CHAPTER 7

Distinctive Characteristics of Madeira Wine Regarding Its Traditional Winemaking and Modern Analytical Methodologies

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Contents

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Abstract Madeira wine, a fortified wine produced in Madeira Island, is a special wine among all types of wine due its specific winemaking process. The aim of this chapter is to describe important aspects of Madeira winemaking and some scientific research currently carried out in these particular kinds of wines. The first part of the chapter concerns the most important aspects of winemaking technology used in Madeira wine production. The second part, the more extensive, deals with the different groups of compounds and how these are modified during the various steps of the production process, namely the aging period.

If your head is wax, don't walk in the sun.
Benjamin Franklin

He was probably drinking his favorite wine, Madeira, when he said it. And then he got all of his friends to drink Madeira to toast the signing of the Declaration of Independence.

I. INTRODUCTION

Winemaking is a fascinating and complex transformation process of a raw plant material. It starts with the arrival of the harvest at the cellar and ends with the most active and decisive fermentation steps. After this, for some wines, comes the long aging period of the wine, during which the bouquet and taste of the wine are developed and refined.

Together with biochemical reactions catalyzed by enzymes of yeasts and bacteria, chemical reactions also occur between molecules already present in the must, those gradually extracted from the grape solids during fermentation, those derived from metabolism and, possibly, also those released by the wood. For many of them, the temperature and dissolved oxygen parameters related to technological operations of the winery can have dramatic effects and the quality of the final wine depends on the type and intensity of reactions taking place.

Madeira wines exhibits a peculiar winemaking processing, fundamental to the development of its specific characteristics. It is a fortified wine made on the island of the same name of the coast of Portugal. The Madeira Island has a long winemaking history dating back to the Age of Exploration, when Madeira was a standard port of call for ships heading to the New World or

East Indies. To prevent wine from spoiling, neutral grape spirits were added. On the long sea voyages, the wines would be exposed to excessive heat and movement which transformed their properties, including the color and flavor. These changes were found by wine producers when an unsold shipment of wine returned to the islands after a round trip. The experts found that the taste of the wine had improved significantly. Merchants started shipping barrels of Madeira to the Indies with the sole objective of enriching it, and in the process, adding value. It was this wine that, on its entrance into Europe, garnered unprecedented fame. Motivated by the evidence that heat improved quality and added value, by the mid-eighteenth century, wineries invested in *estufagem* (baking process) chambers, a technique that is still used. These conditions facilitate the transformation of young wines, obtained from sensory neutral grapes, into genuine, internationally appreciated wine with an intense and interesting aroma. In fact, for Madeira old wines, the wine bouquet is dictated by the particular aging process rather than by the grape variety used. Madeira wine resultant from baking process is recognized as a product with high value and is esteemed worldwide.

Today, Madeira is noted for its unique winemaking process, which involves heating the wine up to temperatures as high as 50 °C for an extended period and deliberately exposing the wine to some degree of oxidation. Because of this unique process, Madeira is a very robust wine that can be long-lived, even after being opened (Stevenson, 2005).

II. THE HISTORY

In 1419, at the beginning of Portuguese explorations, João Gonçalves Zarco, Tristão Vaz Teixeira, and Bartolomeu Perestrelo discovered an island in the middle of the Atlantic and which they named Madeira. The three Captains had received special privileges from Infante D. Henrique (Henry, the Navigator) and immediately started to cultivate the lands with wheat, vines, and sugarcane (Stevenson, 2005).

At first, all of them thrived, but today only wine continues to play an important role in the island's economy. Historical records in 1450 by the Venetian navigator, Alvise da Mosto, known as Luis de Cadamosto, show that Malvasia Cândida was brought during the first years of colonization. Infante D. Henrique ordered that lands be planted with Malmsey, brought from Candia (the capital of Crete). Throughout the fifteenth century, vineyards expanded steadily. The consequence of this was an increasing in exports, but it is the discovery of America by Christopher Columbus that constitutes a landmark in the history of Madeira wine.

Tales are told involving historical figures, in which the notoriety of Madeira wine abroad was already clear. It is said that, in 1478, George, Duke of Clarence, the brother of Edward IV, the King of England, when

sentenced to death by the High Chamber, chose to be drowned in a butt of Malmsey (Stevenson, 2005).

Throughout the seventeenth century, the production and export of Madeira wine grew steadily. Although major exporters were foreigners, British influence became predominant with the development of colonial markets in America and through commercial concessions made to British merchants. These concessions enabled British merchants living on the island to occupy a privileged position in commercial trade with the Indies and America. This led to a triangular commerce between Madeira, the New World, and Europe (with Great Britain occupying a prominent position). Transportation of goods from the Portuguese and the British colonies back to Europe represented another lucrative trade.

The association of Madeira with the United States is intimate. The Declaration of Independence, on 4th July 1776, was toasted by George Washington with a chalice of Madeira wine. It was the wine preferred by kings, emperors, and statesmen and served during the banquets of European Courts. Thomas Jefferson, and all the other ''Founding Fathers,'' greatly appreciated the most exquisite wines of the time, but preferred Madeira over all.

The beginning of the nineteenth century was marked by an export boom, caused by the Napoleonic Wars. However, this was not to be a favorable century for Madeira wine. The postwar depression devastated European exports.

During the first decade of the twentieth century, and up to the First World War, export markets changed, making Germany the best importer of Madeira wine. This century was equally marked by efforts in terms of regulating Madeira wine production in an attempt to improve quality. Several Portuguese and English wineries merged, changing the industry forever.

The Revolution of 1974, and the subsequent entrance of Portugal into the European Union (EU), brought about significant developments in the Autonomous Region of Madeira RAM - Madeira Island and which had an impact in its vitivinicultural sector. The reinforcement of quality control became one of the priorities of the RAM government, promoting a sustainable development of the wine industry.

Today, growers and all wineries are committed to constantly improving the quality, packaging, and promotion of Madeira wine.

III. THE TRADITION

A. Grape varieties

Currently, there are five main *Vitis vinifera* L. varieties used to produce Madeira wine Boal, Malvasia, Sercial, Verdelho (white grape varieties), named as noble varieties, and Tinta Negra (red grape variety). Occasionally

Terrantez and Bastardo varieties are also used, although they produce wines of excellent and uniqueness quality, they are increasingly rare on the Island. These varieties were highly attacked by oidium and phylloxera epidemic in XX. Since this epidemic, Tinta Negra is the workhorse variety on the Island and is found in various levels in many blends and vintage wines. There are several other varieties recommended (mainly Bastardo, Tinta, Verdelho Tinto) and authorized (mainly Complexa, Deliciosa, Listrão) to produce Madeira wine.

Vineyards occur over much of the Island of Madeira as well as Porto Santo. From the total island area (about 73200 hectares, ha), about 1400 ha produce appellation control wines, such as Madeira and ''Madeirense'' (VRPRD) or Geographical Indication wines, such as ''Terras Madeirenses.'' The main viticulture councils are ''Câmara de Lobos,'' situated on the south coast, with about 125 ha, followed by São Vicente with about 122 ha and Santana with approximately 82 ha, both on the north coast.

Most of these regions occur on slopes of 25° or above. Slopes between 16 and 25°, suitable for agriculture require terraces called *poios*. These are constructed using local basaltic stone. The terraces make mechanization almost impossible. Everything from pruning to harvesting involves manual labor. The most traditional training system is the *latada* or *pergola*. In this system, the vines are guided horizontally along wires and suspended off the ground by stakes. The trellis height varies between 1 and 2 m, and planting densities vary between 2500 and 4000 vines per hectare. During the second half of the twentieth century, the espalier vineyard or *espaldeira* configuration was tried. It can accommodate 4000–5000 plants per hectare with some success.

B. The specificity of the Madeira winemaking process

The initial winemaking steps of Madeira are like those for most other wines, with the grapes being harvested. Harvesting takes place according to well-established rituals from the end of August until mid-October. Everyone is involved in harvesting to speed the process. Grapes are placed in boxes (25 and 50 kg) and transported to the cellars. Each bunch is inspected and rotten grapes are eliminated. After weighing, and determination of their potential alcohol equivalent, a decision is made as to which type of wine is envisaged. Subsequent to crushing, fermentation follows standard procedures. Figure 7.1 summarizes the winemaking and aging processes to obtain Madeira wine.

The fermentation is stopped by the addition of neutral grape spirits (fortification), and depending on the time it occurs, it may be obtained wines with different sweetness. Madeira wine is classified into four basic categories:

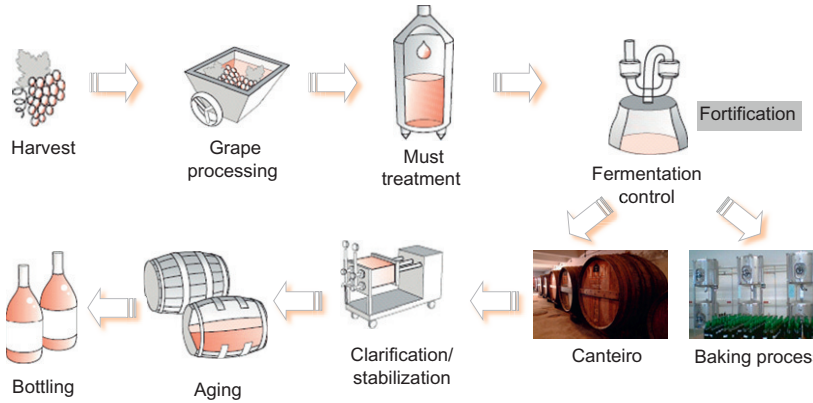

FIGURE 7.1 Schematic diagram of Madeira winemaking process showing the basic key unit operations that characterize Madeira wines.

- *Dry* (*Sercial*): usually fermented down to 25 g L^{-1} residual sugar, giving a dry wine. It is characterized by high-toned colors, almond flavors, and high acidity.
- *Medium sweet* (*Boal*): fermented down to 65 g L^{-1} residual sugar, producing a medium dry wine. It is characterized by a dark color, medium rich texture, and raisin flavors.
- *Medium dry* (*Verdelho*): fermented down to 90 g L^{-1} residual sugar, giving a medium sweet wine. The style is characterized by smoky notes and high acidity.
- *Sweet* (*Malvasia*): traditionally not fermented. It generates a sweet wine with about 110 g L^{-1} residual sugar. The style is characterized by a dark color, and a rich texture with coffee-caramel flavors. Like other Madeira's made from the noble grape varieties, the Malvasia grape used has naturally high levels of acidity in the wine which balances with the high sugar levels so that the wines do not taste cloying sweet.

Viticulture and oenology play an important role in the economy of many countries, and considerable efforts are devoted to improve the quality of products and to match the broadest demands of the market. Many industrial processes are finalized to obtain products with peculiar characteristics. The peculiar characteristics of Madeira wines arise from the specific and singular winemaking process. Once fortified, the wines may be subjected to one of the two different heating processes (Fig. 7.1): *Estufagem* (*baking process*) or *Canteiro* (*wood casks*).

1. The baking process—"Estufagem"

This process confers the uniqueness, peculiarity, and specificity of Madeira wine. It is meant to simulate the effects of a long sea voyage of aging barrels through tropical climates. As noted, the benefits of this exposure were discovered in the seventeenth century, where casks of Madeira were stored in the warm, humid holds of sailing ships for months at a time. It led to the employment of the technique of baking used today.

Under the technological point of view the wine is placed in large coated vats or in stainless steel tanks and heated by immersing rods containing hot water (45 and 50 °C; increased at 5 °C/day) for a minimum of 3 months. After this step the wines are placed in oak casks and subjected to a period of *estagio* or rest for at least 90 days. Bottled wines cannot be sold before 31st October of the second year following the harvest.

2. The wooden casks support—"Canteiros"

This aging process is used for the highest quality Madeira wines, which are aged without the application of the baking process. This term comes from the fact that oak casks are placed on wooden support beams called *canteiros*. In this winemaking procedure, Madeira wine aging usually occurs in the top floors of cellars, where the temperatures (30–35 °C in the summer) and humidity level (70–75%) are high, for a minimum of 2 years, developing complex aromas and intense flavors. Other phenomena common to all types of aging processes, including crystallization and precipitation, chemical reactions between wine components, and extraction of oak constituents from the casks, are responsible for the observed modifications. The extraction of volatiles from the wood during barrel aging is regulated by diffusion kinetics. As a general rule, extraction is highest at the beginning of aging, gradually tapering off with time and barrel age (Morales *et al.*, 2004). Small amounts of compounds, such as guaiacol, 2-furfural, and 5-methyl-2-furfural are present in all wines aged in previously used barrels. On the other hand, amounts of oak lactones increase in the second year inside barrels and begin to decline in the third year (Del Barrio-Galán *et al.*, 2011; Morales *et al.*, 2004). A recent study showed that extraction of volatile substances from wood dropped off sharply after 12 months, though there was high variability between wines.

Traditionally, large oak barrels are used to facilitate the diffusion of oxygen. This is assumed to play a major role in the many chemical reactions occurring during aging. Oxidation reactions promote desired changes in wine phenolics (e.g., anthocyanins, flavonoids, and tartaric esters of hydrocinnamic acids) as well as oak phenolics extracted from the

wood of the casks. The resultant organoleptic modifications are generally described as *oxidized* in dry wines, *rancio* in sweet fortified red wines, and *maderized* in sweet fortified white wines (Chatonnet and Dubourdieu, 1998). Oxidation phenomena are considered desirable, or even indispensable, for the proper development of the bouquet of sweet fortified Madeira wines.

Exposure to extreme temperature and oxygen accounts for the wine's stability. An opened bottle of Madeira wine can remain unharmed for up to a year. Properly bottled, Madeira is one of the longest-lasting wines, surviving for more than 150 years in excellent condition.

C. Production and marketing

The total production of *Vitis vinifera* L. grapes in 2010 in the RAM was 3.730 tones (t) which corresponds to the production of 3.07 million liters of Madeira wine (Fig. 7.2). Marketing of Madeira wine in 2010 reached 3.3 million L with the countries of the EC representing a market share of 69%, the main destiny of this wine, especially France, the United Kingdom, and Germany. The domestic market represents about 14% of the global market of Madeira wine. The most significant part of these sales is registered in Madeira, especially owing to the sales of Madeira wine to

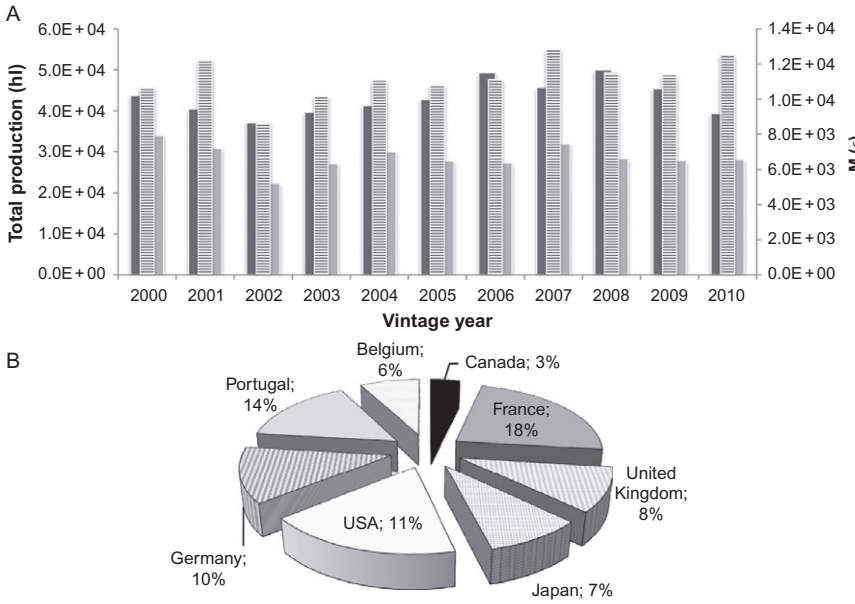

FIGURE 7.2 (A) Evolution of total production and total exportations of Madeira wines during the past decade; (B) major markets for Madeira wines (source: IVV, IP; IVBAM, IP).

tourists visiting the Island. Outside the EC, as seen from the figures stated, the main markets for Madeira wine are to be found in the United States and Japan (source: IVBAM, IP).

IV. CHEMICAL AND SENSORIAL CHARACTERIZATION OF MADEIRA WINE

Wine is one of the most complex and interesting matrices for a number of reasons. It is composed of volatile compounds, some of them responsible for the odor, and nonvolatile compounds which cause taste sensations, such as sweetness (sugars), sourness (organic acids), bitterness (polyphenols), and saltiness (mineral substances; Rapp and Mandary, 1986). With a few exceptions, those compounds need to be present in levels of 1%, or even more, to influence taste. Generally, the volatile components can be perceived in much lower concentrations, since our organs are extremely sensitive to certain aroma substances (Rapp *et al.*, 1986). Carbohydrates (monosaccharides, disaccharides, and polysaccharides), peptides, proteins, vitamins, and mineral substances are among the other wine constituents.

In order to expand the worldwide market, considerable efforts are being devoted to improve the image of Madeira wine. Consequently, their characteristics have to be well defined. So, in order to define and describe the particular characteristics and the authenticity of the product, secondary metabolites of grape and wines mainly linked to a specific variety, must be deeply studied. In Madeira wine, these compounds are mainly included in the chemical classes of mono and sesquiterpenoids C_{13} norisoprenoid higher alcohols, ethyl esters, volatile fatty acids, carbonyl compounds, sulfur compounds, furanic compounds, lactones, and polyphenols.

Due to the lack of scientific studies on the nature and content of polysacharides, peptides, proteins, and vitamins in Madeira wines, they will not be covered in these discussions.

A. Physicochemical parameters

The quality control of the main enological parameters of commercially available wines, according to EC regulation (EC No. 822, 1987), is almost nonexistent in the literature (Nogueira and Nascimento, 1999). The physicochemical and sensorial parameters must also be definitely controlled as a strategy to confirm the authenticity and to prevent or detect possible adulterations (Nogueira and Nascimento, 1999), which contributes to increase consumer confidence. Nogueira and Nascimento (1999) were

the first authors to study the physicochemical parameters of Madeira wines which were divided as acidic, alcoholic and phenolic, glucidic and extract, mineral and sulfur dioxide, and volatile composition. The *acidic* composition showed an average pH value almost invariant for all samples studied, which ranged between 3.26 and 3.42 (20 °C). The *alcoholic and phenolic* composition, taking into consideration the acquired volumetric alcoholic degree, observed by aerometry (20 °C), showed average values up to 18% for younger samples, but a small increment to above 19% could be observed for Madeira wine with 10 years (Nogueira and Nascimento, 1999). The average of total polyphenols measured, using the Folin-Ciocalteu colorimetric index (IFC) adopted by OIV, showed values which increase slightly with the sugar content (Nogueira and Nascimento, 1999). The *glucidic* and *extract* composition, as was expected, showed an average content of total sugar which clearly increased with the sweetness degree, which ranged between 47.98 and 122.50 g L^{-1}. The *mineral* and *sulfur dioxide* composition showed an average content of ash and ash alkalinity very similar for all samples studied. For chlorides, phosphates, and sulphates, the average contents increased slightly with aging, but the same level was observed within each type (Nogueira and Nascimento, 1999). The analytical parameters found in young Madeira wines from different varieties are presented in Table 7.1. The results are similar to those obtained by Nogueira and Nascimento (1999).

B. Volatile and aroma compounds of Madeira wines

1. Extraction techniques and analytical methodologies

The quality of wines is improved by optimizing the winemaking processes, such as harvest and grape processing alcoholic fermentation, malolactic fermentation, and barrel–botle aging. The legislation of the European Community (EC) and of single countries is devoted to protecting consumer health and internal markets from the sometimes harmful effects that may be caused by low-quality products. Legal limits are defined and quality certificates are often required (for pesticides, toxins, ethyl carbamate, etc). In this context, knowledge of the chemical composition of grapes and wines is essential.

The great development of analytical techniques and instruments has allowed the advance from the first studies focused on the analysis of major volatile compounds to the analysis of compounds present in very low concentrations (even at levels below ng L^{-1}) and with low odor thresholds. Due to the great complexity of the wine matrix, for the analysis of some minor, but *key* aroma compounds, different sample work-up procedures reported to determine volatile and semivolatile constituents,

TABLE 7.1 Global characterization of young Madeira wines (data from IVBAM, IP)

Variety		Density (g mL^{-1} a 20 °C)	pH	Ethanol content (%, v/v)	SO_2 (mg L^{-1})		Acidity (g L^{-1})			Sugars (g L^{-1})		Dry extract (g L^{-1})
					Free	Total	Volatile	Fix	Total	Reducing	Totals	
Boal	Min	1.0123	3.62	16.7	3.10	9.51	0.21	4.1	4.5	32.2	68.6	93.6
	Max	1.0164	3.68	16.9	4.21	12.20	0.55	4.9	5.1	35.7	76.5	104.0
	$\bar{x}$	1.052	3.66	16.8	3.64	10.8	0.42	4.5	4.6	34.1	73.0	99.3
Malvazia	Mín	1.0033	3.39	17.2	3.33	9.0	0.25	5.3	5.8	22.8	54.7	80.1
	Máx	1.0154	3.45	21.1	4.31	13.1	0.38	6.1	6.4	34.6	74.7	100.8
	$\bar{x}$	1.046	3.42	19.3	3.80	11.4	0.32	5.7	6.1	26.7	65.9	91.7
Sercial	Mín	0.9838	3.12	16.8	3.10	7.2	0.41	6.2	6.8	22.4	51.3	24.4
	Máx	0.9865	3.41	17.0	4.20	10.0	0.45	8.0	8.6	27.1	54.9	37.5
	$\bar{x}$	0.9853	3.26	16.9	3.50	8.7	0.43	7.3	7.7	25.7	52.7	32.9
Verdelho	Mín	0.9991	3.28	15.8	2.95	10.0	0.37	3.9	5.5	24.8	38.2	61.3
	Máx	1.0064	3.69	17.9	4.90	11.2	0.52	5.4	5.8	32.5	49.4	73.0
	$\bar{x}$	1.0039	3.41	17.2	3.87	10.6	0.47	4.8	5.7	27.9	44.2	67.8

Mín, minimum value; Máx, maximum value; $\bar{x}$, average.

are usually based on liquid–liquid extraction (LLE) and solid-phase extraction (SPE) (Câmara *et al.*, 2003a,b, 2004b, 2006c; Pozo-Bayón *et al.*, 2001). However, most of these approaches present several disadvantages, typically time- and labor-intensive, uses of significant amounts of environmentally unfriendly and toxic solvents, and involves multistep procedures, which can lead to analyze losses and a reduction in sensitivity. So, numerous efforts have been directed toward the search and development of adequate and valuable alternative extraction techniques that minimize the use of harmful organic solvents and/or even solvent-free procedures, and therefore, more sustainable and easily implemented.

Currently, the trend in the analysis of volatile compounds is more focused in the use of miniaturized sample preparation analytical techniques, namely solid-phase microextraction (SPME) developed by Pawliszyn in the 1990s (Arthur and Pawliszyn, 1990; Musteata *et al.*, 2007; Risticevic *et al.*, 2009), stir bar sorptive extraction (SBSE) (Baltussen *et al.*, 1999), and solid-phase dynamic extraction (SPDE) . These methodologies have been attracted the attention of many scientists due to its many special features over classical approaches (Alves *et al.*, 2005; Câmara *et al.*, 2004a, 2006a,b, 2007). Among the many advantages, use of little or no solvent, minimum sample preparation, increasing of efficiency of analysis, and user-friendly system should be pointed out.

The combination of these microextraction techniques that combine sampling, extraction and pre-concentration into a sigle step, with high sensitive gas chromatograph detectors such as mass spectrometer is the way to determine compounds at levels of ng L^{-1} that could be important for wine aroma characterization.

Recently, considerable research has been dedicated to the combination of independent techniques with the aim of strengthening resolving power (Kidwell and Riggs, 2004; Tranchida *et al.*, 2004). GC × GC combined with ToFMS detection represents a successful example of this combination. GC×GC was developed as a powerful separation method and emerged as an interesting alternative to analyze complex samples or analyze trace target analytes within a single analysis and overcoming the coelution problem (Souza *et al.*, 2009; Perestrelo *et al.*, 2010, 2011; Rocha *et al.*, 2007; Musteata *et al.*, 2007). GC × GC employs two orthogonal mechanisms to separate the constituents of the sample within a single analysis based on the application of two GC columns coated with different stationary phases.

2. Volatile constituents of young Madeira wines

Among the various factors contributing to consumer acceptance and valorization of the wine, its aroma and flavor are probably the most important parameters. Over the past few decades, wine aroma has been

thoroughly studied, resulting in knowledge of about 800 chemically different volatile compounds. However, a much smaller number are odor-active and must be considered for differentiation purposes. These compounds belong to different chemical groups, including higher alcohols, ethyl and isoamyl esters, FA, acetates, carbonyls, thiols, furan compounds, monoterpenoids, C_{13} norisoprenoids, volatile phenols among others, with different polarities, volatilities, and moreover are found in a wide range of concentrations (from ng L^{-1} to mg L^{-1}). They proceed from four major sources: (i) grapes (varietal origin); (ii) processing procedures (crushing, pressing, etc.) involving chemical, enzymatic-chemical, and thermal reactions; (iii) fermentation processes; and (iv) chemical reactions during maturation of wine (involving wood extractives, commonly oak (Alves *et al.*, 2005; Câmara *et al.*, 2006a; Perestrelo *et al.*, 2011; Pozo-Bayón *et al.*, 2001). Table 7.2 showed the volatiles found in Madeira wines with their corresponding odor descriptors and odor threshold.

Numerous studies on the volatile composition of Madeira wines helped to elucidate the basic flavor chemistry in this field of special interest. Enormous efforts were focused on the topic of varietal, aroma compounds (e.g., mono and sesquiterpenoids, and C_{13} norisoprenoids, Alves *et al.*, 2005; Câmara *et al.*, 2004a, 2006a, 2007); prefermentative compounds (e.g., C_6 alcohols and aldehydes; Câmara *et al.*, 2006b; Perestrelo *et al.*, 2010); a large group of secondary or fermentative compounds (e.g., higher alcohols, esters, fatty acids and carbonyl compounds; Alves *et al.*, 2005; Câmara et al., 2006b; Pereira *et al.*, 2010a,b, 2011; Perestrelo *et al.*, 2010), and finally post-fermentative or aging compounds (e.g., volatiles extracted from oak, like volatile phenols, furans, and lactones; Alves *et al.*, 2005; Câmara *et al.*, 2003a,b, 2004b, 2006b,c; Pereira *et al.*, 2010a,b, 2011; Perestrelo *et al.*, 2011).

It has been a long-standing aim of enological research to distinguish analytically between grape varieties or wines on the basis of compositional parameters. Such a differentiation is essential to an understanding of the factors responsible for varietal flavors of wines. Monoterpenoids secondary plant constituents formed by biosynthesis, and C_{13} norisoprenoids, resulting from biodegradation of diterpenes and carotenoids, form an important part of the grape bouquet. As these compounds are not significantly affected by the fermentation stage it has been suggested their use for the varietal characterization of wines.

Monoterpenoids have been described as being responsible for the varietal aroma of some wines. The characteristic composition of this chemical class in several grape varieties is claimed to be only marginally influenced by the growing area while other find effect of light exposure. These compounds are responsible for the aroma profile of the Muscat

TABLE 7.2 Madeira wine volatiles isolated by HS-SPME using a DVB/CAR/PDMS fiber and identified by gas chromatography–mass spectrometry, their principal *m/z* signals,[a] and the corresponding odor descriptors and odor thresholds

Chemical groups/volatiles	*m/z*[a]	Odor description[a]	Odor threshold[a] (ng mL^{-1})
Terpenoids			
(*E*)-Linalool oxide	59, 43, 68	Floral, green, rose, sweet	190
(*Z*)-Linalool oxide	59, 43, 68	Floral, green, rose, sweet	100
Linalool	93, 121, 136	Lavender, lemon, floral, green, muscat	6
DOD	67, 71, 82	–	–
α-Terpineol	93, 121, 136	Earth, pungent, wood	250
(+)-δ-Cadinene	161, 189, 204	Fresh, wood	–
Citronellol	71, 68, 55, 43	Citrus, clove, floral, fresh, green, rose,	30
Ho-trienol	93, 121, 136	Floral	110
Nerol	93, 121, 136	Rose, lime, floral	400
Geraniol	93, 121, 136	Floral, rose	100
Geranyl acetone	69, 43	Floral, fruity, guava, pear, waxy, wood	60
Nerolidol	69, 93, 41	Waxy, rose, apple, green, citrus	64,000
Farnesol	69, 93, 41	Sweet, mild, oily, floral	20
C_{13}-Norisoprenoids			
Vitispirane I	177, 192	Camphor, eucalyptus, spice, wood	800
Vitispirane II	177, 192	Camphor, eucalyptus, spice, wood	–
TDN	157, 142, 115	Floral, peach, pleasant, strawberry, wine	2.5
β-Damascenone	69,121, 41	Apple, floral, fruity, honey, sweet, tobacco	1.5
Alcohols			
1-Butanol	56, 43, 41	Sweetish, putrid, oil	500
4-Methyl-2-pentanol	69, 45	Pungent, alcohol	50,000

3-Methyl-1-butanol	57, 56, 41	Roasted, wine, onion, fruity	250
1-Hexanol	56, 55, 43	Fruity	2500
(*E*)-3-Hexen-1-ol	67, 82, 55	Green, grass	–
(*Z*)-3-Hexen-1-ol	67, 82, 55	Fresh, green grass-like, leafy	70
(*E*)-2-Hexen-1-ol	67, 82, 55	Walnut, medicinal, cooked butter, green, leafy	–
(*Z*)-2-Hexen-1-ol	67, 82, 55	Fruity, green, caramel	–
2,3-Butanediol	57, 45, 43	Buttery, creamy	668,000
1-Nonanol	69, 57, 41	Citrus	50
Methionol	106, 61, 58, 57	Boiled potato, cooked cabbage, rubber, soup	500
Benzyl alcohol	108, 107, 78	Blackberry, fruity	10,000
Phenylethyl alcohol	91, 122, 92	Floral, herbal, honey, pollen, rose, spice, sweet	750
2-Phenoxyethanol	94, 77, 66	Alcoholic, floral, rose	–
Ethyl esters			
Ethyl hexanoate	88, 101, 99, 43	Anise, fruity, strawberry, sweet, wine	14
Ethyl octanoate	88, 101, 127	Fruity, must, pineapple, soap, sweet, waxy	580
Ethyl nonanoate	88, 101, 141	Apple, banana, cognac, tropical, waxy, wine	–
Ethyl decanoate	88, 101, 155	Apple, fruity, pleasant, soap, sweet, waxy	200
Ethyl benzoate	105, 77, 122	Floral, herbal, honey, lettuce, watermelon	60
Ethyl 9-decenoate	88, 101, 69	Fruity	–
Ethyl benzeneacetate	91, 65	Floral, fruity, honey, spice, sweet	–
Ethyl dodecanoate	88, 101, 183	Soap, sweet	8
Ethyl tetradecanoate	88, 101, 157	Fatty-cognac, oil, waxy, weak	–
Ethyl hexadecanoate	88, 101, 157	Cream, fruity, milk, rancid, sweet, waxy	>2000
Methyl 7,10-octadecadienoate	81, 95, 67	–	–
Ethyl pyruvate	43	Ethereal, fruity, sweet, rum-like	100,000
Ethyl lactate	45, 75	Acidic, ethereal, fruity, strawberry, sweet	14,000

(*continued*)

TABLE 7.2 (*continued*)

Chemical groups/volatiles	*m/z*[a]	Odor description[a]	Odor threshold[a] (ng mL^{-1})
Ethyl 2-hydroxy-3-methylbutanoate	73, 55, 76	Pineapple, strawberry, tea, honey	–
Diethyl succinate	101, 129	Fabric, floral, fruity, potato, sweat, watermelon	200,000
Isopropyl myristate	102, 60, 43	Faint, fatty, oil	–
Ethyl 3-hydroxyhexanoate	71, 117, 43	Citrus, fruity, grape, green, sweet	265
Methyl salicylate	120, 92, 65	Berry, sweet, warm, wine	40
Higher alcohol acetates			
Ethyl acetate	43, 61, 79	Acid, buttery, caramel, fruity, pungent, solvent, sweet	7500
Isoamyl acetate	43, 55, 70	Banana, fresh, fruity, pear, sweet	30
Hexyl acetate	43, 56, 84	Acid, citrus, fruity, green, herbal, rubber, spice, sweet wine, tobacco	1500
2-Phenylethyl acetate	104, 43	Cocoa, floral, honey, rose	250
Acids			
Acetic acid	43, 45, 60	Vinegar, sour, pungent	200,000
Propanoic acid	74, 73, 45	Pungent, acidic, dairy-like	20,000
Dimethylmalonic acid	60, 43	–	–
Butanoic acid	60, 73	Sharp, dairy-like, cheesy, buttery	240
Isovaleric acid	60, 87, 43	Sweaty, cheese, rancid	120
2-Hydroxybenzenepropanoic acid	60, 104, 77	–	–
Hexanoic acid	60, 73, 87	Sweaty, pungent, cheese, goat-like, rancid	3000
2-Ethylhexanoic acid	73, 88, 57	Mild	–
Octanoic acid	60, 73, 43	Fatty acid, cheese, fresh, moss	7000

Nonanoic acid	60, 73, 57	Green, fat, musty, sweaty, sour	3000
Decanoic acid	60, 73, 41	Soapy, fatty	15,000
Benzoic acid	105, 122, 77	Wine-like, very weak, balsamic	–
Dodecanoic acid	60, 73, 43	Dry, metallic, weak, fatty, waxy	10,000
Tetradecanoic acid	60, 73, 55	Very faint, waxy-oily	10,000
Volatile phenols			
4-Ethylguaiacol	137, 152, 122	Clove, leather, phenolic, smokey	50
Eugenol	164, 149, 131, 77	Balsamic, camphoraceous, honey, spice	6
Furans			
2-Furfural	96, 95, 39	Almond, caramel, fruity, sweet, wood	3000
5-Acetoxymethyl-2-furfural	126, 109, 79, 43	–	–
5-Hydroxymethyl-2-furfural	97, 126, 41	Almond, cardboard, paper	–
Pantolactone	71, 43	Caramel, cocoa, liquorices, sweet, toast	50
Butyrolactone	86, 42	Caramel, coconut, cream, peach	35,000
Carbonyl compounds			
Acetaldehyde	44, 29	Pungent, ethereal, fresh, fruity	15
Benzaldehyde	106, 105, 77	Burnt sugar, almond, woody	350
1-(2-Methylphenyl)-ethanone	119, 91, 134	Sweet, anisic, phenolic, burnt, nutty, honey	–

DOD, 2,6-dimethyl-1,7-octadiene-3,6-diol; TDN, 1,1,6-trimethyl-1,2-dihydronaphthalene.
[a] Adapted from Câmara (2004).

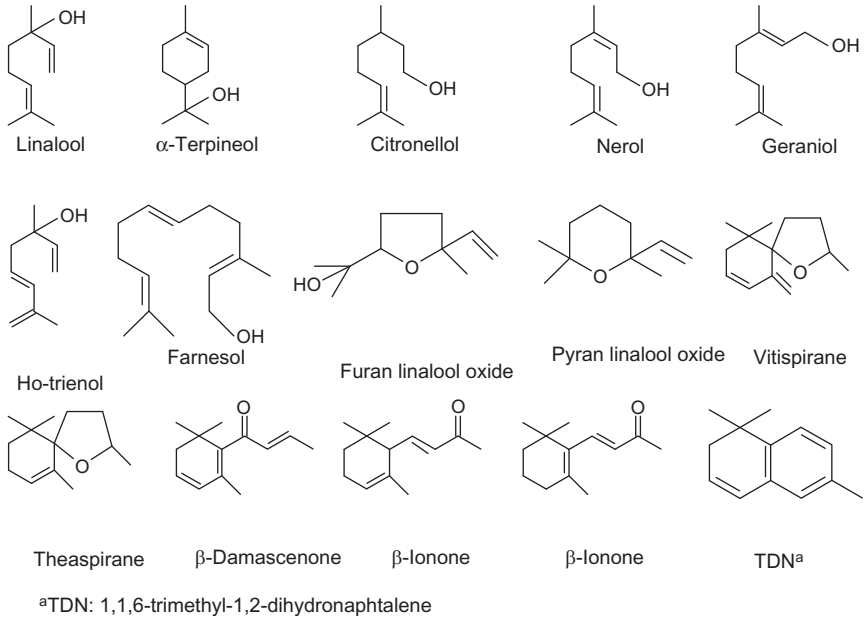

FIGURE 7.3 Major varietal constituents found in *Vitis vinifera* L. varieties used in the production of Madeira wines.

varieties, but some of non-Muscat grape varieties such as Riesling, Sylvanner, and Gewürztraminer also contain higher levels of monoterpenes. The major varietal constituents found in *Vitis vinifera* L. varieties used in the production of Madeira wines are illustrated in Fig. 7.3.

Câmara *et al.* (2004a) used SPME in headspace mode (HS-SPME) combined with gas chromatography–quadrupole mass spectrometry (GC–qMS) methodology to study the varietal composition of musts and young (1–3 years old) Madeira wines. The content of monoterpenoids and C_{13} norisoprenoids have been determined in a Malvasia, Boal, Sercial, and Verdelho must samples over three consecutive vintages (1998–2000; Fig. 7.4). Linalool, α-terpeniol, citronellol, and β-damascenone are the predominant varietal compounds in these samples, and according to the authors, these volatiles are present at levels lower than their odor threshold. The Malvasia wine showed the highest monoterpenoid content, whilst the Verdelho wine exhibited the lowest one (Fig. 7.4A). Principal component analysis (PCA) and stepwise linear discriminant analysis (SLDA) analyzes provided good differentiation among the four varieties studied. Among C_{13} norisoprenoids, β-damascenone is the most prevalent. With concentrations above its odor threshold value (45 ng L^{-1} according to Ribéreau-Gayon *et al.*, 2000) it may be considered as a

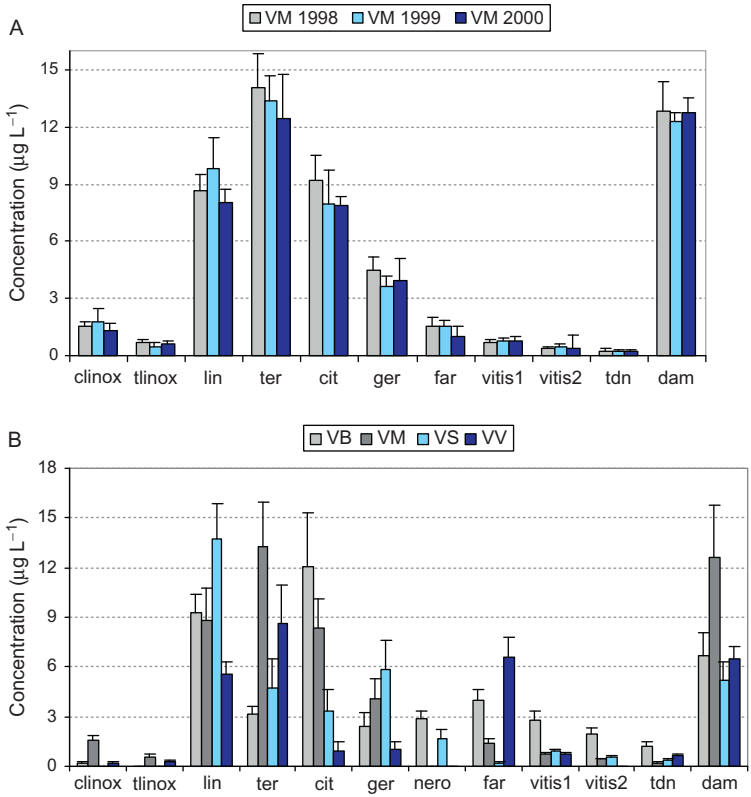

FIGURE 7.4 Profile of compounds from varietal origin (monoterpenoids and C_{13} norisoprenoids) found in young Madeira wines, according to (A) vintage year (1998, 1999, and 2000), and (B) grape variety (VB, Boal wine; VM, Malvasia wine; VS, Sercial wine; VV, Verdelho wine). Legend: clinox, (*E*)-linalool oxide; tlinox, (*Z*)-linalool oxide; lin, linalool; ter, α-terpineol; cit, citronellol; ger, geraniol; far, farnesol: vitis1, vitispirane (isomer 1); vitis2, vitispirane (isomer 2); tdn, 1,1,6-trimethyl-1,2-dihydronaphthalene; dam, β-damascenone; adapted from Câmara, 2004).

potential impact odorant, contributing with notes of violets, exotic fruit, and/or exotic flowers for the overall aroma of young Madeira wines. In addition, during three consecutive vintages, the content of monoterpenoid compounds remained relatively constant (Fig. 7.4B).

In order to obtain a deep characterization and differentiation of Madeira wines according to main grape varieties (Câmara *et al.*, 2006a,b,c), multivariate analysis was applied to varietal, prefermentative, and fermentative data on terpenoids, C_6 alcohols, higher alcohols, fatty acids, ethyl esters, and carbonyl compounds. The results showed that Malvasia wines had the

highest content of monoterpenoids, whereas Boal wines were distinguished by the highest levels of C_{13} norisoprenoids, higher alcohols, and carbonyl compounds. Sercial wines presented significant levels of acetates, fatty acids, ethyl esters and volatile phenols, and Verdelho wines were characterized by the highest content of ethyl esters and furan compounds. These data also allowed the statistic differentiation between these wines. From 42 variable used in the multivariate analysis, ethyl octadecanoate, (*Z*)-3-hexen-1ol, benzoic acid, and ethyl benzeneacetate are those most correlated with Malvasia wines, whereas benzyl alcohol, (*E*)-3-hexen-1ol, benzaldehyde, and (*E*)-2-hexen-1ol are highly associated to Boal wines. For Sercial wines, the 2-methylpropan-1-ol is the most discriminant variable, whereas for Verdelho, 5-ethoxymethyl-2-furfural, nonanone, ethyl 9-decenoate, and 5-hydroxymethyl-2-furfural are the most discriminant. The knowledge of the odor thresholds (Table 7.2) of the wine volatile constituents allowed obtaining the odor activity values (OAVs) of the aroma compounds found in wines. OAV was obtained by dividing the concentration of the compound in a matrix by its odor threshold in that matrix. Although this parameter provides a rough pattern of the sensory importance of the odorants, it allows turning the quantitative data into sensorial information. So it is generally assumed that the odorants with higher OAVs contribute in a stronger manner to the overall aroma. Thus, the most potent odor-active impact compounds on the aroma of young Madeira wines were quantified (Fig. 7.5).

There are seven compounds with OAV > 1 in the Madeira wines analyzed. These compounds are displayed in a spider-web (Fig. 7.5).

Tinta Negra Madeira wines were fully studied by Perestrelo *et al.* (2006). By using LLE/GC–qMS, it was identified more than 80 volatile compounds, belonging mainly to higher alcohols, ethyl esters, acids, and lactones. Higher alcohols are composed of isoamyl alcohols (e.g., 3-methylbutan-1-ol), C_6 alcohols, and aldehydes (related to lipoxygenase activity of grapes), and aromatic alcohols (e.g., benzyl alcohol and 2-phenylethanol). Short-chain alcohols and aldehydes, such as (*E*)-3-hexenol and (*E*)-3-hexenal, are associated with flavors described as ''green,'' or ''grassy,'' and are the products of lipid degradation. Upon tissue disruption, fatty acids come in contact with lipoxygenase enzyme (LOX), and the volatiles are subsequently released. The biosynthetic pathway of volatiles derived from fatty acids is illustrated in Fig. 7.6.

Ethyl hexanoate and octanoate were the predominant FA esters found in Tinta Negra wines, whereas diethyl succinate and ethyl lactate were the most abundant diprotic acid ethyl esters. According to the authors, the most sensory important flavors seem to be attributed to 3-methylbutan-1-ol, 2-phenylethanol, isoamyl acetate, diethyl succinate, 2-phenylethylacetate, phenylacetaldehyde, γ-nonalactone, ethyl hexanoate, ethyl octanoate, hexanoic acid, and octanoic acid.

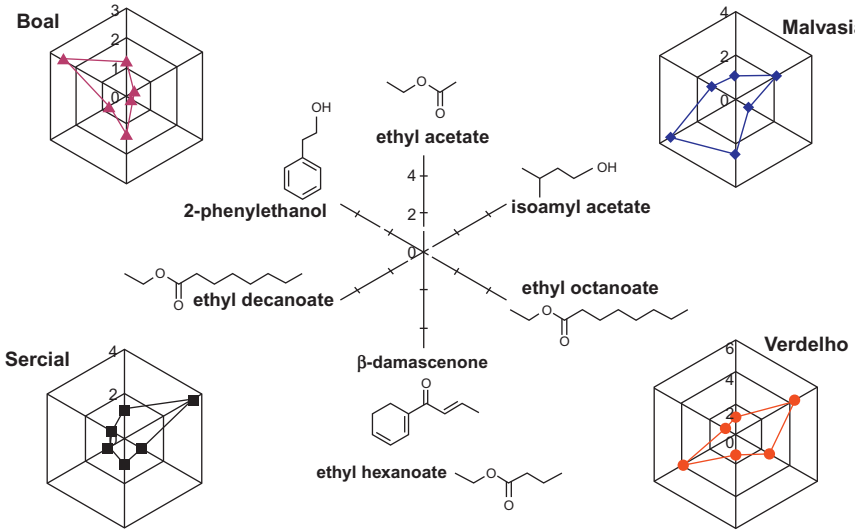

FIGURE 7.5 Spider-web for the odorants with OAV 1 for the four young Madeira wines studied (adapted from Câmara, 2004).

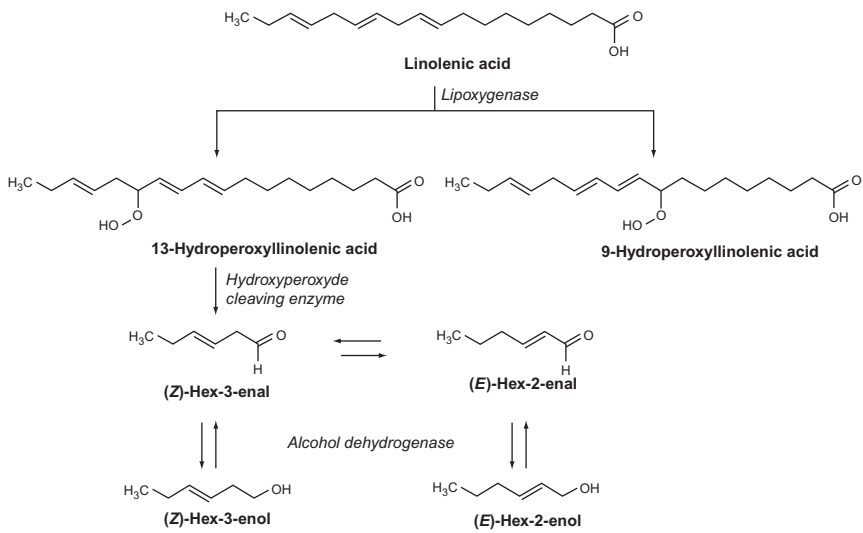

FIGURE 7.6 Short-chain aldehydes and alcohols produced from the degradation of fatty acids in grapes via the lipoxygenase (LOX)/hydroperoxide lyase (HPL) pathway during the prefermentative stages of vinification (adapted from Câmara, 2004).

The powerful potentialities of SBSE followed by thermal desorption and GC–qMS methodology to characterize Madeira wine was also explored by Perestrelo *et al.* (2009). This methodology provided higher ability for profiling traces and ultratraces of compounds in Madeira wines, including esters (80.7–89.7%), higher alcohols (3.5–8.2%), C_{13} norisoprenoids (1.7–6.5%), carboxylic acids (1.6–4.2%), aldehydes (0.9–3.7%) pyrans (0.2–1.7%), lactones (0.3–2.7%), and mono (0.1–1.4%), and sesquiterpenoids (0.1–0.8%). The authors reported that the concentration of some of them is above their odor threshold, and therefore can probably play a remarkable impact on the aroma complexity of the corresponding wines.

3. Evolution of volatile compounds during Madeira wine aging

The aging process promotes several modifications in Madeira wine composition. Such changes are essentially the result of the baking process, oxidative conditions (due to oxygen diffusion through pores in wood cooperage, affecting both intrinsic wine components and those extracted from the oak) and, to a lesser extent, phenomena common to all types of aging processes. These include crystal precipitation, chemical reactions between wine components, and substances extracted from wooden cooperage. All these processes can modify the wine's volatile composition. Many of these changes are subtle and, in some cases, so small that their impact on the sensory properties of wine is not noticeable. On the other hand, certain reactions have a noticeable effect on the various sensory attributes of wine, and they play a significant role in wine aging and hence wine aroma. Also microorganisms, derived from the wine or barrels, especially lactic bacteria and yeasts, can produce important impact volatile compounds, such as vinylphenol and vinylguaiacol.

The release of oak volatiles into the wine during the barrel aging of wine, one of the primary reasons why wines are made to undergo aging in the wood, is regulated by diffusion kinetics. As a general rule, extraction of volatiles is highest at the beginning (few month) of aging, gradually tapering off with time and barrel age (Cerdán and Ancín-Azpilicueta, 2006). This makes it necessary to take into account the different factors that modulate the release of volatile components from the oak to the wine. These factors include the type of oak employed and its geographical origin (Simón *et al.*, 2003; Perez-Coello *et al.*, 1999), the drying treatment to which the oak has been subjected (Doussot *et al.*, 2002; Masson *et al.*, 2000), the degree of toasting (Cadahía *et al.*, 2003; Chatonnet, 1999; Hale *et al.*, 1999), and the time that wine is in the barrel, along with barrel age and usage, that is, the number of times a barrel has been used (Pérez-Prieto *et al.*, 2002; Singleton, 1995). The volatiles arising from oak have a distinct impact on the aroma of the wine. Their nature

depends on the quantity potentially extractable, and the duration of contact time. Among those released from the wood aromatic aldehydes (namely vanillin), and γ-lactones (notably the oak lactones (*E*)- and (*Z*)-β-methyl-γ-octalactones) can have a great influence on the aroma of the wine. 2-Furfural and 5-methyl-2-furfural have little influence. γ-Lactones are formed by cyclization from their corresponding β-hydroxycarboxylic acids. The basic biosynthetic mechanisms are summarized in Figure 7.7 (Haffner *et al.*, 1996). Their fragrance is characterized as coconut-like and fruity-like (γ-hexalactone), coconut-like (γ-octalactone), peach-like and milky (γ-decalactone), and fruity, sweet floral (γ-dodecalactone).

The specific oxidative conditions to which Madeira wine is submitted can lead to an increase in aldehydes, mainly acetaldehyde, and acetals (Belitz and Grosch, 1999). Due to the increased acetaldehyde content, acetalization between acetaldehyde and glycerol (a major wine constituent) is highly favored at wine pH values. The result is the formation of four heterocyclic acetals: (*E*)- and (*Z*)-5-hydroxy-2-methyl-1,3-dioxane ((*E*)-dioxane and (*Z*)-dioxane) and (*E*)- and (*Z*)-4-hydroxymethyl-2-methyl-1,3-dioxolane ((*E*)-dioxolane and (*Z*)-dioxolane). These compounds have been identified in several wines (Müller *et al.*, 1978; Silva Ferreira *et al.*, 2002; Simpson, 1980; Williams and Strauss, 1978), and their evolution in Port wine and Madeira wine has been described in detail by Silva Ferreira *et al.* (2002) and Câmara *et al.* (2003a,b), respectively. The influence of the baking process and aging on dioxanes and dioxalanes content was evaluated by Câmara *et al.* (2003a,b). A linear correlation of the investigated acetals with wine age has been observed. It permits

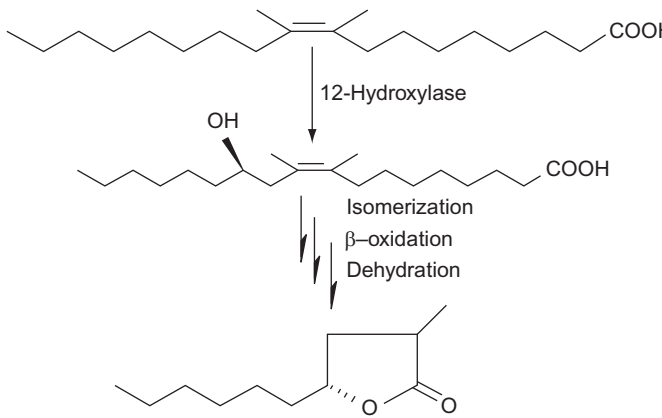

FIGURE 7.7 Formation of γ-decalactone from unsaturated fatty acid (oleic acid; adapted from Haffner and Tressl, 1996).

differentiation of young from old Madeira wines and can be used as an indicator of Madeira wine age. The (*E*)-5-hydroxy-2-methyl-1,3-dioxane is the isomer present at highest level in every of the Madeira wines analyzed after the first 3 years of aging.

Câmara *et al.* (2004a,b, 2006c) identified over 120 volatile compounds in aged Madeira wines. The most predominant ones were found in higher alcohols (mainly in isoamyl alcohols and 2-phenylethanol), ethyl esters of medium-chain acids (hexanoic and octanoic acids), and furanic derivatives. Other components included C_{13} norisoprenoids, isomers of vitispiranes, β-damescenone and TDN, monoterpene oxides ((*E*)-furan linalool oxide and (*Z*)-furan linalool oxide), fatty acids, aldehydes, furan derivatives, γ-lactones, such as the (*E*)- and (*Z*)-isomers of whisky lactone, isomers of dioxanes and dioxolanes, and some enolic derivatives. Only two sulfur-containing compounds were identified: methionol and benzothiazol. From 120 volatiles, a linear correlation ($r > 0.91$) between concentration and age was achieved only for Sotolon, 2-furfural, 5-methyl-2-furfural, 5-hydroxymethyl-2-furfural, 5-ethoxymethyl-2-furfural, (*E*)-dioxane, and (*E*)-dioxolane (Câmara *et al.*, 2006c). Thus, these volatiles appear to be good indicators of Madeira wine age.

The major volatile changes in Madeira wines involved a marked decrease in the concentration of ethyl esters from fatty acids (C_6–C_{16}) and acetates. In contrast, the concentration of the EE of diprotic acids, such as ethyl lactate and diethyl succinate, increased markedly. Figure 7.8 shows the comparative profile of the volatile compounds of Malvasia wines with 1 and 25 years old.

The increase in 3-methylbutan-1-ol and 2-phenylethanol content contribute to fruit and floral odors of aged Madeira wines, whereas limited development of C_6 alcohols avoids the generation of herbaceous and vegetal aspects. The decrease of FAEE during aging could explain the absence of freshness and fruitiness in old Madeira wines. Other observations include marked decreases in the levels of medium and long-chain acids (e.g., hexanoic and octanoic acid), whilst the levels of short-chain acids (e.g., butanoic and isobutanoic acid) increase. Furan content was directly correlated with age and residual sugar content of the wines. Figure 7.9 illustrates the evolution of the major chemical groups identified in Madeira wines during wine aging.

Monoterpenoids (Ter), C_{13} norisoprenoids (C13), and ethyl esters from fatty acids (EE) showed a significant decrease with wine aging. Conversly, fatty acids (FA), ethyl esters from fix acids (EEFA), lactones (Lac), volatile phenols (VP), and furan compounds (Fur) exhibited a great increase during aging, whilst higher alcohols and acetates showed an irregular behavior (Fig. 7.9).

The establishment of potential age markers is important to detect frauds and to ensure the authenticity of the wine. Further, the economic

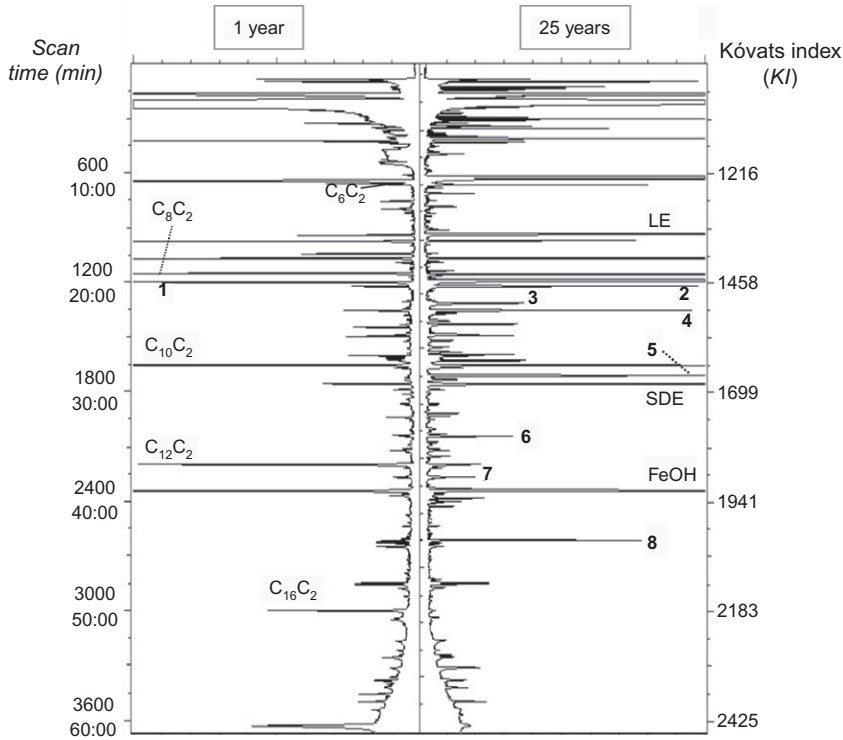

FIGURE 7.8 Typical GC–qMS chromatograms of a dichloromethane extract from two Malvasia wine samples aged 1 and 25 years . *Peak identification*: (1) acetic acid; (2) 2-furfural; (3) 1,1-diethoxyethane; (4) benzaldehyde; (5) ethyl benzoate; (6) ethyl benzeneacetate; (7) benzyl alcohol; (8) ethyl 3-hydroxyhexanoate; LE, ethyl lactate; SDE, diethyl succinate; FeOH, 2-phenylethanol; C6C2, ethyl hexanoate; C8C2, ethyl octanoate; C10C2, ethyl decanoate; C12C2, ethyl dodecanoate; C16C2, ethyl hexadecanoate (adapted from Câmara *et al.*, 2006a,b,c).

value of Madeira wine is highly associated with its age. Some volatile compounds that belong to furans, lactones, volatile phenols, and acetals have been reported as potential aging markers in Madeira wines (Câmara *et al.*, 2003a,b, 2006a,b,c; Pereira *et al.*, 2010a,b,c). These studies revealed the complexity of Madeira wine matrix. Recently, Perestrelo *et al.* (2011) used the GC × GC–ToFMS combined with HS-SPME methodology in order to obtain a deep characterization of the chemical groups, namely, furans, lactones, volatile phenols, and acetals potentially related with aging of Madeira wines from different varieties (Malvasia, Boal, Sercial, Verdelho, and Tinta Negra), types (sweet, medium sweet, dry, and medium dry), and ages (Vintage and blended wines). Considering the chemical groups of furans, lactones, volatile phenols, and acetals, one hundred and three

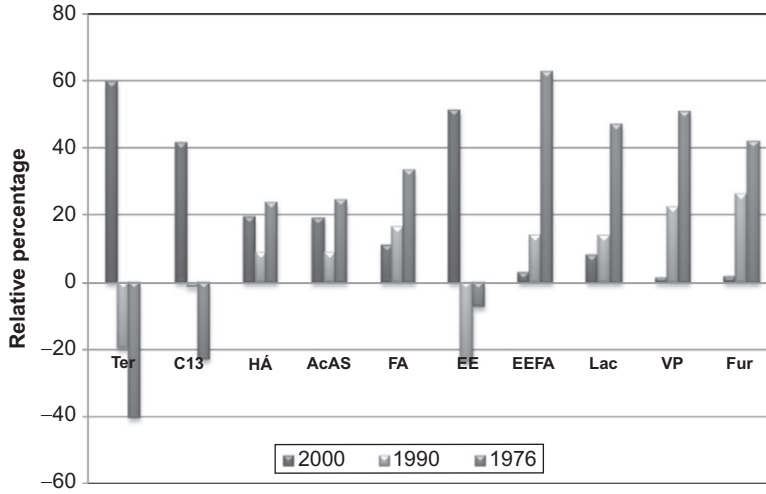

FIGURE 7.9 Evolution of some chemical families of volatile compounds during Madeira wine aging (adapted from Câmara, 2004).

volatile compounds were tentatively identified, among these 71 have been reported for the first time in Madeira wines. The chemical groups that could be used as potential age markers were predominantly acetals, namely diethoxymethane, 1,1-diethoxyethane, 1,1-diethoxy-2-methyl-propane, 1-(1-ethoxyethoxy)-pentane, *trans*-dioxane and 2-propyl-1,3-dioxolane, and from the other chemical groups, 5-methylfurfural and *cis*-oak-lactone, independently of the variety and the type of wine.

In order to evaluate the best temperature and time of baking process, Silva *et al.* (2008) used an expert panel to analyze seven descriptors, including dried fruit, nutty, baked, oak, mushroom, and brown sugar. The optimal temperature and time of baking process respecting the specificity of Madeira winemaking are considered 45 °C for 4 months. On the basis of aroma extract dilution analysis (AEDA), several Maillard by-products, such as Sotolon, 2-furfural, 5-methyl-2-furfural, 5-ethoxy-methyl-2-furfural, methional, and phenylacetaldehyde, were identified in both Malvasia and Sercial wines under study which may explain the baked, brown sugar, and nutty odor descriptors.

4. Impact odorants in Madeira wines

The aroma of wine is one of the most complex existing in nature for a number of reasons. Firstly, because there are many different wines showing distinctive aromas, secondly because the aromas of even a single wine change with time, while it is stored in the bottle and in the glass before being consumed. Finally, in most cases, wines do not have a simple

characteristic aroma. In contrast, they have a palette of subtle aromas which are very difficult to define. They are also perceived idiosyncratically by different consumers or expertises. Such sensorial complexity is, of course, caused by the chemical complexity of the wine's aroma.

Madeira wine is often noted as possessing a complex and unique fragrance. During aging, the wines lose its fresh and fruity character being replaced by more complex descriptors, such as nuts, dried fruits, toasty, brown sugar, almonds, and mushrooms (Alves *et al.*, 2005). In addition, according to Silva *et al.* (2008), Madeira wines were also characterized as maderized, spicy, lacquer, candy, and nutty descriptors. The lowest scores were related to spicy descriptors, whereas the highest detected related to candy, toasty, maderized, and dried fruits and spicy aspects. These attributes depend on grape variety and winemaking process including aging (Campo *et al.*, 2006; Silva *et al.*, 2008). Features related to dried fruits and toasty were rather homogenous for all categories of Madeira wines, suggesting that these descriptors are characteristic of all Madeira wines, independent of grape variety. The burnt sugar and caramel characteristics are defined by the presence of 2-hydroxy-3-methyl-cyclopentanone (cyclotene) and 3-hydroxy-2-methyl-pyranone (maltol) (Fig. 7.10). These compounds have been previously identified in toast oak by Dubois (1989). The former is formed from the less basic Amadori intermediates and can be produced at lower temperature than maltol (main product of the thermal degradation of 1,4-disaccharides).

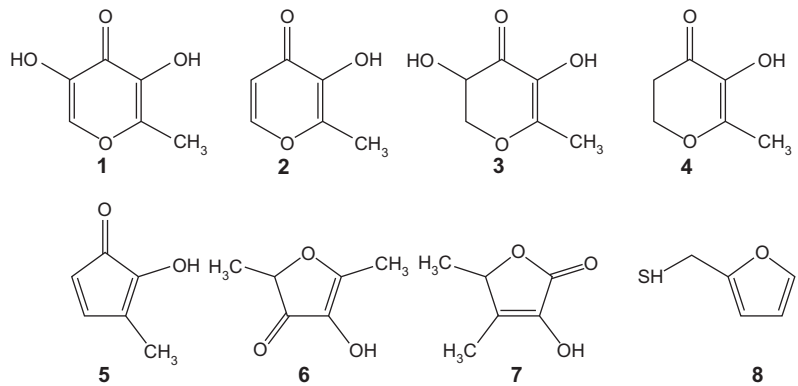

FIGURE 7.10 Structures of volatile compounds characterized from "toasty caramel" aroma released in wine from toasted woods during aging. (1) 3,5-dihydroxy-2-methyl-4*H*-pyran-4-one; (2) 3-hydroxy-2-methyl-4*H*-pyran-4-one; (3) 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP); (4) 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (furaneol); (5) 2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one (dihydromaltol); (6) 2-hydroxy-3-methyl-2-cyclopenten-1-one (or cyclotene) (Cutzach *et al.*, 1997); (7) 3-hydroxy-4,5-dymethyl-2(5*H*)-furanone (Sotolon; Câmara *et al.*, 2006a,b,c); (8) 2-furanmethanethiol (furfurylthiol; Tominaga *et al.*, 2000).

4-Hydroxy-2,5-dimethyl-3(2*H*)-furan-3-one (furaneol) has an intense and persistent fruity-toasty aroma, whereas 2,3-dihydro-3,5-dihydroxy-2-methyl-4(*H*)-pyranone (DDMP) present a toasty character with fruity-caramel overtones. These molecules can be produced in fair extension by the heat breakdown of single or complex sugars in the cask wood. In Madeira wines, much larger quantities can be formed when the sugars are heated in the presence of an amino acid residue during the baking process (*estufagem*) by Maillard reactions. The structures of some of the volatile compounds responsible for toasty and caramel odors are shown in Fig. 7.10. Formation of these molecules in the presence of amino acids allows to infers that Maillard reactions occur.

A powerful odorant with a strong roast coffee aroma (Tominaga *et al.*, 2000), 2-furanmethanethiol (2-furfurylthiol), was detected only in Sercial wines, contributing probably to their typical aroma (Câmara, 2004). The aroma profile of these wines were enriched in Sotolon, phenylacetaldehyde, wood extractable aromas, and lacked the important varietal aromas, such as monoterpenoids (e.g., linalool), cysteine-derivative thiols (e.g., 3-mercaptohexyl acetate), and pyrazines (e.g., 2-methoxypyrazines; Silva *et al.*, 2008). Although 2-furfural, 5-methyl-2-furfural, 5-hydroxymethyl-2-furfural, 5-ethoxymethyl-2-furfural are often formed during aging of sweet wines (Table 7.3), they were not detected by gas chromatography–olfactometry (GC-O). The data obtained suggest that these furans are not relevant to aroma attributes of Madeira wines (high odor threshold), even if they are quantitatively important.

Pereira *et al.* (2010c,d) found that the levels of 5-hydroxymethyl-2-furfural and 2-furfural in sweet wines (Malvasia, Boal) increased slight for sweet (Malvasia) when *estufagem* was conducted at 30 °C. In dry wines (Sercial), their content was found below to its detection threshold. At higher temperatures (45 and 55 °C) a continuous increase was observed. Thus, the presence of 5-hydroxymethyl-2-furfural can be easily controlled during baking process by adjusting the temperature.

Another impressive flavor compound, recognized as the key molecule on the typical aroma of barrel aged Port wines (Baumes *et al.*, 1986) which present a certain degree of similarity with old Madeira wines, is the 3-hydroxy-4,5-dymethyl-2(5*H*)-furanone (Sotolon). Their odor threshold was evaluated at 19 μg L^{-1} in Port wine, according to Silva Ferreira *et al.* (2002). It contributes significantly to the characteristic sensorial impression of several foods (Silva Ferreira *et al.*, 2002, 2003) as well as other liquor wines like flor-sherry and *Botrytised* wines (Ferreira *et al.*, 1997; Simpson, 1978), Port wine (Silva Ferreira *et al.*, 2003), Jura wines (Blanck *et al.*, 1992) ''vin jaunes,'' ''vins doux naturelles'' (Guth and Grosch, 1994), and Tokay wines. On the other hand, different authors suggested that Sotolon can contribute to the typical aged aroma of wines. According to its contents, Sotolon can influence differently the aroma of wines.

TABLE 7.3 Values of furan compounds determined in different types of Madeira wines

Wines		Furanic compounds (relative amount)			
		Furfural	5-methyl-furfural	HMF[a]	EMF[b]
Malvazia	Min[c]	0.5	0.02	5.7	0.0
	Max[d]	23.3	3.9	100.3	13.2
	$\bar{x}^{e}(n = 22)^{f}$	*9.7*	*1.7*	*44.9*	*3.6*
Boal	Min	0.8	0.02	2.9	0.0
	Max	24.1	1.9	74.3	10.9
	$\bar{x}(n = 26)$	*8.6*	*0.7*	*29.1*	*2.4*
Verdelho	Min	0.3	0.05	0.7	0.0
	Max	21.0	2.3	46.6	11.9
	$\bar{x}(n = 21)$	*7.5*	*0.9*	*14.7*	*1.5*
Sercial	Min	0.2	0.0	1.2	0.0
	Max	19.0	2.9	39.1	5.9
	$\bar{x}(n = 17)$	*5.7*	*0.6*	*10.4*	*0.5*

	LOP (mg L^{-1})	Odor description
Furfural	150	Paper, green
5-Methyl-2-furfural	20	Curry, nut
HMF	100	Aldehydes
EMF	–	Spice, curry

Sensory thresholds (in beer) and odor descriptors.
[a] 5-Hydroxymethyl-2-furfural.
[b] 5-Ethoxymethyl 2-furfural.
[c] Minimum.
[d] Maximum.
[e] Mean value.
[f] Number of samples.

Less than 300 μg L^{-1}, Sotolon takes part of a *plume* aroma, whereas between 300 and 600 μg L^{-1}, it is responsible for the *dried prickly-pear*, *dried fruit* aroma. More than 600 μg L^{-1}, the wines are characterized by *rancio* character.

Kobayashi (1989) reported the formation of Sotolon in wines by an aldol condensation of acetaldehyde and α-ketobutiric acid (derived from threonine) followed by lactonization (Fig. 7.11). During aging, ethanol is converted into acetaldehyde, thus allowing the formation of Sotolon (Silva Ferreira *et al.*, 2003).

On the other hand, several authors reported a relation between Sotolon levels and the presence of sugar, which are present in great quantities in Madeira wines (Câmara *et al.*, 2006c). It is important to note that the fortified Madeira wines were always aged in thermal/oxidizing environment without yeast ''flor.''

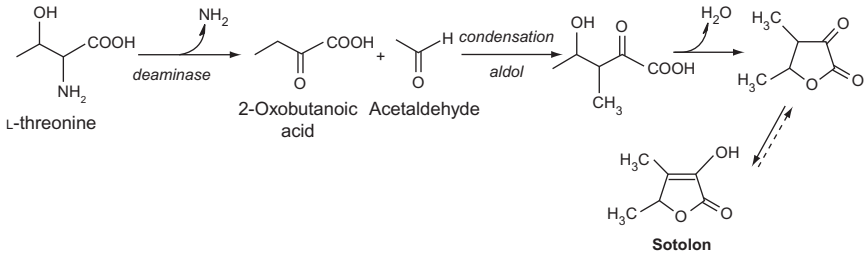

FIGURE 7.11 Formation of Sotolon (3-hydroxy-4,5-dimethyl-2(5*H*)-furanone) in wines by aldol condensation of acetaldehyde and 2-oxobutanoic acid followed by lactonization (Kobayashi, 1989).

C. Organic acids

The organic acid profile of a wine is an important parameter since it can provide information relative to the fermentation process, a wine physicochemical stability and sensory attributes (e.g., color, flavor, and taste), microbial state, and geographic authenticity (Klampfl *et al.*, 2000). Relatively few organic acids of wine present enough volatility to contribute to its odors. The most common acids in wines, tartaric, malic, and citric acids, come from the grapes, whereas succinic, lactic, and acetic acids result from alcoholic and malolactic fermentations. A particularly odorous acid is acetic acid (vinegar odor). It confers a disagreeable sensation in the mouth. For that reason, volatile acidity is one of the most important analytical parameters to characterize in enology. Noticeable acetic acid may be due to the development of lactic disease, or because yeasts produce more acetic acid than normal by hydrolysis of acetyl-CoA. Pereira *et al.* (2010c) performed the first study of organic acids in Madeira wines. The tartaric, malic, and lactic acids are the predominant organic acids in all Madeira wines, whereas citric acid was only found in one sample. The overall range varied from 0.06 to 6.27 g L^{-1}, with oxalic acid occurring at the lowest concentration with the exception of acetic acid their amounts in wines are usually below their respective odor threshold.

D. Amino acids and biogenic amines

Together with proteins and peptides, amino acids constitute the main components of the nitrogenous fraction of musts and wines. They are also the most studied and best known nitrogenated components in wines. Free amino acids in musts are of paramount importance. They constitute a source of nitrogen for yeasts in alcoholic fermentation, for lactic acid bacteria in malolactic fermentation, and can also be a source of aromatic compounds (Košir and Kidrič, 2001). In certain cases, some amino acids

can produce undesirable compounds in wines, such as ethyl carbamate, biogenic amines, ochratoxin A (from 2-phenylalanine) and carbolines (from tryptophan; Herraiz and Ough, 1993; Herraiz *et al.*, 1993).They serve as nutrients for yeasts in alcoholic fermentation and can also be metabolized by the lactic acid bacteria responsible for the process of malolactic fermentation. According to Bisson (1991) and Rapp and Versini (1991), the concentration and composition of amino acids in wines and musts can also have an important effect on the aromatic complexity of wines.

Biogenic amines are low molecular weight compounds derived from aromatic or cationic amino acids and all of them have one or more positive charge and a hydrophobic skeleton. The chemical structure of biogenic amines can be aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine), or heterocyclic (histamine, tryptamine). In foods, they are mainly synthesized in fermentative processes, and during aging and storage, may be produced by microbial decarboxylation of the corresponding amino acid precursors.

If they accumulate, they are undesirable compounds in all foods and beverages. At high concentrations, they have the potential to generate headaches, respiratory distress, heart palpitation, hypotension, and several allergenic disorders. Their toxicology is increased in the presence of alcohol and acetaldehyde (Bauza *et al.*, 1995; Lehtonen *et al.*, 1992; Lonvaud-Funel, 2001; Pereira *et al.*, 2008; Santos, 1996). Problems related to biogenic amine formation affect numerous fermented food products consumed more frequently than wine, such as cheese, beer, some fermented sausages, and meat products among others (Fernandez-Garcıa *et al.*, 1999; Izquierdo-Pulido *et al.*, 2000; Kaniou *et al.*, 2001), which have higher levels of these compounds. However, in alcoholic drinks, especially wine, biogenic amines have received more attention, because ethanol can increase the effects on health by directly or indirectly inhibiting the enzymes responsible for the detoxification of these compounds (Maynard and Schenker, 1996). Other amines, such as tyramine and phenylamine, can cause hypertension and other symptoms associated with vasoconstriction, caused by the release of noradrenaline. Although putrescine and cadaverine are not themselves toxic, they can increase the toxicity of histamine, tyramine, and phenylethylamine, since they interfere in detoxification reactions. Moreover, putrescine and cadaverine can have negative effects on wine aroma, giving them flavors of putrefaction or rotting flesh, respectively.

The concentration of amino acids and biogenic amines in wines depend on several parameters, such as grape variety, fertilization, season, ripeness, and enologic practices (Gómez-Alonso *et al.*, 2007). Pereira *et al.* (2008) quantified the level of amino acids and biogenic amines in Madeira wines. Phenylalanine, arginine, and γ-aminobutyric acid (GABA) were the most abundant amino acids, whereas biogenic amines were present at residual levels, below their limit of detection (LOD). This

result was not unexpected as most biogenic amines are a by-product of malolactic fermentation. This fermentation rarely occurs in Madeira wines.

E. Mineral composition

Several minerals and trace elements are vital to the human organism and must be ingested with daily food in sufficient amounts. Wine can contribute not only minerals containing potassium, calcium, and magnesium but also numerous essential trace elements, such as chromium, cobalt, iron, fluorine, copper, selenium, and zinc, among others. The contents found in wine are very low ranging from mg L^{-1} to μgL^{-1}, sometimes even lower.

Minerals appear to be the best way to identify geographical origin due to their direct correlation with soil composition (Medina, 1996), nevertheless this correlation was not always obtained, and the use of mineral profile as a traceability tool is not consensus among expertises. Moreover, knowledge of the mineral composition of wines is of interest because of their influence on wine-making, where minerals such as potassium, calcium, iron, and copper can produce precipitates and cloudiness (Trujillo *et al.*, 2011). Low levels for some minerals are set by the international community due to their potentially toxic effects, such as disrupting the dietary intake of many essential minerals (Mayer *et al.*, 2003). The mineral content could also affect geographic authentication (Trujillo *et al.*, 2011) or negatively influence the wines organoleptic properties (Ibanez *et al.*, 2008; Mayer *et al.*, 2003). Thus, their determination is very important. Their mineral concentration in wines depends on features such as the soil, viticulture practices, aging, environmental contamination, and adulteration (Ibanez *et al.*, 2008; Mayer *et al.*, 2003). Although few data are available on metallic composition of Madeira wines, Trujillo *et al.* (2011) determined that the majority of metals present are in agreement with most values reported in literature, except for sodium. Its higher concentration may be due to the effect of marine spray on the vines. The wines had a significantly higher mean content in Fe, Cu, Zn, and Mn and a significantly lower Rb content in comparison to Azores and Canary Islands wines. PCA provided differentiation of the samples according to their winemaking process and/or equipment employed, whereas linear discriminant analysis (LDA) allowed classification and validation of the wines according to origin.

F. Polyphenols in table Madeira wine

In the past decades, the increased consumption of table grapes and wines has been encouraged by the amply demonstrated beneficial effects of these substances on human illness, such as cardiovascular diseases, brain degeneration, and certain carcinogenic diseases (Caillet *et al.*, 2006; Cheng *et al.*, 2007).

Plants synthesize a vast range of secondary metabolites with a significant portion consisting of phenolic and flavonoid compounds (Crozier *et al.*, 2006). They are structurally diverse, and many are distributed among a very limited number of species within the plant kingdom. These metabolites can be categorized as (i) flavonoids and (ii) nonflavonoid phenolic compounds. To date, several hundreds of different flavonoids have been described and the number continues to increase. This group comprises compounds with 15 carbons, with two aromatic rings connected by a 3-carbon bridge (Fig. 7.12). According to the modifications of the central C-ring, they can be divided into different structural classes including flavonols (represented mainly by quercetin, kaempferol, and myricetin), flavones (represented by apigenin and luteolin), flavan-3-ols

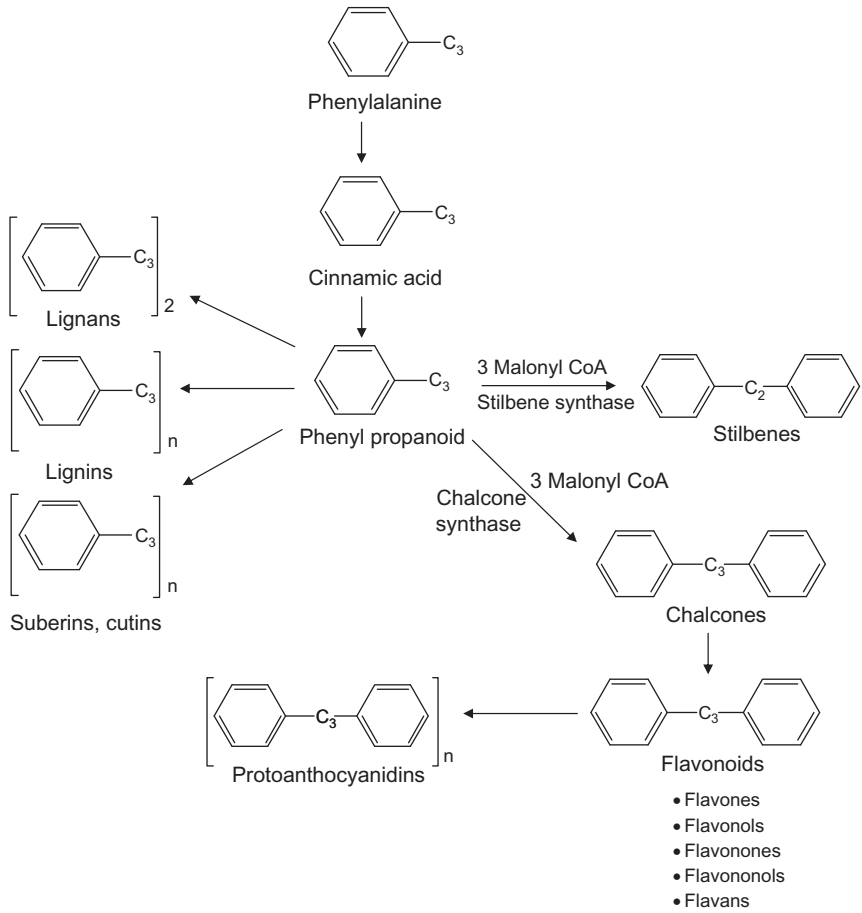

FIGURE 7.12 Overview of the biosynthesis of the main polyphenols from the phenylalanine precursor (adapted from Naczk and Shahidi, 2004).

(ranging from the simple monomers (+)-catechin, and its isomer (−)-epicatechin to the oligomeric and polymeric proanthocyanidins), flavanones, isoflavones, and anthocyanidins.

The main nonflavonoid phenolic compounds (Fig. 7.12) of dietary significance are the C_6–C_1 phenolic acids (gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids), the C_6–C_3 hydroxycinnammates (*p*-coumaric, caffeic, and ferulic acids, frequently accumulate as their respective tartrate esters, coutaric, caftaric, and fertaric acids) and their conjugated derivatives, and the polyphenolic C_6–C_2–C_6 stilbenes (phytoallexins produced by plants in response to disease, injury, and stress). Arising biogenetically from either the shikimate/phenylpropanoid pathway (Fig. 7.12) or ''polyketide'' acetate/malonate pathway, or both, producing monomeric and polymeric phenols and polyphenols, and which fulfill a very broad range of physiological roles in plants. Apparently, they act as defense (against herbivores, microbes, viruses, or competing plants) and signal compounds (to attract pollinating or seed dispersing animals), as well as protecting the plant from ultraviolet radiation and oxidants.

For many years, considerable attention has been directed toward human behavior that could either be considered risk factors or even protective elements for developing chronic pathologies. In particular, much effort has been devoted to elucidating the role of diet in preventing cardiovascular diseases (Scalzo *et al.*, 2005). Moderate consumption of red table wine has been putatively associated with lowering the risk of developing coronary heart disease, and other biological properties, including the inhibition of platelet aggregation (Varache-Lembège *et al.*, 2000), vasorelaxion (Mattace Raso *et al.*, 2001), modulation of lipid metabolism, inhibition of low-density lipoprotein oxidation (Fauconneau *et al.*, 1997), and may be active on treatment of many forms of cancer and aging process (Packer *et al.*, 1999), due to wine antioxidant and anti-inflammatory properties.

These beneficial effects are mainly attributed to the occurrence of polyphenol compounds, such as anthocyanins, catechins, proanthocyanidins, stilbenes, and other phenolics. These compounds are usually present in the higher plants and reach a higher concentration in red wine grapes than white varieties. They play a very important role in wine quality, since they contribute to the wine organoleptic characteristics, such as color (anthocyanins) and flavor, astringency (tannins), bitterness, haze formation, and interaction with proteins during wine oxidation (Delgado *et al.*, 2004; Segade *et al.*, 2008). Moreover, they act as potent antioxidants, reinforcing antioxidant system against reactive oxygen (ROS) and nitrogen (RNS) species.

These compounds have a strong influence on the quality and character of the wine and are therefore important not only for the wine characterization but also reflects the history of the wine producing process, including the grape variety, the yeast strain, the containers used for fermentation and storage, and the enological practices. Their nature and

content can vary significantly according to different intrinsic and extrinsic factors such as plant genetics and cultivar, soil composition, and growing conditions, maturity state, and postharvest conditions, among other (Câmara *et al.*, 2006a,b,c; Ferreira *et al.*, 2002a,b).

The health benefits explain the growth in interest concerning the characterization and evaluation of phenolics and antioxidant capacity in food-related products. The chemical structures of the main polyphenols found in Madeira table wines are summarized in Fig. 7.13.

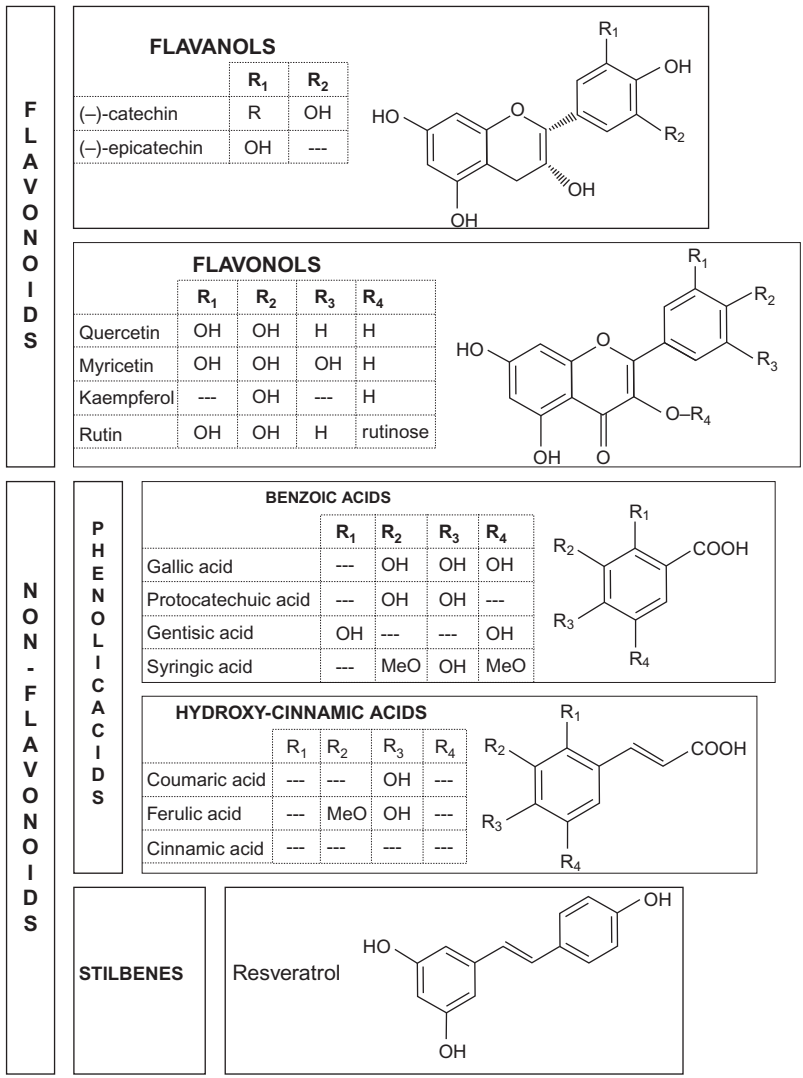

FIGURE 7.13 Chemical structures of some polyphenols identified in Madeira wines.

The content of phenolic compounds found in the Madeira wine samples assayed is represented in Table 7.4. As can be easily observed, the phenolics analyzed are about six times more abundant in red than in white wines. The fact that polyphenols content is higher in red wines was widely described before in the literature (Kuroda and Hara, 1999).

Palheiros red wine (PT red wine produced from Cabernet Sauvignon, Merlot, and Touriga Nacional grape varieties), was by far the one that showed higher polyphenolic content considering the sum of the polyphenols quantified (almost 3.93 μg mL^{-1}), followed by Terras de Lava wine (TL wine produced from Merlot, Cabernet Franc, Cabernet Sauvignon, and Saborinho grape varieties), with polyphenolic composition around 3.06 μg mL^{-1}. In white wines, the polyphenolic content is significantly lower, varying from about 3.74 ng mL^{-1} in the Vignatico wine (VGB wine produced from Boal variety) up to 54.9 μg mL^{-1} in the Seiçal wine (SB produced from Verdelho and Arnsburguer grape varieties). This fact makes white wines less prone to be effective in health protection against oxidative damage as the protective effects associated to moderate wine consumption have been attributed to their content in polyphenols.

Regarding to the individual polyphenols, protocatechuic acid were the most abundant polyphenol in all wines studied, except in the SB, Madeira Island wine. The second most represented polyphenol is (−)-catechin, although it is not present in PT red wine, SB, and LB (Latadas wine produced from Verdelho grape variety) white wines.

V. MADEIRA WINE AUTHENTICITY

EC legislation, as well as those of each member, are intended to protect consumer health, rather than the market, from the introduction of low-quality products. This goal is achieved by accurate foods controls. Consequently, quality certificates are often required for exporting wine and enological products. To prevent fraud and to confirm product identity and authenticity, accordance with actual product characteristics and producer declarations (e.g., variety, geographic origin, quality and vintage) have to be verified. Researchers and control organizations are active in developing new analytical methods. These methods are applied to verify the product origin, as well as detect illegal additions, adulteration (sugar beet, cane sugar, or ethanol addition, watering), and the presence of contaminants.

In this context, physicochemical parameters (Nogueira and Nascimento, 1999), volatile profile (Câmara *et al.*, 2003a,b, 2004b, 2006b, c; Pereira *et al.*, 2010a,b, 2011), phenolic constituents (Paixão *et al.*, 2008; Pereira *et al.*, 2010c,d), organic acids (Pereira *et al.*, 2010d), amino acids (Pereira *et al.*, 2008), biogenic amines (Pereira *et al.*, 2008), and minerals (Trujillo *et al.*, 2011) have been determined in Madeira wines.

TABLE 7.4 Concentration[a] of polyphenol compounds found in table Madeira wines

		Red wine							White wine					
		BT	ET	TLT	PT	TL	VT	TT	SB	LB	RB	EB	TB	VGB
Polyphenols	Gallic acid	17.3 ± 2.5	15.6 ± 0.7	17.1 ± 2.4	13.9 ± 4.5	13.4 ± 3.5	8.86 ± 1.8	7.41 ± 6.3	12.2 ± 0.7					
	Protocatechuic acid	19.3 ± 1.3	13.9 ± 2.3	11.0 ± 2.4	14.5 ± 0.5	3.36 ± 3.7	12.9 ± 2.4	9.18 ± 1.3		9.90 ± 1.4	3.64 ± 3.0	9.83 ± 0.9	2.11 ± 1.2	3.74 ± 2.6
	(−)-Catechin	<LOQ	<LOQ	<LOQ		69.5 ± 1.1	43.0 ± 4.5	<LOQ			<LOQ	<LOQ	<LOQ	<LOQ
	Gentisic acid												19.8 ± 2.5	
	(−)-Epicatechin													
	Syringic acid	15.1 ± 3.1	12.4 ± 1.3	22.6 ± 1.3	9.82 ± 1.2	12.6 ± 1.8	18.8 ± 2.5	30.8 ± 1.2					2.76 ± 3.5	
	p-Coumaric acid		31.7 ± 3.2				23.2 ± 1.4	8.07 ± 0.4	6.90 ± 3.6	6.02 ± 4.2	7.04 ± 1.4	8.40 ± 1.6		
	Ferulic acid		5.66 ± 2.2											
	m-Coumaric acid		11.8 ± 6.3							<LOQ				
	Rutin		4.41 ± 1.5	50.4 ± 0.5										
	Myrcetin		5.37 ± 1.4						<LOQ	<LOQ				
	Quercitin + cinnamic acid		<LOQ		24.4 ± 1.0		<LOQ				<LOQ		<LOQ	
	Kaempferol		<LOQ				<LOQ							<LOQ

Wine Codes: BT: Basalto wine; ET: Enxurros wine (red); TLT: Terras de Lava wine; PT: Palheiros wine; TL: Terras de Lava wine; VT: Vignatico wine; TT: Torcaz wine; SB: Seiçal wine; LB: Latadas wine; RB: Rocha Branca wine; EB: Enxuros wine (white); TB: Terrantez; VGT: Vignatico wine; LOQ: Lower than limit of quantification.

[a] The content of each of the 13 polyphenols analyzed in the wine samples tested is the mean of three replicates ± RSD and indicated as μg mL^{-1}. The gray-shadow boxes refer to polyphenols not detected in the respective wine sample.

They are precise means by which the authenticity of the grape varieties used to produce the wine, their origin, the vintage, and genuineness of the samples can be assessed. Statistics analysis, namely univariate analysis of variance (ANOVA), multivariate analysis (PCA, SLDA, partial least squares discriminant analysis (PLS-DA), partial least squares regression (PLS-regression), among others, have proved to be useful tools in establishing the authenticity of Madeira wine. Pereira *et al.* (2010a,b) characterized the volatile profile of Madeira wines of different ages and varieties to ensure wine identity and authenticity. From all Madeira wines studied, Malvasia wines were the best characterized. This is likely attributable to their higher sugar content and more complex volatile profile. Sercial and Verdelho wines were identifiable, based on higher alcohol and ester content, whereas Malvasia and Boal had the most obviously based lactones and carbonyl contents. The results obtained provide a useful procedure to identify different types of Madeira wines in terms of their aroma profile and age. Additionally data about volatile and phenolic composition of 26 Madeira wines were considered adequate to predict the age of the Madeira wines (Pereira *et al.*, 2011). Rudnitskaya *et al.* (2010) have recently investigated the use of an electronic tongue (ET) to recognize the Madeira wines. Wine age, grape variety, and their interaction were found to be significant for the HPLC data while only effect of wine age was significant for the ET data. The wine age could be predicted with an accuracy of 2.6 and 1.8 years with HPLC and ET data, respectively. Thus, ET is promising as a technique for rapid assessment of the age of Madeira wine as well as quantification of some organic acids and phenolic compounds.

REFERENCES

Alves, R. F., Nascimento, A. M. D., and Nogueira, J. M. F. (2005). Characterization of the aroma profile of Madeira wine by sorptive extraction techniques. *Anal. Chim. Acta* **546**, 11–21.

Arthur, L. and Pawliszyn, J. (1990). Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62**, 2145–2148.

Baltussen, E., Sandra, P., David, F., and Cramers, C. (1999). Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *J. Microcol. Sep.* **11**, 737–747.

Baumes, R., Cordonnier, R., Nitz, S., and Drawert, F. (1986). Identification and determination of volatile constituents in wines from different wine cultivars. *J. Agric. Food Chem.* **37**, 927–934.

Bauza, T., Blaise, A., Mestres, J. P., Teissedre, P. L., Daumas, F., and Cabanis, J. C. (1995). Biogenic amines contents and their variation parameters in Cotes Du Rhone, Vallee Du Rhone and Provence Wines. *Sci. Aliment.* **15**, 367–380.

Belitz, H. D. and Grosch, W. (1999). Food Chemistry. 2nd edn. Springer, Berlin, Heidelberg, New York.

Bisson, L. F. (1991). Influence of nitrogen on yeast and fermentation of grapes. *In:* proceedings of the international symposium on nitrogen in grapes and wine. *Am. Soc. Enol. Vitic.* **323**, 78–89.

Blanck, I., Sen, A., and Grosch, W. (1992). Potent odorants of the roasted powder and brew of Arabica coffee. *Z. Lebensm. Unters. Forsch.* **195**, 239–245.

Cadahía, E., Simón, B. F., and Jalocha, J. (2003). Volatile compounds in Spanish. French and American oak woods after natural seasoning and toasting. *J. Agric. Food Chem.* **51**, 5923–5932.

Caillet, S., Salmiéri, S., and Lacroix, M. (2006). Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. *Food Chem.* **95**, 1–8.

Câmara, J. S. (2004). Analytical Characterization of Madeira Wines. Ph.D. Thesis, University of Madeira, .

Câmara, J. S., Marques, J. C., Alves, A., and Ferreira, A. C. S. (2003a). Heterocycles acetals in Madeira wine. *Anal. Bioanal. Chem.* **375**, 1221–1224.

Câmara, J. S., Marques, J. C., Alves, A., and Ferreira, A. C. S. (2003b). Heterocyclic acetals in Madeira wines. *Anal. Bioanal. Chem.* **375**, 1221–1224.

Câmara, J. S., Herbert, P., Marques, J. C., and Alves, M. A. (2004a). Varietal flavour compounds of four grape varieties producing Madeira wine. *Anal. Chim. Acta* **513**, 203–207.

Câmara, J. S., Marques, J. C., Alves, M. A., and Ferreira, A. C. S. (2004b). 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone levels in fortified Madeira wines: Relationship to sugar content. *J. Agric. Food Chem.* **52**, 6765–6769.

Câmara, J. S., Alves, M. A., and Marques, J. C. (2006a). Changes in volatile composition of Madeira wines during their oxidative ageing. *Anal. Chim. Acta* **563**, 188–197.

Câmara, J. S., Alves, M. A., and Marques, J. C. (2006b). Multivariate analysis for the classification and differentiation of Madeira wines according to main grape varieties. *Talanta* **68**, 1512–1521.

Câmara, J. S., Alves, M. A., and Marques, J. C. (2006c). Development of headspace solid-phase microextraction-gas chromatography–mass spectrometry methodology for analysis of terpenoids in Madeira wines. *Anal. Chim. Acta* **555**, 191–200.

Câmara, J. S., Alves, M. A., and Marques, J. C. (2007). Classification of Boal, Malvazia. Sercial and Verdelho wines based on terpenoid patterns. *Food Chem.* **101**, 475–484.

Campo, E., Ferreira, V., Escudero, A., Marqués, J. C., and Cacho, J. (2006). Quantitative gas chromatography–olfactometry and chemical quantitative study of the aroma of four Madeira wines. *Anal. Chim. Acta* **563**, 180–187.

Cerdán, T. G. and Ancín-Azpilicueta, C. (2006). Effect of oak barrel type on the volatile composition of wine: Storage time optimization. *LWT—Food Sci. Technol.* **39**, 199–205.

Chatonnet, P. (1999). Discrimination and control of toasting intensity and quality of oak wood barrels. *Am. J. Enol. Vitic.* **50**, 479–494.

Chatonnet, P. and Dubourdieu, D. (1998). Comparative study of the characteristics of American white oak (''Quercus alba'') and European oak (''*Quercus petraea* and *Q. robur*'') for production of barrels used in barrel aging of wines. *J. Int. Sci. Vigne Vin.* **32**, 99–104.

Cheng, M., Gao, H. Q., Xu, L., Li, B. Y., Zhang, H., and Li, X. H. (2007). Cardioprotective effects of grape seed proanthocyanidins extracts in streptozocin induced diabetic rats. *J. Cardiovasc. Pharmacol.* **50**, 503–509.

Crozier, A., Clifford, M. N., and Ashihara, H. (2006). Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet. Blackwell Publishing Ltd., Oxford, UK.

Cutzach, I., Chatonnet, P., Henry, R., and Dubourdieu, D. (1997). Identification of volatile compounds with a "toasty" aroma in heated oak used in barrelmaking. *J. Agric. Food Chem.* **45**, 2217–2224.

Del Barrio-Galán, R., Pérez-Magariño, S., and Ortega-Heras, M. (2011). Techniques for improving or replacing ageing on lees of oak aged red wines: The effects on polysaccharides and the phenolic composition. *Food Chem.* **127**, 528–540.

Delgado, R., Martín, P., Álamo, M., and González, M. (2004). Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *J. Sci. Food Agric.* **84**, 623–630.

Doussot, F., De Jéso, B., Quideau, S., and Pardon, P. (2002). Extractives content in cooperage oak wood during natural seasoning and toasting: Influence of tree species, geographic location and single-tree effects. *J. Agric. Food Chem.* **50**, 5955–5961.

Dubois, P. (1989). Apports du fût de chêne neuf a l'arôme des vins. *Rev. Fr. Oenol.* **120**, 19–24.

Fauconneau, B., Waffo-Teguo, P., Huguet, F., Barrier, L., Decendit, A., and Merillon, J. (1997). Scavenger and antioxidant properties of astringin and phenolic compounds extracted from *Vitis vinifera* cell cultures. *Life Sci.* **61**, 2103–2110.

Fernández-García, E., Tomillo, J., and Nuñez, M. (1999). Effect of added proteinases and level of starter culture on the formation of biogenic amines in raw milk Manchego cheese. *Int. J. Food Microbiol.* **52**, 189–196.

Fernández de Simón, B., Cadahía, E., and Jalocha, J. (2003). Volatile compounds in a Spanish red wine aged in barrels made of Spanish, French, and American oak wood. *J. Agric. Food Chem.* **51**, 7671–7678.

Ferreira, V., Escudero, A., Fernandez, P. E., and Cacho, J. (1997). Changes in the profile of volatile compounds in wines stored under oxygen and their relationship with the browning process. *Z. Lebensm. Unters. Forsch.* **205**, 392–396.

Ferreira, A. C. S., Barbe, J. C., and Bertrand, A. (2002a). Heterocyclic acetals from glycerol and acetaldehyde in Port wines: Evolution with aging. *J. Agric. Food Chem.* **50**, 2560–2564.

Ferreira, V., Ortín, N., Escudero, A., López, R., and Cacho, J. (2002b). Chemical characterization of the aroma of Grenache rose wines. Aroma extract dilution analysis, quantitative determination and sensory reconstitution studies. *J. Agri. Food Chem.* **50**, 4048–4054.

Gómez-Alonso, S., Hermosín-Gutiérrez, I., and García-Romero, E. (2007). Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. *J. Agric. Food Chem.* **55**, 608–613.

Guth, H. and Grosch, W. (1994). Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **42**, 2862–2866.

Haffner, T. and Tressl, R. (1996). Biosynthesis of (*R*)-γ-decanolactone in the yeast *Sporobolomyces* odorus. *J. Agric. Food Chem.* **44**, 1218–1223.

Hale, M. D., Mccafferty, K., Larmie, E., Newton, J., and Swan, J. S. (1999). The influence of oak seasoning and toasting parameters on the composition and quality of wine. *Am. J. Enol. Vitic.* **50**, 495–502.

Herraiz, T., Huang, Z., and Ough, C. S. (1993). 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid and 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in wines. *J. Agric. Food Chem.* **41**, 455–459.

Herraiz, T. and Ough, C. S. (1993). Chemical and technological factors determining tetrahydro-β-carboline-3-carboxylic acid content in fermented alcoholic beverages. *J. Agric. Food Chem.* **41**, 959–964.

Ibanez, J. G., Carreon-Alvarez, A., Barcena-Soto, M., and Casillas, N. (2008). Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis. *J. Food Compos. Anal.* **21**, 672–683.

Izquierdo-Pulido, M., Marine-Font, A., and Vidal-Carou, M. C. (2000). Effect of tyrosine and tyramine formation during beer fermentation. *Food Chem.* **70**, 329–332.

Kaniou, I., Samouris, G., Mouratidou, T., Eleftheriadou, A., and Zantopoulos, N. (2001). Determination of biogenic amines in fresh unpacked and vacuum-packed beef during storage at 4 °C. *Food Chem.* **74**, 515–519.

Kidwell, D. A. and Riggs, L. A. (2004). Comparing two analytical methods: Minimal standards in forensic toxicology derived from information theory. *Forensic Sci. Int.* **145**, 85–96.
Klampfl, C. W., Buchberger, W., and Haddad, P. R. (2000). Determination of organic acids in food samples by capillary zone electrophoresis. *J. Chromatogr. A* **881**, 357–364.
Kobayashi, A. (1989). FlaVour chemistry trends and developments. ACS Symposium Series 388 American Chemical Society, Washington, DC, p. 49.
Košir, I. J. and Kidrič, J. (2001). Identification of amino acids in wines by one- and two-dimensional nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.* **49**, 50–56.
Kuroda, Y. and Hara, Y. (1999). Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutat. Res.* **436**, 69–97.
Lehtonen, P., Saarinen, M., Vesanto, M., and Riekkola, M. L. Z. (1992). Determination of wine amines by HPLC using automated precolumn derivatisation with o-phthalaldehyde and fluorescence detection. *Lebensm. Unters. Forsch.* **194**, 434–437.
Lonvaud-Funel, A. (2001). Biogenic amines in wines: Role of lactic acid bacteria. *FEMS Microbiol. Lett.* **199**, 9–13.
Masson, E., Baumes, R., Moutounet, M., and Puech, J. L. (2000). The effect of kiln drying on the levels of ellagitannins and volatile compounds of European oak (*Quercus petraea* Liebl.) stave wood. *Am. J. Enol. Vitic.* **51**, 201–214.
Mattace Raso, G., Meli, R., Carlo, G., Pacilio, M., and Carlo, R. (2001). Inhibition of inducile nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. *Life Sci.* **68**, 921–931.
Mayer, H., Marconi, O., Floridi, S., Montanari, L., and Fantozzi, P. (2003). Determination of Cu(II) in beer by derivate potentiometric stripping analysis. *J. Inst. Brew.* **109**, 332–336.
Maynard, L. S. and Schenker, V. J. (1996). Monoamine-oxidase inhibition by ethanol in vitro. *Nature* **196**, 575–576.
Medina, B. (1996). Wine authenticity. *In* ''Food Authentication'', (P. R. Ashurst and J. M. Dennis, Eds), Vol. 1996, pp. 60–107. Blackie, London, UK.
Morales, M. L., Benitez, B., and Troncoso, A. M. (2004). Accelerated aging of wine vinegars with oak chips: Evaluation of wood flavour compounds. *Food Chem.* **88**, 305–315.
Müller, C., Kneper, R., and Webb, A. (1978). 1,3-Dioxanes and 1,3-dioxolanes as constituents of the acetal fraction of Spanish fino sherry. *Am. J. Enol. Vitic.* **29**, 207–212.
Musteata, M. and Pawliszyn, J. (2007). Bioanalytical applications of solid-phase microextraction. *Trends Anal. Chem.* **26**, 36–45.
Naczk, M. and Shahidi, F. (2004). Extraction and analysis of phenolics in food. *J. Chromatogr. A* **1054**, 95–111.
Nogueira, J. M. F. and Nascimento, A. M. D. (1999). Analytical characterization of Madeira wine. *J. Agric. Food Chem.* **47**, 566–575.
Packer, L., Rimbach, G., and Virgili, F. (1999). Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*pinus maritima*) bark, pycnogenol. *Free Radic. Biol. Med.* **27**, 704–724.
Paixão, N., Pereira, V., Marques, J. C., and Câmara, J. S. (2008). Validation of a methodology for quantification polyphenols with potential antioxidant properties in wines by reverse phase HPLC. *J. Sep. Sci.* **31**, 2189–2198.
Pereira, V., Pontes, M., Câmara, J. S., and Marques, J. C. (2008). Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldeyde derivatization procedure. *J. Chromatogr. A* **1189**, 435–443.
Pereira, A. C., Reis, M. S., Saraiva, P. M., and Marques, J. C. (2010a). Analysis and assessment of Madeira wine ageing over an extended time period through GC–MS and chemometric analysis. *Anal. Chim. Acta* **660**, 8–21.
Pereira, A. C., Reis, M. S., Saraiva, P. M., and Marques, J. C. (2010b). Aroma ageing trends in GC/MS profiles of liqueur wines. *Anal. Chim. Acta* **659**, 93–101.

Pereira, V., Albuquerque, F. M., Ferreira, A. C., Cacho, J., and Marques, J. C. (2010c). Evolution of 5-hydroxymethylfurfural (HMF) and furfural (F) in fortified wines submitted to overheating conditions. *Food Res. Int.* **44**, 71–76.

Pereira, V., Câmara, J. S., Cacho, J., and Marques, J. C. (2010d). HPLC-DAD methodology for the quantification of organic acids, furans and polyphenols by direct injection of wine samples. *J. Sep. Sci.* **33**, 1204–1215.

Pereira, A. C., Reis, M. S., Saraiva, P. M., and Marques, J. C. (2011). Madeira wine ageing prediction based on different analytical techniques: UV–vis, GC–MS HPLC-DAD. *Chemometr. Intell. Lab. Syst.* **105**, 43–55.

Perestrelo, R., Barros, A. S., Câmara, J. S., and Rocha, S. M. (2011). In-depth search focused on furans, lactones, volatile phenols, and acetals as potential age markers of Madeira wines by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry combined with solid phase microextraction. *J. Agric. Food Chem.* **59**, 3186–3204.

Perestrelo, R., Fernandes, A., Albuquerque, F. F., Marques, J. C., and Câmara, J. S. (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. *Anal. Chim. Acta* **563**, 154–164.

Perestrelo, R., Nogueira, J. M. F., and Câmara, J. S. (2009). Potentialities of two solventless extraction approaches–stir bar sorptive extraction and headspace solid-phase microextraction for determination of higher alcohol acetates, isoamyl esters and ethyl esters in wines. *Talanta* **80**, 622–630.

Perestrelo, R., Petronilho, S., Câmara, J. S., and Rocha, S. M. (2010). Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry combined with solid phase microextraction as a powerful tool for quantification of ethyl carbamate in fortified wines. The case study of Madeira wine. *J. Chromatogr. A* **1217**, 3441–3445.

Perez-Coello, M. S., Sanz, J., and Cabezudo, M. D. (1999). Determination of volatile compounds in hydroalcoholic extracts of French and American oak woods. *Am. J. Enol. Vitic.* **50**, 162–165.

Pérez-Prieto, L. J., López-Roca, J. M., Martínez-Cutillas, A., Mínguez, F. P., and Gómez-Plaza, E. (2002). Maturing wines in oak barrels. Effects of origin, volume, and age of the barrel on the wine volatile composition. *J. Agric. Food Chem.* **50**, 3272–3276.

Pozo-Bayón, M. A., Pueyo, E., Martín-Álvarez, P. J., and Polo, M. C. (2001). Polydimethylsiloxane solid-phase microextraction–gas chromatography method for the analysis of volatile compounds in wines: Its application to the characterization of varietal wines. *J. Chromatogr. A* **922**, 267–275.

Rapp, A. and Mandery, H. (1986). Wine aroma. *Experientia* **42**, 873–884.

Rapp, A. and Versini, G. (1991). Influence of nitrogen compounds in grapes on aroma compounds of wine. *In:* proceedings of the international symposium on nitrogen in grapes and wine. *Am. Soc. Enol. Vitic.* **323**, 156–164.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., and Dubourdieu, D. (2000). Handbook of Enology. The Chemistry of Wine. Stabilization and Treatment, Vol. 2. Wiley, New York p. 187.

Risticevic, S., Niri, V. H., Vuckovic, D., and Pawliszyn, J. (2009). Recent developments in solid-phase microextraction. *Anal. Bioanal. Chem.* **393**, 781–795.

Rocha, S. M., Coelho, E., Zrostlíková, J., Delgadillo, I., and Coimbra, M. A. (2007). Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry of monoterpenoids as a powerful tool for grape origin traceability. *J. Chromatogr. A* **1161**, 292–299.

Rudnitskaya, A., Rocha, S. M., Legin, A., Pereira, V., and Marques, J. C. (2010). Evaluation of the feasibility of the electronic tongue as a rapid analytical tool for wine age prediction and quantification of the organic acids and phenolic compounds. The case-study of Madeira wine. *Anal. Chim. Acta* **662**, 82–89.

Santos, M. H. S. (1996). Biogenic amines: Their importance in foods. *Int. J. Food Microbiol.* **29**, 213–231.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., and Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition* **21**, 207–213.

Segade, S. R., Vázquez, E. S., and Losada, E. D. (2008). Influence of ripeness grade on accumulation and extractability of grape skin anthocyanins in different cultivars. *J. Food Compos. Anal.* **21**, 599–607.

Silva Ferreira, A. C., Guedes de Pinho, P., Rodriques, P., and Hogg, T. (2002). Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. *J. Agric. Food Chem.* **50**, 5919–5924.

Silva Ferreira, A. C., Hogg, T., and Guedes de Pinho, P. (2003). Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **51**, 1377–1381.

Silva, H. O., Pinho, P. G., Machado, B. P., Hogg, T., Marques, J. C., Câmara, J. S., Albuquerque, F., and Ferreira, A. C. S. (2008). Impact of forced-aging process on Madeira wine flavor. *J. Agric. Food Chem.* **56**, 11989–11996.

Simpson, R. F. (1978). Aroma and compositional changes in wine with oxidation, storage end aging. *Vitis* **17**, 274–287.

Simpson, R. F. (1980). Volatile aroma components of Australian port wines. *J. Sci. Food Agric.* **31**, 214–222.

Singleton, V. L. (1995). Maturation of wines and spirits: Comparisons, facts, and hypotheses. *Am. J. Enol. Vitic.* **46**, 98–115.

Souza, P. O., Cardeal, Z. L., Augusti, R., Morrison, P., and Marriott, P. J. (2009). Determination of volatile compounds in Brazilian distilled cachaça by using comprehensive two-dimensional gas chromatography and effects of production pathways. *J. Chromatogr. A* **1216**, 2881–2890.

Stevenson, T. (2005). The Sotheby's Wine Encyclopedia. Dorling Kindersley, UK, pp. 340–341.

Tominaga, T., Blanchard, L., Darriet, P., and Dubourdieu, D. (2000). A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **48**, 1799–1802.

Tranchida, P. Q., Dugo, P., Dugo, G., and Mondello, L. (2004). Comprehensive two-dimensional chromatography in food analysis. *J. Chromatogr. A* **1054**, 3–16.

Trujillo, J. P. P., Conde, J. E., Pont, M. L. P., Câmara, J., and Marques, J. C. (2011). Content in metallic ions of wines from the Madeira and Azores archipelagos. *Food Chem.* **124**, 533–537.

Varache-Lembège, M., Waffo-Téguo, P., Richard, T., Monti, J. P., Deffieux, G., Vercauteren, J., Mérillon, J. M., and Nuhrich, A. (2000). Structure–activity relationship of polyhydroxystilbene derivatives extracted from *Vitis vinifera* cell culture as inhibitors of human platelet aggregation. *Med. Chem. Res.* **10**, 253–267.

Williams, P. J. and Strauss, C. R. (1978). Studies of volatile components in the dichloromethane extract of Australian flor sherries. The identification of the isomeric ethylideneglycerols. *J. Inst. Brew.* **84**, 144–147.