of long-chain fatty acids, important components of yeast membrane [106]. Their aroma goes from vinegar to pungent, rancid, and soapy, sweetie, fruit and butter [106] (**Table 5**). One of the major problematic volatile acids is acetic acid. It can be formed as a by-product of AF, MLF, or as a product of the metabolism of acetic bacteria. Acetic acid affects the quality of certain types of wine when it is present above a given concentration [107] due to its unpleasant vinegar aroma.

Terpenes are one of the major grape components that contribute to wine aroma. This is especially valid to wines of Gewürztraminer and Muscat varieties, but these flavor compounds are also present in other grape varieties, where they supplement other varietal flavors and aromas. They are present in two forms: a free volatile and a non-volatile sugar-conjugated [108]. Geraniol (geranium flowers aroma) and linalool (rose or lavender-like aroma) are considered to be the most important of the monoterpene alcohols as they are present in higher levels and have lower perception thresholds than other major wine monoterpenes [109]. Monoterpenes can be released from their glycosides either by acid or by enzymatic hydrolysis. Hydrolysis during winemaking is caused by grape [110] or microorganisms enzymes taking part in the process [111]. In the yeasts that were selected in the past years, glycosidase activities have been used for the hydrolysis of glycoconjugated aromatic precursors in order to enhance wine sensorial quality [112]. Fungi are considered a promising genetic source for commercial production of recombinant β-glucosidase [113]. In a work by Zietsman et al. [114], an yeast strain (*S. cerevisiae* VIN13) was built to express and secrete the *Aspergillus awamori* encoding a B-type α-l-arabinofuranosidase (AwAbfB) in combination with either the β-glucosidases BGL2 from *Saccharomycopsis fibuligera* or the BGLA from *Aspergillus kawachii*. Coexpression of AwAbfB and BGL2 in VIN13 increased free monoterpenes in wines. Panelists confirmed wine aroma profile improvement, mainly in floral character [114]. Recently, Pardo et al. [109] found that the expression of *Ocimum basilicum* (sweet basil) geraniol synthase (GES) gene in an *S. cerevisiae* wine strain greatly changed terpene profile of wine made from a non-aromatic grape variety.

3.2. Lactic acid bacteria metabolites: beyond malolactic fermentation

The complexity and diversity of LAB metabolic activities in wine illustrates that MLF is more than a mere decarboxylation of L-malic acid into L-lactic acid, and it may affect positively and/or negatively the quality of wine [123] (**Table 6**). Besides to the decrease in acidity, MLF also improves sensorial characteristics and increases wines microbiological stability that undergone this important second fermentation [124, 125].

Aromatic modifications are due to L-lactic acid, less aggressive, and due to the increase of a number of other compounds such as diacetyl, acetoin, 2,3-butanediol, esters mainly ethyl lactate and diethyl succinate, and some higher alcohols and aromatic aglycones released by the action of β-glucosidases [126–128]. Sumby et al. [129] have verified the impact that different strains of *O. oeni* had on wine aroma and related that to their ester hydrolysis and synthesis abilities. For the aromatic complexity of wines, the production of volatile sulfur compounds,

particularly 3-methylsulfanyl-propionic acid with chocolate and toasted odors [130], and the activity of taninoacil hydrolase enzyme, commonly termed tannase, reducing wine astringency and turbidity [131], also contribute.

Compounds	Odor description	Det. threshold (μg/L)	References
4-Ethylguaiacol	Bacon, spice, clove, or smoky aromas	33	Dai et al. [26]; Bartowsky [123]
4-Ethylphenol	Horse and barnyard odor	440	Barthelmebs et al. [147], [148]
Tetrahydropyridines	Mousy off-odor	60	Swiegers et al. [149]; Harrison and Dake [150]
3- Methylsulfanyl-propionic acid	Chocolate and toasted odors	244	Pripis-Nicolau et al. [151]
Ethyl lactate	Lactic, raspberry	154–636	Ferreira et al. [118]; Bartowsky [152]
Diethyl succinate	Fruity, melon	1200	Peinado et al. [119]; Bartowsky [152]
Diacetyl	Butter	200–2800	Martineau and Henick-Kling [153]; Bartowsky and Henschke [154]
Acetoin	No negative organoleptic influence. Unpleasant buttery flavor at concentrations higher than threshold	150	Swiegers et al. [155]; Ehsani et al. [156]
2,3-Butanediol	Neutral sensory qualities	150	Swiegers et al. [155]; Romano and Suzzi [157]

Table 6. Major LAB aromatic compounds, odor description, and detection thresholds in wine.

Concerning to negative effects on wine quality, LAB may be responsible for the formation of ethyl carbamate by the degradation of arginine [124] and for the formation of biogenic amines such as histamine, tyramine, and putrescine by the degradation of precursor amino acids [132, 133]. Also, although less frequent nowadays, bitterness by acrolein formation from glycerol

degradation [134], butter aroma due to excessive production of diacetyl [135], flocculent growth [136], mannitol taint [137], ropiness [138], tartaric acid degradation [137], mousy off-odor by acetamide production of tetrahydropyridines [139], the geranium off-odor [140], and the formation of 4-ethilguaiacol and 4-ethylphenol volatile phenols [141, 142] are spoilage phenomenons that may occur after malolactic fermentation. Nevertheless, it is thought that the time between the completion of alcoholic fermentation and the start of malolactic fermentation is the most likely time that *Brettanomyces* multiplies and produces "Brett character," 4-ethylphenol of flavor, in wine [143].

As what happens to other food products, some researchers defend the use of autochthones LAB strains, more adapted and efficient to regional vinification conditions, for keeping the typicity of wines, instead of using universal ones that may impart similar characteristics and thus leading to final products that are too similar and also for preserving the local microbial biodiversity [144, 145]. According to Marcobal and Mills [146], the knowledge of some wine LAB whole genome, including the PSU1 *O. oeni* strain, allows deeper phylogenetic analyses and their relation with key pathways involved in carbon and nitrogen metabolism, which will foster modeling of *O. oeni* growth and metabolism in order to predict optimum strategies for efficiently performing the MLF with a desired flavor outcome.

4. Composition of grapes and wines: new analytical techniques

Several different analytical approaches are increasingly used to profile the volatile, non-volatile, and elemental composition of grapes and wines (see recent reviews, e.g., [158, 160]).

According to a review made by Ebeler [159], we can group these analytical approaches in (i) targeted analysis of compounds, (ii) non-targeted analysis and profiling of metabolites, (iii) elemental analysis, and (iv) relating chemical composition and sensory attributes (**Table 7**).

Therefore, wine composition and hence wine origin are possible by combining several analytical techniques (**Table 7**) that offer significant advantages for trace quantification of important aroma-active volatiles [174], [175] and taint compounds [163]. It is also possible to comprehensively profile metals [178], including those that affect chemical stability and oxidative reactions, and to characterize aroma qualities of complex mixtures [182]. Each of these tools, alone and in combination, is providing significant new insights into variables influencing grape and wine composition and flavor. Moreover, concerning to specific grape compounds, in past years, several methodologies were also developed focused on the identification, quantification, and also in extraction techniques. For example for phenolic compounds, substantial developments for individual phenolic analysis, such as benzoic and cinnamic acid, coumarins, tannins, lignins, lignans, and flavonoids, have occurred over the last 25 years. Thus, several extraction techniques have been employed namely for grape phenolic compounds, such as ultrasounds and microwaves [183], supercritical fluid extraction [184], subcritical water extraction [185], high hydrostatic pressure extraction [186], pulsed electric fields [187], and enzymatic treatment [188].

Analytical approaches	Analytical techniques	Examples and references
Targeted analysis of compounds (i)	Selected ion monitoring and tandem mass spectrometric, MS/MS or MS^n	Analysis of trace analytes, with important sensory properties—Ebeler [160] and Robinson et al. [161, 162]—such as 2,4,6-trichloroanisole (TCA)—Hjelmeland et al. [163]
	Combination of liquid chromatography, LC with mass spectrometry, MS. MS/MS is the combination of two mass analyzers in one mass spectrometry instrument, LC-MS/MS/LC-MS/MS. Supercritical fluid chromatography (SFC)	Smoke-derived volatile phenols—guaiacol and their glycoside precursors, and anthocyanins from grapes and wines—Kennison et al. [164–166], Hayasaka et al. [167], and Pati et al. [168]. Polyphenols from grape seed extracts—Kamangerpour et al. [169]
Non-targeted analysis and profiling of metabolites (ii)	Ultra-high performance liquid chromatography, UHPLC wish operates in the 20,000 psi range, combined with quadrupole time-of-flight mass spectrometry, qTOF and UHPLC-qTOF-MS	Varietal classification of wines—Vaclavik et al. [170] and Flamini [171]
	Ion cyclotron resonance mass spectrometry, ICR-MS	Characterization of Pinot Noir grapes and wines and chemodiversity comparison of different appellations: Vintage vs terroir effects—Roullier-Gall et al. [172, 173]
	Gas chromatography combined with time-of-flight mass spectrometry, GC GC-TOF-MS	Identification of over 350 volatile compounds in Australian Cabernet Sauvignon wines—Robinson et al. [174, 175]
	Nuclear Magnetic Resonance, NMR	^{1}H NMR metabolite profiling to relate chemical composition to sensory perception of body and mouthfeel of white wines—Kogerson et al. [176]
Elemental analysis (iii)	Inductively coupled plasma mass Spectrometry, ICP-MS	Relating elemental composition of wines to the vineyard that the grapes were grown or in wish winery they were made—Hopfer et al. [177]. Leaching of metals from stainless steel containers and from closures—Hopfer et al. [178]
Relating chemical composition and sensory attributes (iv)	Categorical principal components analysis, CATPCA; principal components analysis, PCA and partial least squares analysis, PLS	One or more compounds that correlate with specific aroma or flavor attributes—Polaskova et al. [179] and development of a flavor lexicon using new statistical nonparametric approaches—Vilela et al. [180] and Monteiro et al. [181]
	In-instrument gas chromatography recomposition-olfactometry, GC-RO	Perceptual characterization and analysis of aroma mixtures—Johnson et al. [182]

Table 7. Analytical approaches, analytical techniques used to profile the volatile, non-volatile and elemental composition of grapes and wines.

5. Final remarks

The study of the grape berry physiology and metabolome will provide a deep understanding of the primary metabolites including sugars, organic acids and amino acids, and some secondary metabolites accumulated in the grape berries such as phenolic compounds. This issue is of particular importance for viticulturists and oenologists in order to know how grape composition could affect wine quality. In addition, biotechnological approaches for wine quality, practiced during wine AF and MLF, are also a promising tool available for oenologists that improve wine quality, namely, their sensorial value.

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