Universidade de Aveiro Departamento de Química
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Sílvia Lancha Petronilho

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica da Doutora Sílvia Maria da Rocha Simões Carriço, Professora Auxiliar do Departamento de Química da Universidade de Aveiro, e do Doutor Manuel António Coimbra Rodrigues da Silva, Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro.

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"O verdadeiro conhecimento, como qualquer outra coisa de valor, não é para ser obtido facilmente. Deve-se trabalhar por ele, estudar por ele, e mais que tudo, rezar por ele."

Thomas Arnold

Ao meu pai, mãe e namorado (noivo)

o júri

presidente

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professora auxiliar da Escola de Ciências e Tecnologia da Universidade de Évora

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OBRIGADA!

palavras-chave

Viticultura sustentável, *Vitis vinifera* L., Região Demarcada da Bairrada, características do ano de colheita e da parcela de vinha, potencial enológico da casta, estudo integrado, rede de aroma.

resumo

A viticultura sustentável de uma região passa, entre outros aspetos, pela maximização das potencialidades das castas, minimizando posteriores intervenções ao nível da vinificação, contribuindo para a produção de vinhos de qualidade mantendo a sua tipicidade e racionalizando custos. O conhecimento detalhado das especificidades de cada região, nomeadamente as características da parcela de vinha (tipos de solo e dados topográficos) e as condições climatéricas do ano, é fundamental para a sustentabilidade neste setor. Assim, em linha com esta tendência atual, o ponto de partida para o desenvolvimento da presente tese de doutoramento consistiu em avaliar o potencial enológico de diferentes castas cultivadas na Região Demarcada da Bairrada em Portugal. Ao longo da maturação as uvas das diferentes variedades sofrem várias alterações, tais como, os bagos tornam-se mais doces e menos ácidos, e desenvolvem as suas propriedades de sabor, aroma e cor. O desenvolvimento dessas características é essencial para definir o potencial enológico das uvas, ou seja, para estimar a possibilidade da sua utilização para a produção de vinhos com características específicas. Foi desenhado um plano de amostragem de três anos para avaliar o efeito do ano de colheita e das características da parcela na composição das uvas *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional e Sousão. Para cada casta foram selecionadas 3 parcelas com diferentes características. Foram avaliados diversos parâmetros físico-químicos, ao longo da maturação: peso do bago, pH, acidez titulável, conteúdo de açúcar e compostos fenólicos totais, atividade anti-radicalar e composição volátil (fração livre). Para as uvas maduras foi também feita a análise da fração glicosidicamente ligada. A análise abrangente aplicada aos resultados obtidos para as uvas maduras, permitiu avaliar a significância dos efeitos do ano de colheita e das características da parcela na composição de cada casta. Considerando todos os parâmetros em estudo, foi possível destacar algumas diferenças significativas. De acordo com os resultados obtidos ao longo da maturação, foi possível concluir que as uvas Arinto, Bical e Sauvignon Blanc provenientes das parcelas com solos argilo-arenosos e argilo-calcários tinham maior teor em compostos fenólicos e maior atividade anti-radicalar. Por outro lado, a casta Sauvignon Blanc apresentou uma composição volátil semelhante para as uvas provenientes das 3 parcelas, enquanto que as uvas Arinto e Bical provenientes dos solos argilo-arenosos e argilo-calcários apresentaram maior teor em compostos voláteis. Para as castas tintas Baga, Castelão e Touriga Nacional, uvas com teores mais elevados de compostos fenólicos totais, de atividade anti-radicalar e de compostos voláteis foram obtidos a partir das parcelas com solos argilosos e argilo-calcários. Para a Touriga Nacional, a altitude das parcelas também parece modular a composição das uvas. Para além do efeito da parcela, as condições do ano de colheita também influenciaram a composição das uvas: a colheita de 2011 está relacionada com menor teor em compostos fenólicos totais e menor composição volátil, bem como menor atividade anti-radicalar. Para as uvas maduras, a análise de variância-análise simultânea de componentes (ASCA) foi aplicada combinando todos os parâmetros em estudo, a fim de avaliar a influência das características da parcela e do ano de colheita no potencial enológico de cada casta. Os resultados obtidos com esta abordagem global estão estritamente relacionados com os resultados observados ao longo da maturação e revelaram que o ano de colheita é o principal fator que influencia a composição uvas (53% a 68% da variabilidade total dos dados), seguido das características da parcela que explicaram 15% a 19% da variabilidade total dos dados. O potencial enológico de cada casta é diferente de uma parcela para outra, ou seja, um ambiente relacionado com solos argilo-arenoso e argilo-calcários parece favorecer a composição das castas brancas Arinto e Bical, no entanto para as castas tintas a composição das uvas parece ser favorecidas pelos solos argilosos e argilo-calcários. Para além disto, altitudes mais elevadas parecem também favorecer a composição das uvas Touriga Nacional. O Sauvignon Blanc parece ser uma casta bem adaptada às diferentes características das parcelas.

Com o intuito de ir mais além na valorização destas castas, as propriedades de aroma de 6 vinhos monovarietais foram estudadas usando uma rede de aroma, que combina dados moleculares relacionados com a composição volátil e as moléculas ativas chave do aroma. Esta abordagem permitiu identificar diferentes propriedades no aroma dos vinhos e inferir sobre a perceção sensorial do consumidor. Verificou-se que as propriedades de aroma diferem entre castas: enquanto os vinhos Arinto e Sauvignon Blanc exibiram mais aromas a frutos de árvore, doces e florais, relacionados essencialmente com ésteres e norisoprenóides em C_{13}, o oposto foi obtido para o vinho Bical. Estas perceções sensoriais de aroma foram corroboradas pelo painel treinado. Os vinhos da casta Sauvignon Blanc também exibiram mais aromas tostados, relacionados com tióis, principalmente com o 2-metil-3-furantiol. O vinho tinto da casta Touriga Nacional exibiu mais aromas a frutos de árvore, tropicais e a bagas (descrito sensorialmente como fruta doce), e mais aromas tostados e florais, enquanto estes são semelhantes para os outros vinhos tintos em estudo. Para além dos vinhos Portugueses da Bairrada, esta abordagem de rede de aroma é uma ferramenta que pode ser usada para explicar as propriedades de aroma dos vinhos em todo o mundo.

No âmbito da presente tese de doutoramento, os dados obtidos para as uvas e vinhos das castas em estudo, provenientes da Região Demarcada da Bairrada, mostram o caráter único de cada casta e podem ser utilizados pelos produtores de uvas e vinhos como um suporte para a tomada de decisões com base em critérios objetivos, aumentando a sustentabilidade neste sector. Por exemplo, é possível tirar proveito dos recursos naturais e produzir vinhos com características diferentes, obtidos a partir da mesma casta, minimizando os custos durante o processo de vinificação.

keywords

Sustainable viticulture, *Vitis vinifera* L., Bairrada Appellation, harvest and vineyard parcel characteristics, grape variety oenological potential, comprehensive study, aroma network.

abstract

The sustainable viticulture of a region passes, among other aspects, for maximizing the varieties potential minimizing subsequent interventions during winemaking, which should contribute to the production of quality wines maintaining their typicity and rationalizing costs. The detailed knowledge of each Appellation specificities, namely vineyard parcel (soil type and topographical peculiarities) and harvest climatic conditions is crucial for sustainability in this sector. Thus, in line with this current trend, the starting point for the development of this PhD thesis was to evaluate the oenological potential of different varieties cultivated throughout Bairrada Appellation (Portugal). During maturation several changes in grape varieties occur, namely berries become sweeter, less acidic, and they develop flavour, aroma and colour properties. The development of these characteristics is essential to define grapes oenological potential, i.e. to estimate the possibility of their usage to produce specific wines. A three years sampling plan was designed to evaluate the effect of harvest year and parcel characteristics on *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional, and Sousão grapes composition. For each variety, 3 parcels with different characteristics were selected. Several physicochemical parameters were evaluated, during maturation: berry weight, pH, titratable acidity, sugar and phenolic contents, antiradical activity, and volatile composition (free fraction). Special attention was devoted to grapes at technologic maturity, since, besides these parameters, glycosidically-linked fraction was also considered. By using the results obtained at technologic maturity, a comprehensive approach was applied to identify the significance of harvest and parcel characteristics effects on each variety composition.

Considering all the parameters under study, it may be highlighted some significant differences. According to the obtained results determined during maturation, it was possible to conclude that Arinto, Bical and Sauvignon Blanc grapes from parcels with clay-sandy and clay-calcareous soils have higher phenolic content and antiradical activity. Otherwise, Sauvignon Blanc presented similar volatile composition for grapes cultivated in the 3 parcels, while Arinto and Bical exhibited higher volatile content in grapes from clay-sandy and clay-calcareous soils. For Baga, Castelão and Touriga Nacional red varieties, grapes with higher phenolic content, antiradical activity, and volatile content were obtained from clayey and clay-calcareous soils. Furthermore, for Touriga Nacional, parcels altitude seems also to modulate grapes composition. Beyond parcel effect, harvest year conditions also influence grapes composition: 2011 harvest was related with lower phenolic and volatile contents, as well as lower antiradical activity.

For grapes collected at technologic maturity, analysis of variance-simultaneous component analysis (ASCA) was applied combining all the parameters under study, in order to assess the influence of harvest and parcel characteristics on each variety oenological potential. The results obtained using this comprehensive approach is closely related with those observed during maturation and revealed that harvest was the main factor that influenced grapes composition (53% to 68% of the total data set variance) followed by parcel characteristics, explaining ca. 15-19% of the total data set variance. The oenological potential of each variety may be different from one parcel to another, i.e., clay-sandy and clay-calcareous related-environments seem to favour Arinto and Bical white grapes composition, but for the red varieties, grapes composition was favoured by clayey and clay-calcareous soils. Besides, also higher altitude seems to favour Touriga Nacional grapes composition. Sauvignon Blanc seems to be a variety well adapted to the different parcel characteristics.

In order to go forward in the valuation of these varieties, the aroma properties of 6 monovarietal wines were studied based on an aroma network-approach, linking molecular data related to volatile composition and aroma data about the key odor active molecules. This approach allowed to identify different wine aroma properties and to infer about the consumer’s sensory perception. It was found that aroma properties differ from one wine variety to another: while Arinto and Sauvignon Blanc wine exhibited higher tree fruity, sweety and flowery aromas, related essentially with ester compounds and C_{13} norisoprenoids, the opposite was obtained for Bical wine, corroborating the aroma sensory perceptions of the trained panel. Sauvignon Blanc also exhibited higher toasted aromas (related with thiols, mainly with 2-methyl-3-furanthiol). Touriga Nacional red wine exhibited higher tree, tropical, and berry fruits notes (sensory described as sweet fruits), toasted and flowery aromas, while these are similar for the other red wines under study. Besides Portuguese Bairrada wines, this aroma network approach is a tool that can be used to explain the aroma properties of wines worldwide.

The grape and wine data generated under the present PhD thesis, in the context of Bairrada Appellation, shows the unique character of each variety, and may be used by growers and wine producers as a support for decision-making based on objective criteria, increasing the sustainability in this sector. For instance, it is possible to take advantage of the natural resources and produce products with different characteristics obtained from the same variety, minimizing costs during the winemaking process.

Contents

SUPPLEMENTARY DATA IS PROVIDED IN A COMPACT DISC RECORDABLE (CD-R) APPENDED TO THIS THESIS

Publications related to this PhD thesis

Book Chapter:

1. **Sílvia Petronilho,** António S. Barros, Manuel A. Coimbra, Sílvia M. Rocha. Efficient Use of Non-renewable Natural Resources for Quality Wine through Sustainable Viticulture. A. Raza (Ed.), "Agricultural Systems in the 21st Century - Global Agriculture Developments". Nova Science Publishers Inc, New York, 2013, Chapter 9, pp. 195-230, ISBN: 978-1-62948-026-8.

Publication in international journals with Referee:

1. **Sílvia Petronilho**, Manuel A. Coimbra, Sílvia M. Rocha. Review: A critical review on extraction techniques and gas chromatography based analysis for sesquiterpenic compounds from *Vitis vinifera* L.. Anal. Quim. Acta, 846 (2014) 8-35.

Poster communications in international scientific meetings:

1. **Sílvia Petronilho**, Manuel A. Coimbra, Sílvia M. Rocha. The evaluation of the variety adequacy as a strategy for the sustainable viticulture and oenology: *Vitis vinifera* L. cv. Verdelho. 34th International Symposium on Capillary Chromatography and 7th GC×GC Symposium, Riva del Garda, Italy, May 30 to June 4, 2010, PK47.

2. **Sílvia Petronilho**, Manuel A. Coimbra, Sílvia M. Rocha. Variety adequacy as a strategy for the sustainable viticulture in Bairrada Appellation: Bical and Touriga Nacional *Vitis vinifera* L. varieties. Macrowine 2012 - Macrovision of viticulture, wine-making and markets, Sustainable Viticulture section, Bordeaux, France, June18 to 21, 2012.

Poster communications in national scientific meetings:

1. **Sílvia Petronilho**, Ricardo Lopes, Vicente Ferreira, Manuel A. Coimbra, Sílvia M. Rocha. Combining sensorial and instrumental analysis to explain differences in aroma properties of Bairrada white wines: *Vitis vinifera* cv. Arinto, Bical and Sauvignon Blanc. 7º Encontro Nacional de Cromatografia da Sociedade Portuguesa de Química, Faculdade de Ciências, Universidade do Porto, Porto, 9 a 11 de Janeiro de 2012.

2. **Sílvia Petronilho**; Manuel A. Coimbra; Sílvia M. Rocha. Variety adequacy as a strategy for sustainable viticulture based on chemical data from Bairrada Appellation. XXIII Encontro Nacional da Sociedade Portuguesa de Química, Universidade de Aveiro, Aveiro, 12 a 14 de Junho de 2013.

Abbreviations and symbols

^{1}D: First chromatographic dimension

1D-GC: One-dimensional gas chromatography

$^{1}t_{R}$: Retention time on the first dimension

^{2}D: Second chromatographic dimension

$^{2}t_{R}$: Retention time on the second dimension

A_{blank}: Absorbance of the blank

ANOVA: Analysis of variance

A_{sample}: Absorbance of each tested sample

ASCA: Analysis of variance-simultaneous component analysis

A-TD: Adsorption-thermal desorption

CAR: Carboxen

CCA: Canonical correlation analysis

CQW: Controlled quality wines

CW/DVB: Carbowax/divinylbenzene

CW: Carbowax

DHS: Dynamic headspace

DPPH$^{\bullet}$: 2,2-Diphenyl-1-picrylhydrazyl radical

$DPPH_{rem}$: Remaining 2,2-diphenyl-1-picrylhydrazyl radical

DVB/CAR/PDMS: Divinylbenzene/Carboxen/Polydimethylsiloxane

DVB: Divinylbenzene

E: Residual error

$E_{a,tot}$: Explained variance of the sub-model a in the total model

EIOVI: Environmental impact of organic viticulture indicator

F: Frequency

FID: Flame ionization detector

GAE: Gallic acid equivalents

GC: Gas chromatography

GC×GC: Comprehensive two-dimensional gas chromatography

GC×GC–ToFMS: Comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyzer

GPS: Global positioning system

HCA: Hierarchical cluster analysis

HPLC: High performance liquid chromatography

HS: Headspace

I: Intensity

IEC: Ion extraction chromatography

IM: Immersion

LD: Liquid desorption

LLE: Liquid-liquid extraction

LVI: Large volume injection

MDGC: Multidimensional gas chromatography

MF: Modified frequency

MS: Mass spectrometry

NCI: Negative chemical ionization

OAV: Odor activity values

PA: Polyacrylate

PCA: Principal component analysis

PDMS: Polydimethylsiloxane

PLS-DA: Partial least squares-discriminant analysis

QWPSR: Quality wine produced in specified region

RI: Retention index

RSD: Relative standard deviation

SBSE: Stir bar sorptive extraction

SD: Standard deviation

SDE: Simultaneous distillation-extraction

SIM: Single ion monitoring

SLE: Solid liquid extraction

SPE: Solid phase extraction

SPME: Solid phase microextraction

TD: Thermal desorption

ToF: Time of flight

Chapter I - Introduction

Introduction

Overview

This Chapter compiled the aims of this PhD thesis and the state of the art of the most relevant topics related to the field under study. The sustainable viticulture concept and the environmental factors that influence viticulture, namely the climatic conditions modulated by the harvest year and the vineyard parcels characteristics, such as soil and topographical conditions, were discussed in detail. Aiming to develop an application of this concept to Bairrada Appellation, its characteristics were described. The methodologies of extraction and gas chromatographic analysis used for the determination of the volatile composition of *Vitis vinifera* L. related products were reviewed using sesquiterpenic compounds as model analytes. To understand the variety oenological potential regarding the region characteristics, and mainly considering the uncontrollable environment conditions, advanced data processing was considered.

This chapter was written based on two published works:

1. **Book chapter** - Sílvia Petronilho, António S. Barros, Manuel A. Coimbra, Sílvia M. Rocha. Efficient Use of Non-renewable Natural Resources for Quality Wine through Sustainable Viticulture. A. Raza (Ed.), “Agricultural Systems in the 21st Century - Global Agriculture Developments”. Nova Science Publishers Inc, New York, 2013, Chapter 9, pp. 195-230, ISBN: 978-1-62948-026-8;
2. **Review article** - Sílvia Petronilho, Manuel A. Coimbra, Sílvia M. Rocha. Review: A critical review on extraction techniques and gas chromatography based analysis for sesquiterpenic compounds from *Vitis vinifera* L.. Anal. Quim. Acta, 846 (2014) 8-35.

I. 1. PhD thesis aims

Sustainable viticulture appeared as a breakthrough approach aiming to enhance grape varieties quality as an efficient use of non-renewable natural resources. This leads to wine quality enhancing while maintaining an economically viable production. The detailed knowledge of the natural specificities of each region is crucial for sustainability. Even within each Appellation, heterogeneity can be observed regarding the characteristics that may influence grape and wine composition and quality. In line with this actual trend, the evaluation of the variety oenological potential regarding the Appellation conditions, as a strategy for sustainable viticulture, was the starting point for the development of this PhD thesis.

This research study was developed in collaboration with a wine and grape grower company: Manuel dos Santos Campolargo Herdeiros. This company is located in Bairrada Appellation (Portugal), having 170 hectares of vineyard in different locations corresponding to differences in soil types, altitudes, and sunlight exposures. This company produces their wines potentiating the grape varieties attributes, avoiding oenological products, justifying the several international awards won by its wines. Thus, the vineyards of Manuel dos Santos Campolargo Herdeiros at Bairrada Appellation were used to assess the oenological potential of different grape *Vitis vinifera* L. varieties (Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional and Sousão), belonging to different vineyard parcel characteristics over 3 consecutive harvests (2010-2012).

The quality of grapes is influenced by several environmental factors, namely the vineyard parcels characteristics such as type of soil and topography, and climatic conditions which are conditioned by the harvest year, among others. In order to understand the effects of the environment in specific grape variety and, consequently, in wine quality, it is essential to evaluate its effects on physical and chemical parameters, such as berry weight, pH, titratable acidity, sugar and phenolic contents, and volatile composition. Thus, for the determination of vineyard parcel characteristics and harvest year effects on the oenological potential of the different varieties, the following specific aims were established:

1. Determination of the physicochemical parameters (berry weight, pH, sugar content, titratable acidity, total phenolic content, and antiradical activity), for each variety, at 3 vineyard parcels, during maturation, in 3 consecutive harvests (2010-2012).

2. Evaluation of the volatile composition, for each variety, at 3 vineyard parcels, during maturation, in 3 consecutive harvests (2010-2012).

3. Evaluation of the aroma potential, for each variety, based on the free and glycosidically-linked volatile components, at 3 vineyard parcels, at technologic maturity, in 3 consecutive harvests (2010-2012).

4. Combination of the physicochemical parameters with the volatile pattern (free and glycosidically-linked), for each grape variety, for systematic assessment of variety oenological potential, in order to study the influence of harvest and parcel characteristics.

5. Application of statistical tools that may support winemaker's decisions.

6. Taking advantage of the collaboration with the Laboratory of the Analysis of Aromes and Oenology of the University of Zaragoza (Spain), the wines produced from the varieties under study were studied combining sensory and instrumental analyses (aroma network construction), in order to establish the aroma properties of each wine variety, helping to explain the wine aroma perceptions by the consumers.

I. 2. Sustainable viticulture: concept

Sustainability is a visionary development paradigm that, at the beginning of the 21st century, is widely recognized by world leaders, and is a common topic of discussion by society all over the world (Lamastra *et al.*, 2010; Zucca *et al.*, 2009). This term was established in 1987 by the Brundtland Commission that defined the sustainable development as a progress that "meets the needs of the present without compromising the ability of future generations to meet their own needs” (United Nations General Assembly, 1987). This definition acknowledges that while development may be necessary to meet human needs and improve the quality of life, it must occur without depleting the capacity of the natural environment to meet present and future needs. The concept of sustainable development emerged as an attempt to bridge the gap between environmental concerns about the increasingly evident ecological consequences of human activities, and socio-political concerns about human development issues (Robinson, 2004).

Sustainable development represents an actual concern of the society and influences the market trends and also some local and international policies. Thus, this concept was extended to several fields, including agriculture. The adaptation of this important concept to the agriculture sector defines sustainable agriculture as an integrated system of plant and animal production practices having a site-specific application that over the long-term will: i) satisfy human food and fibre needs, ii) enhance environmental quality and the natural resources based upon which the agriculture economy depends, iii) make the most efficient use of non-renewable and on-farm resources and integrate, where appropriate, natural biological cycles and controls, iv) sustain the economic viability of farm operations, and v) enhance the quality of life of the society as a whole (Lamastra *et al.*, 2010; Lichtfouse *et al.*, 2009). Permaculture is another area that adopts sustainable development concept. This offers a unique approach to the practice of sustainable farming, ranching, gardening and living. Permaculture integrates plants, animals, landscapes, structures and humans into symbiotic systems where the products of one element serve the needs of another, and once established, it can be maintained using a minimum of materials, energy and efforts. This system is designed to be diverse, so when one element fails, the system has enough stability and flexibility to prosper (Mollison, 1988).

In line with the actual trends, sustainable viticulture emerged as a breakthrough approach for improving the quality of environmental and natural resources, namely grape varieties, based on an integrated and efficient use of non-renewable resources, integrating environmental, economic and social issues (Lamastra *et al.*, 2010) (Figure 1). Sustainability is a particularly interesting challenge in wine sector, as it tends to grow up in an equilibrium between tradition and innovation (Jackson and Lombard, 1993).

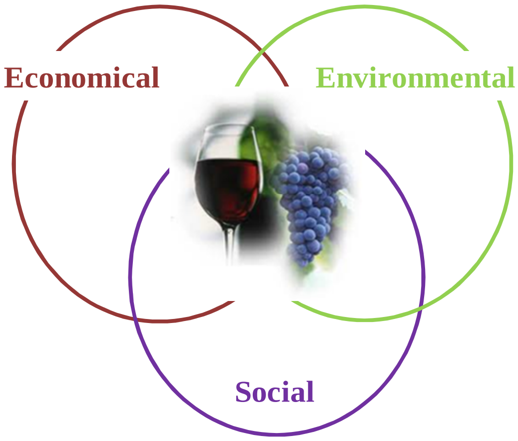


Figure 1. Graphical definition of sustainable viticulture, adapted from Lamastra *et al*. (2010).

The sustainable viticulture is not yet a global practice due to several factors. This concept and its advantages are not fully known by some winegrowers and wine producers (a broad topic that means many things to many people, because people often misunderstand the term ‘sustainable’). Global education and the definition of the limits and advantages of the sustainable viticulture should be done as a base for the development of an integrated platform of global sustainability. The wine producers of each region need to know the potentialities of their vineyards and varieties to go ahead in this field. Achieving sustainable practices is viewed as a process requiring small, realistic, and step by step improvements.

Several examples may be pointed out in different places around the world. Two examples were selected in two continents (America and Europe) to highlight the concept under discussion. In California, sustainable viticulture began in the early 1990s, as a result of the efforts of growers and winemakers in the central valley of California around the town of Lodi. The Lodi–Winegrape Commission established a sustainable winegrowing program where a range of sustainable viticultural practices were implemented and tracked over time. This program involved work with a core group of 40 Lodi growers and about 15 pest control advisors in 60 different vineyards. Various sustainable viticultural practices were implemented in these vineyards, including pest monitoring and vineyard inputs such as water, fertilizers and pesticides, so that growers could see the effects of the sustainable practices. This sustainable winegrowing program has been adapted by several other regional winegrowing associations and regions in Californian (Zucca *et al.*, 2009). Also, in Italy, a sustainable winegrowing program (SOStain) was developed. This program constituted a framework for viticultural and winemaking practices that protected the environment while efficiently and economically produced premium grapes and wines. In this program, the assessment and the interpretation of the results occurred through the use of agro-environmental indicators, the EIOVI (Environmental Impact of Organic Viticulture Indicator) in this case. EIOVI took into account the different agronomical practices used in viticulture (pest, disease, fertilizer, irrigation and soil management, and machinery used) and estimated the effect of vineyard management on soil organic matter and on the biodiversity. The indicator helped decision-makers by informing them of the linkages between viticulture activities and environmental impacts and it could provide an early indication of potential changes in the state of the environment (Lamastra *et al.*, 2010).

The grapes characteristics and composition and wine quality depend on a complex network of variables known to influence viticulture, including environmental conditions and agricultural practices (Figure 2). Thus, the detailed knowledge of these specificities for each region is crucial for the sustainability in this sector. With this perspective on sustainable viticulture it is possible to maximize the potential of the varieties, minimizing further interventions during winemaking process, which should contribute to the production of high quality wines, reducing costs and also environment impact.

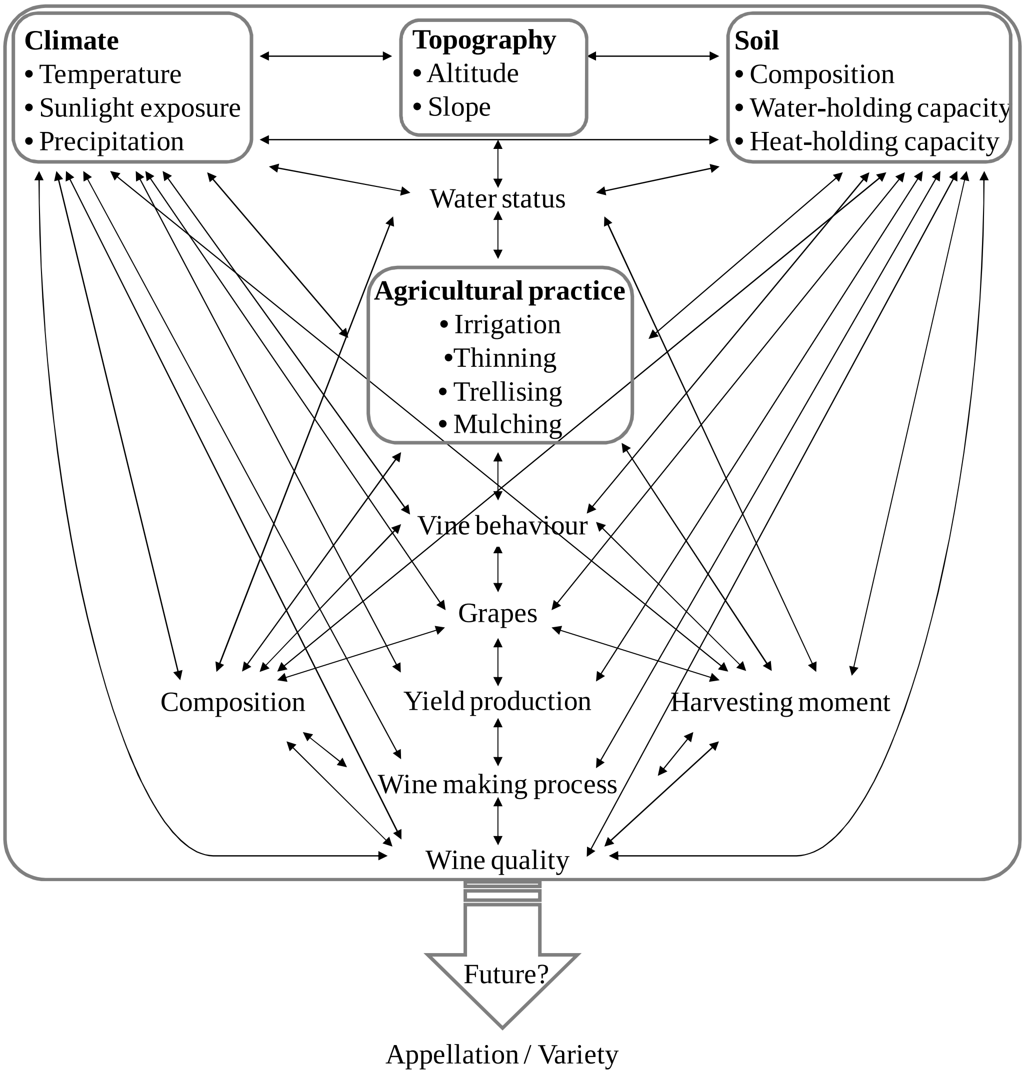


Figure 2. Environmental factors, agricultural practices and winemaking processes that influence wine composition and quality.

In viticulture, the sustainability concept may be explored in different areas, namely i) in the adaptation of agricultural practices and winemaking technologies to each variety and region, reducing costs and environmental impacts, ii) in the implementation of more eco-friendly agriculture with respect to environment, iii) in the development of tools that may support winemakers and local/national/international agencies decisions and policies, iv) in the development of marketing and communication with the consumers. Thus, in line with these subjects, the central focus of the present PhD thesis is the development of tools that may support winemaker's decisions, taking advantages from each variety potential, through the knowledge of its characteristics to the Appellation specificities. As a complex network of variables influences grapes characteristics and composition (Figure 2), in the present PhD thesis two main factors were considered: harvest and vineyard parcel characteristics, which are greatly related to climatic conditions, soil and topographical characteristics. Beyond these factors, agricultural practices have also great impact on grapes composition and quality (Figure 2). However, as similar agricultural practices were performed by the grape grower that supplied the samples for all the vineyards under study, this factor was not considered.

I. 3. Environmental factors that influence viticulture

Wine is produced around the world in different locations, using several hundreds of varieties selected for each local, where varieties characteristics are influenced by soil type and topographical features of the vineyards, and also with climatic conditions of the harvest. Thus, it may be possible to find information about *Vitis vinifera* L. varieties produced in a wide range of conditions, even in extreme ones. Indicatively, some ranges may be considered: temperatures (from < 0 °C to 38 °C), sunlight exposure (1200 to 2800 hours per year), precipitation (0.1 to 20 dm), soils (clay-sandy, clayey, clay-calcareous, volcanic, loam, silt, silt-loam, clay-loam), and topographical features (6 to 3000 m altitude and slope: terrace until 60 %). In this section, the influence of these parameters on viticulture and consequently on grape and wine composition is discussed and some examples are used to illustrate the topics under discussion.

Grape components are produced by the plant itself, in leaves and in berries (sugars, acids, phenolics, volatiles, among others) during fruit development and maturation. Thus, the growth and the fructification of grapevines in the vineyard are of utmost importance to grapes, and consequently, to wine quality. Grape maturation is a very important and decisive physiological period that starts at *véraison* (characterized by the appearance of colour in red varieties and a translucent skin in white ones) and lasts when the maturity is reached, until fruit is fully ripe. Sugar content and titratable acidity are usually used to define the grapes maturity state destined to produce wine: sugar content tend to increase while acidity tend to decrease and then both stabilize when maturity is reached (Conde *et al.*, 2007). The quality of grapes is influenced by several environmental factors (Lila, 2006), namely type of soil, topography, agro-pedological features, and edaphoclimatic conditions, among others (Coelho *et al.*, 2009; Gómez *et al.*, 1995; Pozo-Bayón *et al.*, 2004; Smart, 2003; Turner and Creasy, 2003; Vaudour and Shaw, 2005). However, the dependence of grape berry attributes on the specific environmental conditions of many Appellations remains uncertain, although the specific and systematic knowledge is crucial for sustainability. There are several physicochemical parameters that are commonly used in order to identify the grapes maturity stage, namely berry weight, pH, sugar content, and titratable acidity (Crippen and Morrison, 1986; del Llaudy *et al.*, 2008). Beyond these, volatile and phenolic composition are also considered in order to understand the effects of environment in specific grape variety, and consequently, in wine quality.

I. 3.1. Climate conditions

Growing any crop with quality and economically sustainable, anywhere in the world, is strongly dependent on harvest climate conditions (Anderson *et al.*, 2012). Climate is a very complex, highly variable from one harvest to another, and also pervasive factor in our natural and human-based systems. It is widely recognized that climate has significant implications for the agricultural sector, including viticulture (Jones *et al.*, 2005). It is well known that climate have impact on grapes and wines quality through the effect of both regional and local-scale climatic conditions during the growing season, generating variations in grapevine growth and in berry composition (Jones *et al.*, 2005; Soar *et al.*, 2008). Climate can express its influence through several elements, namely temperature, sunlight exposure, and precipitation, among others (Holland and Smit, 2010; Jones and Davis, 2000; Mateus *et al.*, 2001a).

I. 3.1.1. Temperature

Among climate variables, temperature is recognized as having the greatest effect on physiological behaviour of the grapevine and on chemical changes in the berry during its formation and maturation, affecting plant vitality, maturation rate, and harvesting date (Due *et al.*, 1993). The effect of infield temperature on grapevine development may be reflected in a network that comprises several mechanisms, namely photosynthesis and respiration.

Temperature influences photosynthesis. A study comparing Trebbiano grapevines, grown in Italy at 20, 27.5 and 35 °C in separated chambers, showed that the lowest photosynthetic rate was observed for the vines grown at the maximum temperature tested (Ferrini *et al.*, 1995). The functional activities of the photosynthetic apparatus of two grapevines (*V. vinifera* cv. Müller-Thurgau and Lagrein) were investigated after low night temperature treatment (*ca* 5 ºC). During daylight, these plants were kept at *ca* 25 °C. The low night temperatures applied caused important reductions of the photosynthetic rate, limiting photosynthesis via inhibition of electron transport and photophosphorylation (Allen and Ort, 2011; Bertamini *et al.*, 2007). A recent study carried out in Australia (Greer, 2012) showed that during the growing season of *V. vinifera* cv. Semillon, the higher photosynthetic rates were observed from 25 to 30 ºC.

Temperature also influences the grape berry respiration, which is a key process in the grape maturation. The organic acids of grapes, namely malic acid, are formed in the respiratory process of sugars (Staden *et al.*, 2005). The organic acids formation represents intermediary steps of the respiratory process, releasing a part of the stored energy in sugars molecules (Popescu-Mitroi *et al.*, 2009). If the berry temperature rises during maturation, simultaneous increase in malic acid respiration occurs, accounting for the low acidity of the sunburned berries. Such phenomenon implicitly suggests up-regulation of respiration as a temperature-sensitive malic acid metabolic pathway due to the involvement of malic acid in this pathway during maturation (Bondada and Keller, 2012). Thus, the influence of the temperature in respiration process regulates the organic acids and sugars content in the grapes.

Temperature regulates the grapevine metabolism and the production and accumulation of metabolites, such as those responsible for aroma and colour. As an example, some studies showed the influence of temperature on the concentration of the grape components associated to colour properties (for example phenolic compounds) (Fregoni and Pezzutto, 2000; Montes *et al.*, 2012). It was found that cold days (15 ºC) during maturation improved colour development (due to anthocyanins increasing) in

Cardinal, Pinot Noir, and Tokay berries, while hot days (35 ºC) significantly reduced the formation of anthocyanins. A cold night temperature (10 or 15 ºC) does not reverse the effect of hot days influence on berry colour (Buttrose *et al.*, 1971; Kliewer and Torres, 1972). Similar results were obtained from *V. vinifera* cv. Cabernet-Sauvignon (Goto-Yamamoto *et al.*, 2009) and Merlot (Spayd *et al.*, 2002), suggesting that higher temperatures than 30 to 35 °C promote significant reductions on phenolic content, especially on anthocyanins.

I. 3.1.2. Sunlight exposure

Berry temperature in the field is largely regulated by the flux density of absorbed radiation and convective heat loss and has been shown to increase linearly with incident radiation. The effects of light on grape composition are heavily dependent upon the extent to which berry temperature is elevated as a result of increased sunlight exposure (Bergqvist, 2001). This effect on grape berry development and composition has been investigated during the last few decades, showing that an increasing of sunlight grape exposure during maturation generally improves grape and wine composition (Bergqvist, 2001; Price *et al.*, 1995; Spayd *et al.*, 2002), namely increasing the content of total soluble solids and phenolics, namely anthocyanins (Dokoozlian and Kliewer, 1996; Spayd *et al.*, 2002). Otherwise, prolonged exposure to direct sunlight, rising temperatures in the field around 30 to 35°C, promoted the reduction on phenols content, namely on anthocyanins (Buttrose *et al.*, 1971; Goto-Yamamoto *et al.*, 2009; Kliewer and Torres, 1972).

Sunlight-exposed grapes have also shown higher levels of sugar content and lower values of titratable acidity, malic acid content, and pH when compared to shaded grapes (Crippen and Morrison, 1986; Dokoozlian and Kliewer, 1996; Macaulay and Morris, 1993) and increased wine colour, anthocyanins and tannin contents, and also total phenolics (Song *et al.*, 2015). Sunlight provides light energy for photosynthesis and other light-stimulated metabolic processes (namely the biosyntheses of phenolic compounds promoted by phenylalanine ammonia lyase), and provides heat, both by direct solar radiation on plant surfaces and by heating the surrounding air (Crippen and Morrison, 1986; Ribéreau-Gayon *et al.*, 2000). Heat from sunlight can influence reaction rates of metabolic processes and can also cause stress, either by direct temperature stress or by dehydration (Crippen and Morrison, 1986). The environment surrounding the vines, namely the presence of trees, the abundance of vegetation, and excessive leaf areas, may affect the sunlight exposure, reducing the levels of light penetration and also reducing the air flow (Smithyman *et al.*, 1997). The effect of surrounding vegetation height on Touriga Nacional grapes, from Douro Appellation, in Portugal, was already evaluated. The results showed that grapes grown in vineyards with higher vegetation height (100 cm) had higher carotenoid levels, while grapes grown in vineyards with lower vegetation height (60 cm) with higher sunlight exposure, had higher weight and sugar content. Furthermore, during the maturation period, a lower decrease in carotenoid degradation was observed in vineyards surrounded with higher vegetation height, explaining their higher carotenoid content (Oliveira *et al.*, 2004). A study carried out on Gewürztraminer grape berries composition showed that the levels of glycosylated monoterpenoids were much higher on the sun-exposed grapes when compared with grapes that remained in the partial or total shadow (Reynolds and Wardle, 1989b). The sunlight also promoted the biosynthesis of carotenoids from the first stage of berry formation until *véraison*, decreasing between *véraison* and maturity, giving rise to the glycosylated C_{13} norisoprenoids and other compounds (Baumes *et al.*, 2002). Furthermore, modifications on grape environment such as the hedging and basal leaf removal, or crop level reduction, increased the level of both free and glycosylated terpenoids (Belancic *et al.*, 1997; Reynolds and Wardle, 1989a; Reynolds *et al.*, 1996a; Reynolds *et al.*, 1996b).

I. 3.1.3. Precipitation

The period and precipitation level and the moment when it occurs influence vine water status. The effect of precipitation during initial vegetative phase, flowering, berry development and prior to harvest are considered in this sub-chapter.

The grapevine vegetative phase is influenced by water availability (Acevedo-Opazo *et al.*, 2010) and, depending on the intensity and period of water stress, different effects may be observed such as shoot growth stopping (Hsiao and Xu, 2000; van Leeuwen *et al.*, 2009) and high reduction of leaves size and number, which increases the risks of berry sunburn during its development (Bondada and Keller, 2012). In these cases, irrigation can increase the shoot growth rates and the leaf area. However, a regulation of the water administration needs to be done, as overdone water amounts can originate denser canopies that decrease the radiation levels and airing inside the canopy. Thus, lately, this can lead to a deficient berry development and an increase of the risk of diseases, having a negative effect in the berry quality.

Water deficit between anthesis (flowering period) and *véraison* decreases flowers formation which leads to the diminishing of berry formation and also of its size, and this is often irreversible even if there is no water shortage after the beginning of maturation (McCarthy, 1997). Cell division of pericarp occurs during the first growth phase of the berries. Early water stress reduces the rate of cell division, which explains the inability of berries to recover in size after an early water deficit during the flowering period. Furthermore, organic acids (as tartaric), phenolics (as tannins) and several other compounds such as minerals, micronutrients, and aroma compounds are accumulated during the first phase of berry growth (Cardoso *et al.*, 2005). Thus, an early water stress reduces the accumulation of these components, affecting grapes quality (Conde *et al.*, 2007). Water stress during the development phase of the berry may also decrease berry weight, but in this case the reduction is related to reduce cell volume or diminished sugars and other solutes in the cells. Nutrient deficiencies and other disorders that reduce photosynthesis may also reduce berry growth or slow maturation by decreasing the supply of sugars to the berries (Ojeda *et al.*, 2001). The water status during berry development influences the berry sugar content, which is yield-dependent. For low yields, vine water deficit enhances berry sugar content (Trégoat *et al.*, 2002; van Leeuwen *et al.*, 2009). However, extreme water stress, due to low precipitation, is harmful to the development of the berries, and may lead to yield and quality losses (Ojeda *et al.*, 2002; van Leeuwen *et al.*, 2009). In this case, the irrigation should be an adequate option. Precipitation just prior to harvest can affect grape sugar content, usually expressed as ºBrix, by diluting the sugar and causing ºBrix to drop, thus it is expecting the production of wines with low alcohol content (van Leeuwen and Seguin, 2006). Excessive precipitation at this period can also increase berry size and promote the decreasing the concentration of organic acids, anthocyanins, and tannins content (Keller *et al.*, 2006). Several vine diseases and grapes rotting can also be observed. Otherwise, vines that experience low amount of water have been found to produce fewer and smaller grapes, but with higher sugar, phenolic and volatile content (Jackson and Lombard, 1993; van Leeuwen and Seguin, 2006). In these cases, it is expected to produce wines with high alcohol content, and more intense aroma and colour. This fact is particularly relevant in red wine production due to the fact that controlled water stress of the vine increases berry colour, specially due to the increasing of the concentrations of anthocyanins and tannins (Koundouras *et al.*, 2006; van Leeuwen and Seguin, 1994).

I. 3.2. Vineyard characteristics

I. 3.2.1. Soil

The soil has an important role for viticulture sustainability, since several environmental factors affecting the vine growing and grape and wine composition are related to the soil properties. Soil acts as a regulator of the climate elements, because soil may affect water and nutrient availability to the plant by its retaining capacity, it may affect the microclimate by its heat-retaining and light reflecting capacity, and may affect the root growth by its penetrability (Jackson and Lombard, 1993; Martinez *et al.*, 2011).

Soil types may be defined according to different criteria, namely, taxonomy, morphology, genesis, and texture, among others (Gerrard, 2000; USDA, 1999). Considering the texture features, soil is usually classified as clayey, clay-calcareous and clay-sandy.

The soil type is highly related to the water status through its water-holding capacity (Oliveira *et al.*, 2003; van Leeuwen *et al.*, 2004). The restriction of water supply plays a significant role in vine behaviour and berry composition. A limitation in vine water uptake reduces shoot growth, berry weight and yield and increases berry anthocyanin and tannin content (Choné *et al.*, 2001; Koundouras *et al.*, 2006; van Leeuwen and Seguin, 1994), which, if not excessive, are favourable to grape quality potential (Kennedy *et al.*, 2002; Roby *et al.*, 2004; van Leeuwen *et al.*, 2009). For example, water stress imposed by some types of soils (namely silt-clay) have been shown to increase the oenological potential of Agiorgitiko red grape variety by: i) accelerating sugar accumulation and malic acid breakdown in the juice, ii) promoting the concentration of anthocyanins and total phenolics in berry skins, and iii) increasing the amount of glyco-conjugates of the main aroma components of grapes (Koundouras *et al.*, 2006).

The influence of soil regarding its texture, depth, chemical composition, and water availability on the characteristics of wines was evaluated (Prado *et al.*, 2007; van Leeuwen *et al.*, 2004). The soil type was found to influence significantly the volatile composition of sparkling wines obtained from Fernão-Pires and Baga varieties from Bairrada Appellation, in Portugal. Sparkling wines produced from the clay-calcareous soil presented the highest content of volatiles related to aroma properties when compared to those obtained from grapes produced in clay soils (Coelho *et al.*, 2009). According to these data, clay-related soils seems to improve wine quality (namely increasing its phenolic and volatile contents), when compared to other kind of soils. However, the outcome observed results from a network of other natural factors, intrinsically related to each region, thus its extension to other vineyards and Appellations is not possible. These studies are helpful and needed for each specific wine region, which has specific environments, agricultural practices, varieties, and other inherently natural parameters.

I. 3.2.2. Topographical features

Altitude and slope can strongly regulate the climatic conditions since they are directly associated to the resulting temperature, precipitation, humidity, vineyards surrounding vegetation height, sunlight exposure and shadow of vineyards, vineyards orientation and trellising, influencing grapevine vigour and grape maturation (Jackson and Lombard, 1993). Scarce information is available about the influence of these topographical features on viticulture.

The effect of altitude and its related climatic conditions on phenolic composition (procyanidin and anthocyanin components) of grapes and wines of Touriga Nacional and Touriga Francesa red varieties, from Douro Appellation, have been reported (Mateus *et al.*, 2002; Mateus *et al.*, 2001a; Mateus *et al.*, 2001b). At berry maturity, low altitude (100 to 150 m above the sea level) was shown to be an important factor favouring the biosynthesis of higher concentrations of grape-skin catechin monomers ((+)-catechin, (-)-epicatechin gallate), procyanidin dimers, trimer C1, as well as total extractable proanthocyanidins, when compared to higher altitudes (250 to 350 m above the sea level), contributing to improve wine quality (Mateus *et al.*, 2001a). Higher altitudes are associated with lower temperatures and high humidity, which affect grape maturation, diminishing its polyphenolic composition (Mateus *et al.*, 2002; Mateus *et al.*, 2001a; Mateus *et al.*, 2001b). Similar trend was observed in Cabernet Sauvignon wines produced at different altitudes (909 m and 1280 m) in Loess Plateau (China). The content of phenolic compounds (flavonoids and flavanols) and antioxidant activity of the wines from the lower altitude vineyard (909 m) were relatively higher than those from the higher altitude vineyard (1280 m) (Jiang *et al.*, 2011).

The altitude effect was also studied using grapes from two varieties harvested in different terraces from Douro Appellation (Touriga Franca: 85, 145 and 180 m, and Touriga Nacional: 90, 155 and 210 m) (Oliveira *et al.*, 2004). Touriga Franca grapes grown at lower altitude (85 m) had the lowest carotenoid concentrations. Conversely, for this variety, grapes grown at higher altitudes (145 and 180 m) had higher carotenoid levels. High altitude, which presented lower temperature and higher humidity, is associated with a lower berry growth, decreasing the carotenoid degradation during the maturation period, and this could explain the higher carotenoid values in the high-elevation sites in Touriga Franca variety. This observation was not so evident in Touriga Nacional grapes. At maturity, the carotenoids content of these grapes produced at 155 m altitude were significantly higher than at 90 and 210 m, suggesting that moderate temperatures on moderate altitude positions with good sunlight exposure are ideal for colour development and also for carotenoid accumulation. The latter, as precursors of aroma compounds, may also contribute positively to the wine sensorial properties. It was also reported that aroma potential, given by monoterpene and norisoprenoid components, showed higher content for *V. vinifera* cv. Veneto grapes grown at lower altitude (Tomasi *et al.*, 2000), being expected to obtain wines with higher aroma quality. According to the selected examples, in the same vineyard, grapevines located at lower altitudes produced grapes with better oenological potential, specially related to phenolic and volatile composition.

The slope of a vineyard has also high influence on the temperature and soil drainage, both of which are critical for the growing of grapevines. The manner by which the vines are trellising is the one that best overcomes the restraints imposed upon them by climate, soil, plant needs, and production goals. Vineyards can be planted on very steep slopes (until 60 %) or on terraces. A slope can reduce exposure to heat or cold depending on its aspect (is the term used to describe the direction that slope faces). The aspect of the slope is important for sunlight interception: more sunlight intercepted leads to warm the vineyard. The contribution of slope and its aspect depends on the region; however, it is common to consider that Southern aspects slopes are preferred due to its highest temperature and higher sunlight interception, being great for grapes maturation. The slope of a vineyard lead to changes in several parameters, namely in grapevine vegetative height and also in grapes chemical composition.

I. 4. Natural characteristics of Bairrada Appellation

The evaluation of the variety adequacy regarding the Appellation characteristics should be considered as a strategy for sustainable viticulture. To demonstrate this concept, Bairrada, a Portuguese Appellation legally established in 1979 (Portaria nº 709-A/79, December 28), was selected. Bairrada Appellation is located in the Beiras region, in northwest of Portugal (Figure 3), located between Vouga and Mondego rivers, at east of Caramulo and Bussaco hills and at west of the Atlantic Ocean (Salvador, 1993).

Figure 3. Location of Bairrada Appellation (Portugal).

The total surface area of this region extends over 108,000 hectares, of which only 12,000 hectares are planted with grapes for wine production, representing the red and white vineyards 70% and 30%, respectively. For each Portuguese Appellation there are specific recommended and authorized grape varieties. According to Decreto-Lei nº 301/2003, for the Bairrada Appellation there are a list of 10 white and 16 red *V. vinifera* varieties. These grape varieties are recommended for QWPSR (quality wine produced in specified region) of Bairrada (Decreto-Lei nº72/98, March 26 and Decreto-Lei 301/2003, December 4). From these, Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional, and Sousão were considered in this PhD thesis based on the representativeness of these varieties to this Appellation and on the opinion of the company involved in this work. Bairrada Appellation presents some natural environmental heterogeneity at several levels, namely climate, soils, and altitude.

Bairrada Appellation climate

The climate of Bairrada Appellation is essentially Atlantic and it may present some Mediterranean characteristics with maximum temperatures ranging between *ca* 25 and 35 ºC during summer (June-September). In this Appellation of remarkable maritime influence, it is important to point out the large temperature range between day and night at the time the grapes ripe (reaching a range of 20 ºC), which contributes to maintain the acidity of the grapes and consequently freshness of the wines. Further, the sunlight exposure of Bairrada varies between 2,300 to 2,600 hours/year. Most areas of this Appellation benefit from about 2,500 to 2,600 hours of sunlight per year (Figure 4), which is favourable for grapes maturation.

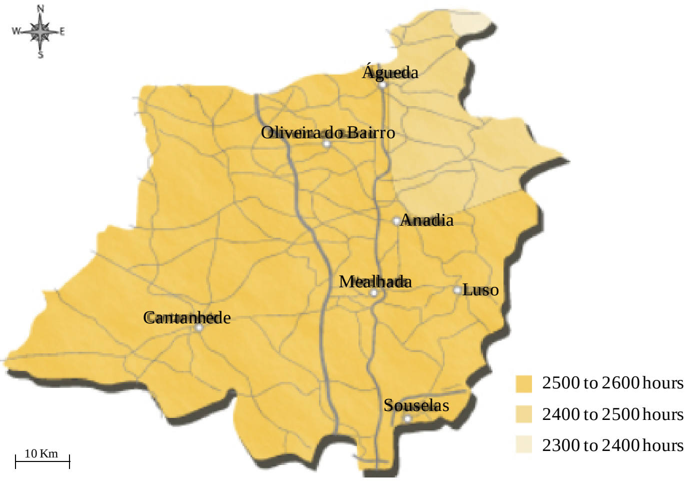


Figure 4. Annual Bairrada sunlight exposure (adapted from http://www.cvbairrada.pt/).

The average annual precipitation characteristic of Bairrada Appellation increases from west to east, varying between 80 to 160 cm per year, being the higher precipitation observed at east (*ca* 120 to 160 cm) (Figure 5). Precipitation amount of this Appellation is irregular throughout the year, peaking during winter season, attaining its maximum at November/December (between *ca* 20 to 25 cm). The occurrence of precipitation in the spring is common until the middle of April or, rarely, until the first days of May, conditioning mainly the early harvesting varieties. In some years, the abundance of rain in the second half of September may cause rotten whose extent depends on the varieties.

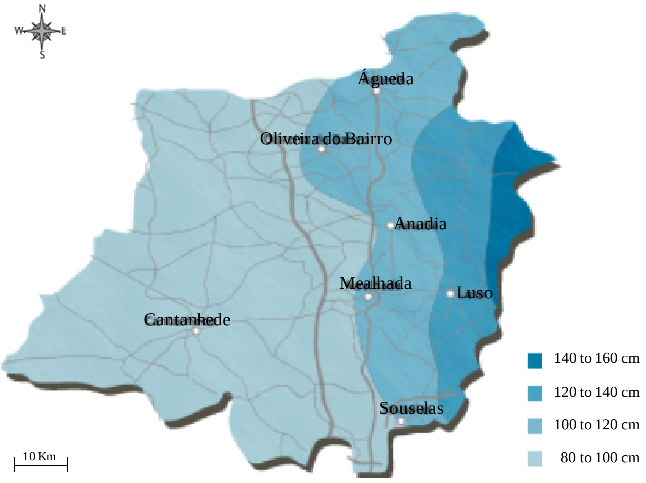


Figure 5. Annual Bairrada precipitation (adapted from http://www.cvbairrada.pt/).

Bairrada Appellation soils

The name “Bairrada” has its roots in the nature of the soils of this region, “*barro*” (Portuguese word for “clay”). The vineyards of Bairrada Appellation were planted mostly in soils from inferior and medium Jurassic, which are clay-calcareous soils. Therefore, the soil in this region has some heterogeneity in its texture and was usually classified into 3 types: i) clayey; ii) clay-calcareous; and iii) clay-sandy. Figure 6 shows the geological map of Bairrada Appellation.

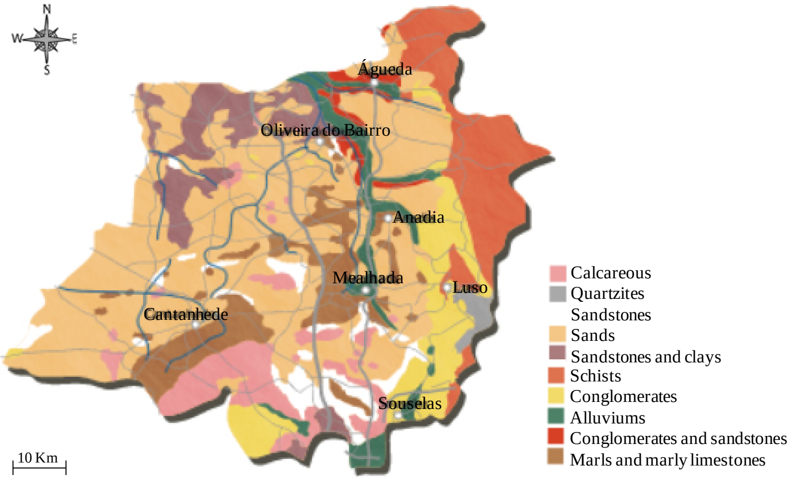


Figure 6. Geological map of Bairrada (adapted from http://www.cvbairrada.pt/).

Bairrada soils are quite varied. Basically, there are large areas of calcareous, clayey and sandy soils. At north of this Appellation there are sands, sandstones and clays while at south are calcareous and sandstones. Schists can be found in the northeast and east, while at west soils are sandier. Furthermore, near the rivers there are areas of alluvia and conglomerates.

Bairrada Appellation altitude

This Appellation presents a wave relief with *ca*. 50 to 150 m of altitude, featuring steep hills and serpentine valleys.

I. 5. Characterization of *Vitis vinifera* L. volatile components

I. 5.1. *Vitis vinifera* L. volatile components

Vitis vinifera L. (common grapevine) is a species of *Vitis* that belongs to the Vitaceae family. This species is a perennial woody vine native to the South-Western Asia and then introduced in Mediterranean region, in Europe, and in other continents, being spread throughout the world (Bombardelli and Morazzoni, 1995). *V. vinifera* is considered one of the major world fruit crops based on the extent of cultivated land and on its economic value. Grapes are used mainly for wine production, but they are also consumed in fresh and as dried fruits or in juice.

There are hundreds of volatile and semi-volatile compounds already reported in *V. vinifera* matrices, namely grapes, musts (grape juices) and wines, representing different chemical families, namely esters, alcohols, acids, aldehydes, ketones, terpenoids, and phenols (Cabredo-Pinillos *et al.*, 2006; Rocha *et al.*, 2007a). Some of these volatile compounds that are responsible for wine aroma could have origin on grapes and must or are formed during alcoholic fermentation by the yeasts (González-Marco *et al.*, 2008; Polášková *et al.*, 2008). These compounds can be present at the free form (volatile) or at the glycosidically-linked one (non-volatile). During grape maturation, the glycosidically-linked fraction is accumulated in the fruit, and may be released during winemaking through the action of endogenous or exogenous β-glucosidases (López-Tamames *et al.*, 1997).

The varietal aroma is directly associated to the grape variety, biosynthesized during grape development, being dependent on harvest and vineyard conditions, and grape maturity state. The terpenic compounds and C_{13} norisoprenoids are associated to the varietal aroma (González-Barreiro *et al.*, 2015; Marais, 1983). The pre-fermentative aromas result from several mechanic or technological operations (transport, crushing, among others) performed before the beginning of the fermentation process. During this, occurs the enzymatic oxidation of fatty acids leading to the formation of C_6 aldehydes and alcohols (Bakker and Clarke, 2011), which are responsible for pre-fermentative aromas. The volatile compounds exhibit different polarities, volatilities, chemical structures, and a wide range of concentrations, which make difficult the establishment of an accurate methodology of analysis. Thus, the establishment of a suitable extraction procedure combined with a gas chromatographic method is always an important challenge to those working on wine chemistry.

The terpenic compounds, namely the sesquiterpenic ones, play a significant role in the varietal aroma of wines due to their flowery, fruity, and fresh odours (Lalel *et al.*, 2003b; Rapp and Mandery, 1986; Williams *et al.*, 1980). Moreover, attending to the complexity of physicochemical characteristics of sesquiterpenic compounds, and also to their importance for the aroma properties of *V. vinifera* related products, sesquiterpenic compounds are selected as a representative analyte model to present the state-of-the-art and the technical know-how about the extraction and gas chromatographic techniques used for their determination on *V. vinifera* related products. The sesquiterpenic compounds present in wines may arise directly from grape and/or may have their origin on the rearrangement processes during winemaking process and/or aging (Rocha *et al.*, 2006a). These secondary metabolites are predominantly formed from farnesyl pyrophosphate. After losing the pyrophosphate residue, different ways of cyclisations are followed. Skeletal rearrangement via carbocation intermediates with hydride or methyl group migration at low pH or temperature conditions (Bülow and König, 2000; Yu and Utsumi, 2009) can give rise to an enormous type of structures (König *et al.*, 1999) (Figure 7).

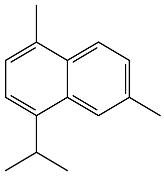
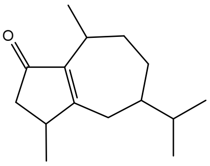
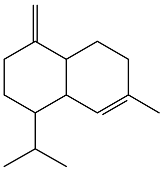
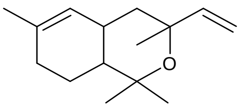
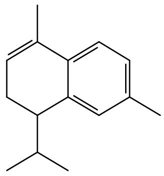
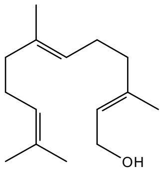
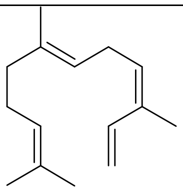
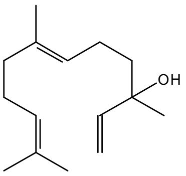
Compound	Chemical Structure	Compound	Chemical Structure
Cadalene		Rotundone	
γ-Cadinene		Cabreuva oxide D	
α-Calacorene		Farnesol	
α-Farnesene		Nerolidol	

Figure 7. Sesquiterpenic compounds identified in *V. vinifera* related matrices, representing the four detected chemical families: hydrocarbons, ketones, oxides and alcohols.

The determination of secondary metabolites of natural products, such as terpenic compounds, represents an essential need for the valuation of these products. Besides *V. vinifera* related matrices, sesquiterpenic compounds have also been found on several natural products of different species of plants (Jalali *et al.*, 2013; Minh *et al.*, 2003; Petronilho *et al.*, 2012; Petronilho *et al.*, 2011; Petronilho *et al.*, 2013; Rafii *et al.*, 1997; Zidorn, 2006; Zielińska and Kisiel, 2000), liverworts (Wurzel and Becker, 1990; Yoyota *et al.*, 1997), fruits (Coelho *et al.*, 2006; Coutinho, 2007; Reis *et al.*, 2009; Rocha *et al.*, 2006a), marine algae (Denys *et al.*, 1993; Elias *et al.*, 1997; Sun *et al.*, 1976), corals (Newberger *et al.*, 2006; Roussis *et al.*, 2000), and sponges (Braekman *et al.*, 2000; Salmoun *et al.*, 2000), among others. The increasing interest on sesquiterpenic compounds comes from the fact that they are present in many natural products claimed as presenting health benefits (Petronilho *et al.*, 2012; Rocha *et al.*, 2006a). For instance, these compounds have been included in cosmetics and functional food products due to their aroma and preservative and bioactive health properties.

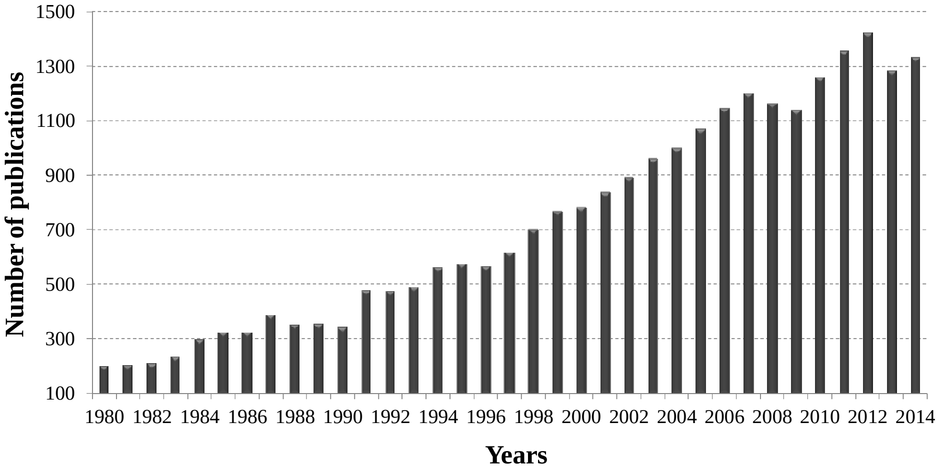


Figure 8. Literature survey of published articles in sesquiterpenic compounds, using a search query with keywords *sesquiterpen** in topic, from 1980 to 2014 via Web of Science^{TM}.

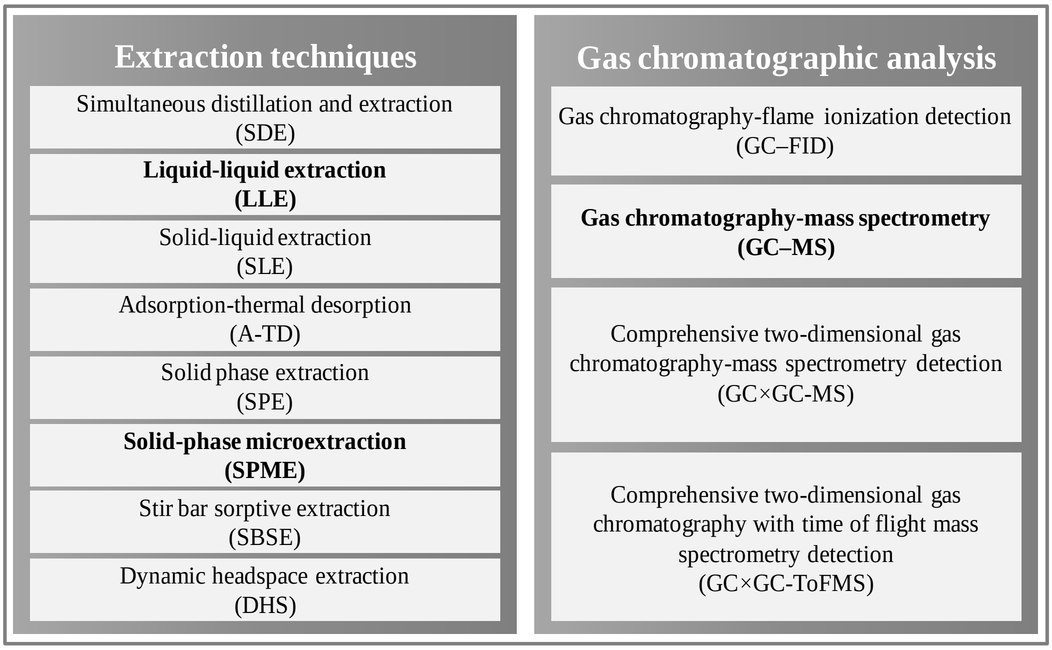
Sesquiterpenic compounds have been the subject of several studies in numerous areas. To date, a total of *ca*. 25,300 publications related to the topic of sesquiterpenic compounds are available on the international scientific databases (search queried with *sesquiterpen**, from 1980 to 2014 via Web of Science^{TM}). The interest on this topic has been increased rapidly over the past 10 years (Figure 8). These studies have been conducted with different purposes, which include several studies mainly in the fields of pharmacology and pharmacy, chemical characterization, molecular biology, plant science, and toxicology. The sesquiterpenic compounds are described as presenting antioxidant (Haraguchi *et al.*, 1997), anti-inflammatory (Lim *et al.*, 2005; Xu *et al.*, 2000), anti-bacterial (Simões *et al.*, 2008; Tamemoto *et al.*, 2001), and anti-cancer (Ahmed *et al.*, 2004; Tatman and Mo, 2002; Wang *et al.*, 2007) properties, among others. Furthermore, as these analytes are hydrophobic and present in several fruits and vegetables, it has been proposed that a diet based on these products, can provide their accumulation in the human body, promoting long and medium-term health beneficial effects (Vinholes *et al.*, 2014).

There are several types of *V. vinifera* related products presenting different characteristics, namely grapes, pomaces, berry skin and pomace distillates, grape skins, musts, and wines (table, fortified, and sparkling), increasing the complexity of sesquiterpenic compounds determination. Furthermore, sesquiterpenic compounds can occur in free form (thus they are volatile and can be more easily detected with adequate extraction and gas chromatographic techniques), or in glycosidically-linked one. In this later case, a previous hot acid and/or enzymatic hydrolysis with β-glycosidases should be performed to release the analytes in order to allow their determination by gas chromatography. Beyond the matrix properties, the physicochemical characteristics of sesquiterpenic compounds should also be taken into consideration. Sesquiterpenic compounds i) are semi-volatile compounds with boiling points ranging, in general, between 250 to 280 ºC (Gildemeister, 1913); ii) present low solubility in water (for example, 0.0023, 0.0108, 1.7, and 2.07 mg L^{-1}, at 25 ºC, for α-bisabolol, β-farnesene, farnesol, nerolidol, respectively) (Petronilho *et al.*, 2012); iii) belong to different chemical families; and iv) can exist at different concentration ranges (from ng to mg L^{-1}) (Baumes *et al.*, 1986; González-Marco *et al.*, 2008).

I. 5.2. Important tools for the determination of sesquiterpenic compounds

The determination of volatile compounds usually includes two steps: i) sample preparation, comprising sample handling, extraction and/or concentration of volatile components, followed by ii) gas chromatographic analysis. Table 1 summarizes a systematic compilation of the sesquiterpenic compounds reported for *V. vinifera* products, organized by chemical families. For each compound the general methodology for extraction and gas chromatographic analysis is indicated (Álvarez *et al.*, 2011; Alves *et al.*, 2005; Bueno *et al.*, 2006; Câmara *et al.*, 2006a; Câmara *et al.*, 2006b, 2007; Câmara *et al.*, 2004; Coelho *et al.*, 2009; Coelho *et al.*, 2008; Coelho *et al.*, 2006; González-Álvarez *et al.*, 2012; Hampel *et al.*, 2005; Kalua and Boss, 2009, 2010; Keyzers and Boss, 2010; López-Vázquez *et al.*, 2010; Lukić *et al.*, 2010; May *et al.*, 2013; May and Wüst, 2012; Oliva *et al.*, 1999; Parker *et al.*, 2007; Perestrelo *et al.*, 2011; Perestrelo *et al.*, 2012; Perestrelo *et al.*, 2006; Piombino *et al.*, 2010; Robinson *et al.*, 2011; Rocha *et al.*, 2006a; Rocha *et al.*, 2000; Rocha *et al.*, 2006b; Ruberto *et al.*, 2008; Salinas *et al.*, 2004; Schmarr *et al.*, 2010; Schreier *et al.*, 1976; Siebert *et al.*, 2008; Tao *et al.*, 2008; Todorova *et al.*, 2010; Versini *et al.*, 1994; Welke *et al.*, 2012). Special attention is devoted to the extraction techniques parameters. The selection of extraction techniques are based mainly on the solubility of sesquiterpenic compounds, especially on non-polar organic solvents (liquid–liquid extraction - LLE, solid–liquid extraction - SLE, and simultaneous distillation-extraction - SDE), and on sorption on a wide range of stationary phases with different design configurations/devices (solid-phase extraction - SPE, solid-phase microextraction - SPME, and stir bar sorptive extraction - SBSE). From these, LLE and

SPME followed by gas chromatography-mass spectrometry (GC–MS) represent the most frequently used methodologies (Table 1 and Figure 9). All of these methodologies allowed the characterization of sesquiterpenic compounds, mainly in free form, as only few reports are available regarding the glycosidically-linked fraction (Perestrelo *et al.*, 2012; Piombino *et al.*, 2010).



Extraction techniques

- Simultaneous distillation and extraction (SDE)
- **Liquid-liquid extraction (LLE)**
- Solid-liquid extraction (SLE)
- Adsorption-thermal desorption (A-TD)
- Solid phase extraction (SPE)
- **Solid-phase microextraction (SPME)**
- Stir bar sorptive extraction (SBSE)
- Dynamic headspace extraction (DHS)

Gas chromatographic analysis

- Gas chromatography-flame ionization detection (GC–FID)
- **Gas chromatography-mass spectrometry (GC–MS)**
- Comprehensive two-dimensional gas chromatography-mass spectrometry detection (GC×GC-MS)
- Comprehensive two-dimensional gas chromatography with time of flight mass spectrometry detection (GC×GC-ToFMS)

Figure 9. Extraction and gas chromatographic techniques used for sesquiterpenic compounds determination in *V. vinifera* related matrices. Bold letters indicate the most commonly used techniques.

Table 1. Sesquiterpenic compounds of *V. vinifera* related matrices and the respective methodologies of analysis.

Compound	Type of Sample	General Methodology	Sample Preparation		References
			Extraction Device [p]	Experimental conditions	
Hydrocarbon-type					
α-Alaskene	Table wine	HS-SPME/GC×GC-ToFMS[a]	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
α-Amorfene	Grape	HS-SPME/GC–MS[b]	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		LLE/GC–MS[c]	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
		SLE/GC–MS[d]	-	Grape berries dipped one by one into acetone (10 mL, 1 min). Resultant extract evaporated to dryness	(Todorova *et al.*, 2010)
	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
Aristolene	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID[e]	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
Aromadendrene	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
	Pomaces	SDE/GC–MS[f]	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID[g]	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
allo-Aromadendrene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
1(5),3-Aromadenedradiene	Table wine	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
α-Bergamotene	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Bicyclogermacrene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)

Bicyclosesquiphellandrene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
α-Bisabolene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
β-Bisabolene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
α-Bourbonene	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
β-Bourbonene (isomer 1)	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
β-Bourbonene (isomer 2)	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
β-Bourbonene (isomer 3)	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
Cadalene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
		HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
	Sparkling wine	SBSE-LD/LVI-GC–MS[h]	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
α-Cadinene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}). SPME fibre exposed to headspace during 60 min	(Coelho *et al.*, 2006)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)

	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
β-Cadinene	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}). SPME fibre exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
	Pomaces distillates	SPE/GC–MS^{i}	Cartridge with 1g Styrene-divinylbenzene (SDVB) polymer	Sample washed with methanol/water (20 mL, 15% v/v) and water (20 mL). Analytes were removed by dichloromethane (30 mL), followed by Vigreux column concentration	(López-Vázquez *et al.*, 2010)
γ-Cadinene	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (85 μm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (85 μm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Pomaces distillates	SPE/GC–MS	Cartridge with 1g Styrene-divinylbenzene (SDVB) polymer	Sample washed with methanol/water (20 mL, 15% v/v) and of water (20 mL). Analytes were removed by dichloromethane (30 mL), followed by Vigreux column concentration	(López-Vázquez *et al.*, 2010)
δ-Cadinene	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Pomaces distillates	SPE/GC–MS	Cartridge with 1g Styrene-divinylbenzene (SDVB) polymer	Sample washed with methanol/water (20 mL, 15% v/v) and water (20 mL). Analytes were removed by dichloromethane (30 mL), followed by Vigreux column concentration	(López-Vázquez *et al.*, 2010)
	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
	Fortified wine	HS-SPME/GC–MS	SPME syringe (85 μm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 2 h, stirring (1200 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2006b)
α-Calacorene	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl	(Coelho *et al.*,

				addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Table wine	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
β-Calacorene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
Calamenene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Table wine	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
Calarene	Table wine	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
Calamene	Table wine	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
α-Caryophyllene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)

		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
β-Caryophyllene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2009)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Foliage	DHS/GC–MS[j]	Dynamic headspace device	Terminal stems were cut and placed into the dynamic headspace device, and the volatile components were sorbed on a Tenax column for 48 h	(Hampel *et al.*, 2005)
α-Cedrene	Table wine	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
α-Chamigrene	Table wine	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
β-Chamigrene	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 µL), under ultrasonic treatment	(Coelho *et al.*, 2008)
α-Copaene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2009)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Pomaces distillates	SPE/GC–MS	Cartridge with 1g Styrene-divinylbenzene (SDVB) polymer	Sample washed with methanol/water (20 mL, 15% v/v) and water (20 mL). Analytes were removed by dichloromethane (30 mL), followed by Vigreux column concentration	(López-Vázquez *et al.*, 2010)
β-Copaene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
α-Cubebene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2009)

		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
β-Cubebene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
α-Curcumene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
Cycloisolongifolene	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
Cyclosativene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Cyperene	Grape	SLE/GC–MS	-	Grape berries dipped one by one into acetone (10 mL, 1 min). Resultant extract evaporated to dryness	(Todorova *et al.*, 2010)
4,5,9,10-Dehydro-isolongifolene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
γ-Elemene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
δ-Elememne	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
Epi-bicyclosesquiphellandrene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
Epizonarene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)

	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Pomaces distillates	SPE/GC–MS	Cartridge with 1g Styrene-divinylbenzene (SDVB) polymer	Sample washed with methanol/water (20 mL, 15% v/v) and of water (20 mL). Analytes were removed by dichloromethane (30 mL), followed by Vigreux column concentration	(López-Vázquez *et al.*, 2010)
α-Farnesene	Grape	HS-SPME/GC–MS	SPME syringe (85 μm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Grape skin	SPE/GC–MS	Column (40 cm × 1 cm) filled with XAD-2 resin (12 cm, 0.15-0.25 mm)	Preparation of grape skins ethanolic extract (15 h). Extract dilution with water and introduction on the column. Analytes were removed by pentane/dichloromethane (80 mL, 2:1 v/v), followed by concentration	(Versini *et al.*, 1994)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Foliage	DHS/GC–MS	Dynamic headspace device	Terminal stems were cut and placed into the dynamic headspace device, and the volatile components were sorbed on a Tenax column for 48 h	(Hampel *et al.*, 2005)
	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 μL) under ultrasonic treatment	(Coelho *et al.*, 2009)
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 μL), under ultrasonic treatment	(Coelho *et al.*, 2008)
α-Farnesene (isomer)	Grape	HS-SPME/GC–MS	SPME syringe (85 μm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
	Grape skin	SPE/ GC–MS	Column (40 cm × 1 cm) filled with XAD-2 resin (12 cm, 0.15-0.25 mm)	Preparation of grape skins ethanolic extract (15 h). Extract dilution with water and introduction on the column. Analytes were removed by pentane/dichloromethane (80 mL, 2:1 v/v), followed by concentration	(Versini *et al.*, 1994)
	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 μL) under ultrasonic treatment	(Coelho *et al.*, 2009)
β-Farnesene	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
Germacrene D	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)

		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Foliage	DHS/GC–MS	Dynamic headspace device	Terminal stems were cut and placed into the dynamic headspace device, and the volatile components were sorbed on a Tenax column for 48 h	(Hampel *et al.*, 2005)
Germacrene B	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Guaiazulene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 µL), under ultrasonic treatment	(Coelho *et al.*, 2008)
3,7-Guaiadiene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Guaia-6,9-diene	Grape	HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
α-Guaiene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
α-Gurjunene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)

		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
β-Gurjunene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
α-Humulene	Grape	LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2009)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
	Foliage	DHS/GC–MS	Dynamic headspace device	Terminal stems were cut and placed into the dynamic headspace device, and the volatile components were sorbed on a Tenax column for 48 h	(Hampel *et al.*, 2005)
Isocalamenene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
Isoledene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-en	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
Muurola-4(14),5-diene	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
α-Muurolene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2009)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)

		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Table wine	HS-SPME/GC–MS	SPME Syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
γ-Muurolene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2009)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 40 ºC, 5 min, stirring (300 rpm), then SPME fibre was exposed to the sample headspace during 30 min	(Kalua and Boss, 2010)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
α-Panasinsen	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
β-Patchoulene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 45 ºC, 10 min, NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 45 min	(Welke *et al.*, 2012)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
Selina-3,7-diene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
Selina-4,6-diene	Grape	HS-SPME/GC–MS	SPME syringe (85 µm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
β-Selinene	Grape	LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
γ-Selinene	Grape	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
δ-Selinene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Sesquichamene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 45 ºC, 10 min, NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 45 min	(Welke *et al.*, 2012)
β-Sesquiphellandrene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
Valencene	Grape	HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)

β-Vetivenene	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
α-Ylangene	Grape	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	Sample was thermostated at 40 ºC, 60 min, stirring (1000 rpm), NaCl addition (200 g L^{-1}), then SPME fibre was exposed to the sample headspace during 60 min	(Coelho *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		HS-SPME/GC–MS	SPME syringe (85 μm PA)	Sample was thermostated at 45 ºC, 30 min, then SPME fibre was exposed to the sample headspace during 10 min	(May and Wüst, 2012)
		HS-SPME/GC–MS	SPME syringe (85 μm PA)	Sample was thermostated at 60 ºC, 30 min, stirring (400 rpm), then SPME fibre was exposed to the sample headspace during 10 min	(May *et al.*, 2013)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
		LLE/GC–MS	-	Must preparation, Pentane-methylene chloride (2:l) extraction, followed by Vigreux column concentration	(Schreier *et al.*, 1976)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
β-Ylangene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
Zonarene	Grape	LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Must preparation, dichloromethane extraction for 23 h, followed by silica column elution with pentane and Vigreux column concentration	(Parker *et al.*, 2007)
Ketone-type					
Rotundone	Grape	SPE/HS-SPME/GC–MS[k]	Cartridge with 0.5g Phenomenex Strata SDB-L solid phase and SPME syringe (65 μm PDMS/DVB)	Must preparation. Sample washed with water and pentane, and eluted with pentane/ethyl acetate (10 mL, 9:1). The recovered extract was extracted by SPME (35 °C, 60 min)	(Siebert *et al.*, 2008)
		SPE/IM-SPME/GC–MS[l]	Cartridge with 0.5g Phenomenex Strata SDB-L solid phase and SPME syringe (65 μm PDMS/DVB)	Must preparation. Sample washed with water and pentane, and eluted with pentane/ethyl acetate (10 mL, 9:1). The recovered extract was extracted by SPME (35 °C, 60 min)	(Siebert *et al.*, 2008)
	Table wine	SPE/HS-SPME/GC–MS	Cartridge with 0.5g Phenomenex Strata SDB-L solid phase and SPME syringe (65 μm PDMS/DVB)	Sample washed with water and pentane, and eluted with pentane/ethyl acetate (10 mL, 9:1). The recovered extract was extracted by SPME (35 °C, 60 min)	(Siebert *et al.*, 2008)
		SPE/IM-SPME/GC–MS	Cartridge with 0.5g Phenomenex Strata SDB-L solid phase and SPME syringe (65 μm PDMS/DVB)	Sample washed with water and pentane, and eluted with pentane/ethyl acetate (10 mL, 9:1). The recovered extract was extracted by SPME (35 °C, 60 min)	(Siebert *et al.*, 2008)
Oxide-type					
Cabreuva oxide D	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
Alcohol-type					
α-Bisabolol	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl	(Robinson *et al.*,

				addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	2011)
α-Cadinol	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Fortified wine	SBSE/TD–GC–MS[m]	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). After this, the stir bar was rinsed in ultra-pure water and dried with paper	(Alves *et al.*, 2005)
δ-Cadinol	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Cubenol	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
2,3-Dihydrofarnesol	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
Epi-α-cadinol	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
γ-Eudesmol	Table wine	HS-SPME/GC–MS	SPME syringe (65 μm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
	Fortified wine	SBSE/TD–GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). After this, the stir bar was rinsed in ultra-pure water and dried with paper	(Alves *et al.*, 2005)
Eudesm-7(11)-en-4-ol	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
Farnesol	Grape	SBSE/TD-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Must preparation. Extraction: at 20 ºC, 6 h, stirring (700 rpm). After this, the stir bar was rinsed in distilled water and dried with a cellulose tissue	(Salinas *et al.*, 2004)
	Grape skin	SPE/ GC–MS	Column (40 cm × 1 cm) filled with XAD-2 resin (12 cm, 0.15-0.25 mm)	Preparation of grape skins ethanolic extract (15 h). Extract dilution with water and introduction on the column. Analytes were removed by pentane/dichloromethane (80 mL, 2:1 v/v), followed by concentration	(Versini *et al.*, 1994)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Must	HS-SPME/GC–MS	SPME syringe (85 μm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring, NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2004)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Dichloromethane extraction (75 mL, 24 h, *ca*. 50 ºC), repeated six times, followed by frozen (-20 °C) and decantation to separate water from organic phase and then dried over anhydrous sodium sulphate. Excess of solvent removed by distillation at low pressure using a trap with liquid nitrogen	(Rocha *et al.*, 2000)
	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Fluorotrichloromethane extraction (100 mL, 24 h), followed by concentration in a microconcentrator	(Bueno *et al.*, 2006)
		HS-SPME/GC–MS	SPME syringe (100 μm PDMS)	Sample was thermostated at 45 ºC, 10 min, stirring, NaCl addition (125	(Tao *et al.*, 2008)

				g L^{-1}), then SPME fibre was exposed to the sample headspace during 15 min	
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 μL), under ultrasonic treatment	(Coelho *et al.*, 2008)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
		HS-SPME/GC×GC-MS^n	SPME syringe (85 μm PA)	SPME fibre was exposed to the sample headspace at 45 ºC, 10 min, stirring	(Schmarr *et al.*, 2010)
		SPE/ GC–MS	Cartridge with Strata-X 33 μm polymeric reversed phase	Sample washed with water (20 mL). Analytes were removed by dichloromethane (10 mL), dried over anhydrous sodium sulphate, followed by N_2 stream concentration	(Álvarez *et al.*, 2011; González-Álvarez *et al.*, 2012)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 45 ºC, 10 min, NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 45 min	(Welke *et al.*, 2012)
	Fortified wine	HS-SPME/GC–MS	SPME syringe (85 μm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 2 h, stirring (1200 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2006b)
		HS-SPME/GC–MS	SPME syringe (85 μm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 2 h, stirring (1250 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2007)
		SPE/GC–MS	Cartridge with 1 g of C_{18} reversed-phase	Free fraction: removed from C_{18} reversed-phase with dichloromethane. The extract was dried over Na_2SO_4, and concentrated in a Kuderna-Danish concentrator and then under a stream N_2. Glycosidically-linked fraction: removed from C_{18} reversed-phase with methanol, followed by vacuum concentration. Residue was dissolved in phosphate-citrate buffer (5 mL, pH 5.0) containing a β-glycosidase enzyme, and incubated at 40 ºC, for 16 h. Extraction of analytes with dichloromethane	(Piombino *et al.*, 2010)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 μL) under ultrasonic treatment	(Coelho *et al.*, 2009)
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 μL), under ultrasonic treatment	(Coelho *et al.*, 2008)
Lanceol	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 45 ºC, 10 min, NaCl addition (300 g L^{-1}) then SPME fibre was exposed to the sample headspace during 45 min	(Welke *et al.*, 2012)
ι-Muurolol	Grape	SLE/GC–MS	-	Grape berries dipped one by one into acetone (10 mL, 1 min). Resulting extract evaporated to dryness	(Todorova *et al.*, 2010)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Fortified wine	SBSE/TD–GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 μL)	Extraction: 20 ºC, 60 min, stirring (800 rpm). After this, the stir bar was rinsed in ultra-pure water and dried with paper	(Alves *et al.*, 2005)
Nerolidol	Grape	HS-SPME/GC–MS	SPME syringe (50/30 μm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 60 ºC, 45 min, stirring (800 rpm), NaCl addition (222 g L^{-1})	(Perestrelo *et al.*, 2011)
		SPE-HS-SPME/GC–MS	Cartridge with 200 mg of Lichrolut EN resin (12 cm, 0.15-0.25 mm) and SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample washed with ethyl acetate (4 mL) and of water (4 mL). Analytes were removed by methanol (2 mL), followed by nitrogen stream concentration. Sample was thermostated at 60 ºC, 5 min, stirring (400 rpm), NaCl addition (300 g L^{-1}) then SPME fibre was exposed to the sample headspace during 45 min	(Perestrelo *et al.*, 2012)
	Berry skin distillates	LLE/GC–MS	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times,	(Lukić *et al.*, 2010)

				followed by nitrogen stream concentration	
		LLE/GC–FID	Parafilm-sealed separatory funnel-	Dichloromethane extraction (5 mL, 90 min), repeated three times, followed by nitrogen stream concentration	(Lukić *et al.*, 2010)
	Pomaces	SDE/GC–MS	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
		SDE/GC–FID	Likens-Nickerson apparatus	Hexane (1mL, 3 h)	(Ruberto *et al.*, 2008)
	Pomaces distillates	SPE/GC–MS	Cartridge with 1g Styrene-divinylbenzene (SDVB) polymer	Sample washed with methanol/water (20 mL, 15% v/v) and of water (20 mL). Analytes were removed by dichloromethane (30 mL), followed by Vigreux column concentration	(López-Vázquez *et al.*, 2010)
	Must	HS-SPME/GC–MS	SPME syringe (85 µm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (1250 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2006a)
	Table wine	HS-SPME/GC–MS	SPME syringe (50/30 µm DVB/CAR/PDMS)	SPME fibre was exposed to the sample headspace at 35 ºC, 90 min, stirring (250 rpm), NaCl addition (300 g L^{-1})	(Keyzers and Boss, 2010)
		HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 10 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006b)
		LLE/GC–MS	Continuous liquid-liquid extraction apparatus	Fluorotrichloromethane extraction (100 mL, 24 h), followed by concentration in a microconcentrator	(Bueno *et al.*, 2006)
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 µL), under ultrasonic treatment	(Coelho *et al.*, 2008)
		HS-SPME/GC–MS	SPME syringe (100 µm PDMS)	Sample was thermostated at 45 ºC, 10 min, stirring, NaCl addition (125 g L^{-1}), then SPME fibre was exposed to the sample headspace during 15 min	(Tao *et al.*, 2008)
		HS-SPME/GC–MS	SPME syringe (65 µm CW/DVB)	SPME fibre was exposed to the sample headspace at 40 ºC, 60 min, stirring (200 rpm), NaCl addition (200 g L^{-1})	(Rocha *et al.*, 2006a)
		A–TD/GC–MS^{o}	Tube with Tenax TA (60-80 mesh).	Analytes were isolated by purging with helium (20 min)	(Oliva *et al.*, 1999)
		HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 µm DVB/CAR/PDMS)	Sample was thermostated at 30 ºC, 10 min, stirring (500 rpm), NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 2 h, stirring (600 rpm)	(Robinson *et al.*, 2011)
		HS-SPME/GC×GC-MS	SPME syringe (85 µm PA)	SPME fibre was exposed to the sample headspace at 45 ºC, 10 min, stirring	(Schmarr *et al.*, 2010)
		SPE/ GC–MS	Cartridge with Strata-X 33 µm polymeric reversed phase	Sample washed with water (20 mL). Analytes were removed by dichloromethane (10 mL), dried over anhydrous sodium sulphate, followed by N$_2$ stream concentration	(González-Álvarez *et al.*, 2012)
	Fortified wine	SBSE/TD–GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). After this, the stir bar was rinsed in ultra-pure water and dried with paper	(Alves *et al.*, 2005)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 2 h, stirring (1250 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2006a)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 2 h, stirring (1200 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2006b)
		HS-SPME/GC–MS	SPME syringe (85 µm PA)	SPME fibre was exposed to the sample headspace at 40 ºC, 2 h, stirring (1250 rpm), NaCl addition (300 g L^{-1})	(Câmara *et al.*, 2007)
		LLE/GC–MS	–	Dichloromethane extraction (5 mL, 90 min), repeated two times, blended and dried over anhydrous sodium sulphate, followed by roto-evaporator concentration and under a stream of pure N$_2$	(Perestrelo *et al.*, 2006)
	Sparkling wine	SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm × 10mm, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm). Back-extraction: pentane (200 µL) under ultrasonic treatment	(Coelho *et al.*, 2009)
		SBSE-LD/LVI-GC–MS	PDMS stir bar (0.5 mm thick; 10mm long, 24 µL)	Extraction: at 20 ºC, 60 min, stirring (800 rpm), addition of ethanol (10%, v/v). Back-extraction: pentane (200 µL), under ultrasonic treatment	(Coelho *et al.*, 2008)

α-Santalol	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 45 ºC, 10 min, NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 45 min	(Welke *et al*., 2012)
β-Santalol	Table wine	HS-SPME/GC×GC-ToFMS	SPME syringe (50/30 μm DVB/CAR/PDMS)	Sample was thermostated at 45 ºC, 10 min, NaCl addition (300 g L^{-1}), then SPME fibre was exposed to the sample headspace during 45 min	(Welke *et al*., 2012)

[a] Headspace-solid phase microextraction-Comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyzer; [b] Headspace-solid phase microextraction-gas chromatography-mass spectrometry; [c] Liquid–liquid extraction-gas chromatography-mass spectrometry; [d] Solid–liquid extraction-gas chromatography-mass spectrometry; [e] Liquid–liquid extraction-gas chromatography-flame ionization detector; [f] Simultaneous distillation-extraction-gas chromatography-mass spectrometry; [g] Simultaneous distillation-extraction-gas chromatography-flame ionization detector; [h] Stir bar sorptive extraction-liquid desorption-large volume injection coupled to gas chromatography–mass spectrometry; [i] Solid phase extraction-gas chromatography-mass spectrometry; [j] Dynamic headspace extraction-gas chromatography-mass spectrometry; [k] Solid phase extraction followed by headspace-solid phase microextraction-gas chromatography-mass spectrometry; [l] Solid phase extraction followed by Immersion-solid phase microextraction-gas chromatography-mass spectrometry; [m] Stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry; [n] Headspace-solid phase microextraction-comprehensive two-dimensional gas chromatography-mass spectrometry; [o] Adsorption-thermal desorption-gas chromatography-mass spectrometry; [p] PDMS: polydimethylsiloxane; CAR:carboxen; PA:polyacrylate; CW:carbowax; DVB:divinylbenzene.

I. 5.3. Extraction techniques towards gas chromatographic analysis

Several extraction techniques have been used to extract sesquiterpenic compounds from *V. vinifera* matrices, such as distillation (López-Vázquez *et al.*, 2010; Ruberto *et al.*, 2008) or solvent extraction (Perestrelo *et al.*, 2006; Rocha *et al.*, 2000; Schreier *et al.*, 1976), which are typically time- and labour-intensive (Polášková *et al.*, 2008). Over the last two decades, microextraction techniques (i.e. SPME and SBSE) (Pawliszyn, 2000; Vas *et al.*, 1998; Vas and Vékey, 2004) have been developed to address the need to facilitate rapid and efficient sample preparation. These solvent-free or solventless techniques promote high extraction efficiency and also address the actual concerns about health and environment sustainability.

Headspace techniques based on the sorption of the sesquiterpenic compounds on Tenax phase (2,6-diphenyl-*p*-phenylene oxide) followed by GC analysis were also used to study these analytes in wine (only nerolidol was identified) (Oliva *et al.*, 1999) and *V. vinifera* foliage (four hydrocarbon sesquiterpene type were detected) (Hampel *et al.*, 2005). Tenax is a hydrophobic adsorbent considered appropriate for wine volatile compounds extraction due to its low water- and ethanol-adsorbing capacity (Díaz-Regañon *et al.*, 1998; Salinas *et al.*, 1994). However, its saturation with the major analytes, as for example the esters, when reached, prevents the adsorption of the minor ones, such as the sesquiterpenic components (Fernandez and Diaz-Marta, 1997). It seems to be appropriate for extraction of sesquiterpenic compounds released by grape foliage, as mono- and sesquiterpenic components are the major ones. As a few bibliography is available about *V. vinifera* applications by using this technique, no further specific details will be discussed about.

I. 5.3.1. Solvent extraction methods

Liquid-liquid and solid-liquid extraction – LLE and SLE

The solvent extraction technique is the first crucial step for the preparation of an extract that should be truly representative of the sample composition (Blanch *et al.*, 1991; Gupta *et al.*, 2012; Mamede and Pastore, 2006; Romanik *et al.*, 2007). Liquid-liquid extraction (LLE) has been widely applied for the determination of sesquiterpenic components from *V. vinifera* related products namely berry skin distillates (Lukić *et al.*, 2010), grapes (extracted before must preparation) (Parker *et al.*, 2007; Rocha *et al.*, 2000;

Schreier *et al.*, 1976), and fortified (Perestrelo *et al.*, 2006) and table wines (Bueno *et al.*, 2006), while solid-liquid extraction (SLE) was only applied to grapes (Todorova *et al.*, 2010).

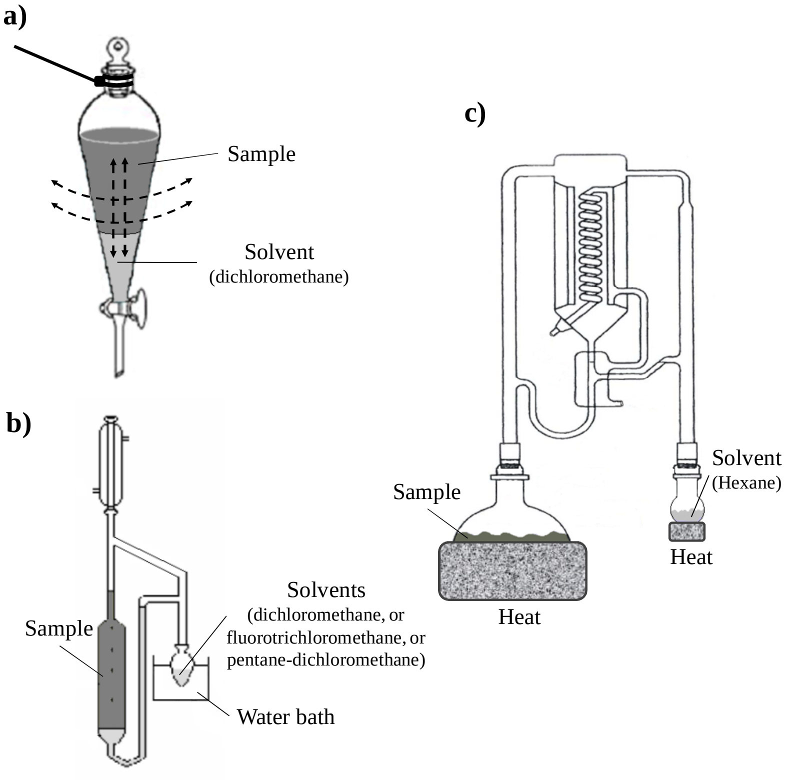


Figure 10. Schematic examples of apparatus used for solvent extraction based techniques: a) separatory funnel (LLE), b) liquid-liquid continuous extraction apparatus (LLE), and c) Likens-Nickerson apparatus (SDE).

LLE and SLE are used to extract components from liquid and solid matrices, respectively, based on their affinities to the organic solvents. Non-polar solvents (dielectric constant $\varepsilon<20$), such as dichloromethane ($\varepsilon=9.1$), fluorotrichloromethane (Freon 11, $\varepsilon\approx2.3$), and pentane ($\varepsilon=1.8$)/ dichloromethane, and also a polar one ($\varepsilon>20$), acetone ($\varepsilon=21$), have been used to extract sesquiterpenic compounds, being dichloromethane the most commonly used (Table 1). Separatory funnel (Figure 10a) and liquid-liquid continuous extraction apparatus (Figure 10b) represent the devices usually used. With this technique, a wide range of chemical structures may be simultaneously extracted. However, the required solvent evaporation prior to GC injection can result in loss or degradation of some analytes with formation of artefacts (Mamede and Pastore, 2006). If the solvent evaporation is performed under vacuum and using a trap with liquid nitrogen for more efficient condensation, it is possible to decrease the evaporation temperature, preventing and/or reducing the degradation and formation of artefacts. For instance, a Vigreux column concentration system under vacuum may be used connected to a liquid nitrogen trap. By using LLE, 15 to 100 mL of solvents have been used, with long extraction times (90 min to 24 h) (Table 1). Large sample volumes (50 to 3,000 mL) are also required, which may represent a problem if there is not enough sample amount available. Besides, as this technique is only applied to liquid samples, the sesquiterpenic components of solid samples, such as grapes and pomaces, may be extracted using SLE.

Simultaneous distillation-extraction – SDE

SDE combines the characteristics of solvent extraction (LLE and SLE) with distillation, being used to extract solid and liquid samples. For several decades, the volatile components of *V. vinifera* related products have been extracted using distillation based techniques (Blanch *et al.*, 1991; Caven-Quantrill and Buglass, 2006; Núñez *et al.*, 1984; Sánchez-Palomo *et al.*, 2009), although only for pomaces the presence of sesquiterpenic compounds (Ruberto *et al.*, 2008), using hexane (ε=1.9) as solvent (Table 1), has been reported.

SDE is a technique used worldwide due to its simplicity, being Likens-Nickerson apparatus one of the most common devices (Figure 10c). Basically, the sample with water is boiled in one flask, while the extracting organic solvent, less dense and immiscible with water, is simultaneously boiled in another flask, both generating steams. The solvent vapours are mixed in a central chamber allowing the organic solvent to extract the soluble analytes. Then, the water and the organic solvent condensate forming two immiscible phases that are able to be separated due to their different densities, returning to their own flasks (Reid, 2003). Due to this apparatus configuration, one of its advantages is the low ratio of the volume of solvent to the quantity of sample. The distillation and extraction processes are continuously repeated along the extraction time (3 h for the pomaces extraction). SDE promotes high extraction efficiency due to the continuous and simultaneous distillation and extraction that allow enrichment of the solvent extract. Using this SDE apparatus, it may be taken into consideration the control of temperature to avoid or reduce the loss and thermal degradation of the sample components. However, this methodology has not been highly exploited for sesquiterpenic compounds extraction from *V. vinifera*. Thus, it is not well demonstrated its theoretical advantages.

I. 5.3.2. Sorptive extraction methods

Solid phase extraction – SPE

SPE is an extraction technique based on the partitioning of solutes between two phases: the sample (liquid sample or solvent extract obtained from the solid sample) and the solid sorbent phase (Piñeiro *et al.*, 2004; Żwir-Ferenc and Biziuk, 2006). This extraction technique has been used to characterize sesquiterpenic compounds from grape pomace distillates (López-Vázquez *et al.*, 2010), grape skin (Versini *et al.*, 1994), and table (González-Álvarez *et al.*, 2012) and fortified wines (Piombino *et al.*, 2010). Free and/or glycosidically-linked sesquiterpenic components are achieved.

The general procedure is to load a sample onto the solid phase, washing away undesired components, and then eluting the target analytes with appropriate solvent into the collection tubes (Castro *et al.*, 2008; Zhou *et al.*, 1996). Before the extraction of the hydrophobic sesquiterpenic compounds, water (ε=80) may be used to eliminate interfering substances, such as sugars (Figure 11a). This is particularly important considering the posterior study of the glycosidically-linked fraction, which involves the use of β-glucosidases that may be partially inhibited by glucose.

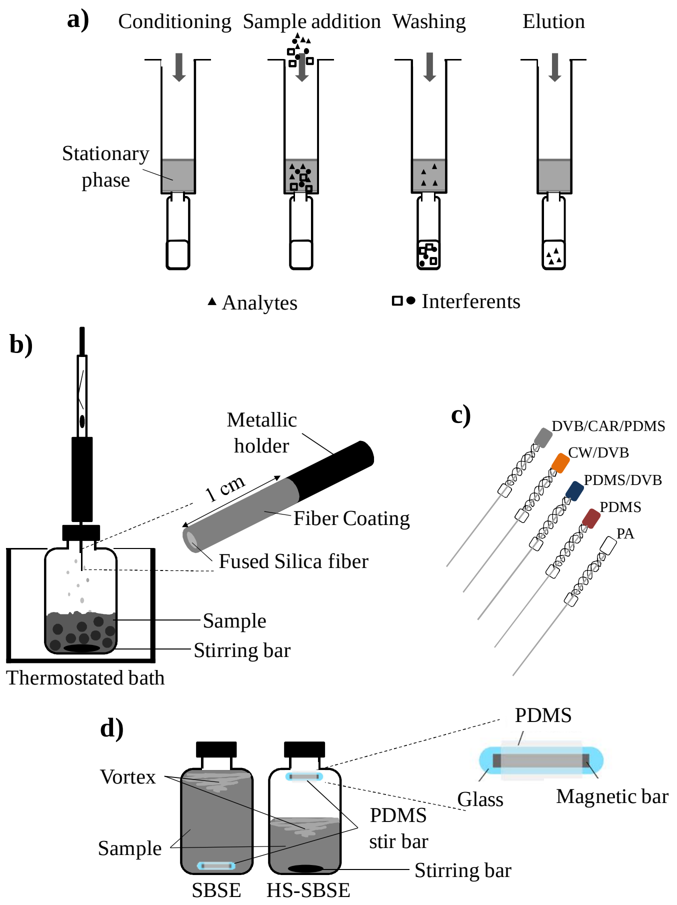


Figure 11. Schematic examples of solid phase operating extraction modes: a) solid phase extraction using cartridges, b) HS-SPME using manual syringe extraction holder, and c) the corresponding most common stationary phases, and d) SBSE with PDMS stationary phase, in immersion or headspace (HS) sampling modes. PDMS – 100 μm Polydimethylsiloxane; PA - 85 μm Polyacrylate; PDMS/DVB – 65 μm Polydimethylsiloxane/Divinylbenzene; CW/DVB – 65 μm Carbowax/Divinylbenzene; DVB/CAR/PDMS - 50/30 μm Divinylbenzene/ Carboxen/ Polydimethylsiloxane

Due to the hydrophobic characteristics of sesquiterpenic compounds, the commonly materials used are polymer-based sorbents such as Amberlite XAD-2, LiChrolut-EN and SDVB, and carbon-based sorbents such as C_{18} reversed-phases (Table 1). The sesquiterpenic compounds (free and glycosidically-linked) are adsorbed at the hydrophobic solid phase surface, and a selective elution is then performed using an appropriate solvent. For elution of the free fraction, non-polar solvents (dichloromethane, ethyl acetate (ε=6), pentane/dichloromethane mixture) have been employed, while the polar solvent methanol (ε=33) has been used for elution of the glycosidically-linked ones (Table 1). The non-polar extract containing the free fraction, after drying and concentration steps, can be directly analysed by GC, whereas the polar extract, containing the glycosidically-linked fraction, needs to be submitted to an hot acid and/or enzymatic hydrolysis to release the analytes prior to GC analysis. Few reports are available regarding the sesquiterpenic glycosidically-linked fraction from *V. vinifera* (Perestrelo *et al.*, 2012; Piombino *et al.*, 2010). The methodology used to obtain this fraction is time-consuming as it involves multi-steps.

Solid phase microextraction – SPME

The SPME is a sample preparation technique based on sorption (absorption and/or adsorption), used for simultaneous sampling, extraction, and pre-concentration of the analytes. Using SPME, only a small fraction of the analytes is extracted (non-exhaustive extraction), which should be representative of the overall composition of analytes in the free form (Vas and Vékey, 2004). SPME can be directly applied to extract analytes from solid, liquid or gaseous samples. Since its development, in the beginning of the 1990 decade (Arthur and Pawliszyn, 1990; Zhang and Pawliszyn, 1993), it has been successfully applied to a wide variety of matrices (Jalali *et al.*, 2013; Petronilho *et al.*, 2013; Reis *et al.*, 2009), including *V. vinifera* related products, such as grapes (Coelho *et al.*, 2006; Kalua and Boss, 2009, 2010; May and Wüst, 2012; Perestrelo *et al.*, 2011), musts (Câmara *et al.*, 2006a; Câmara *et al.*, 2004; Keyzers and Boss, 2010), and table (Rocha *et al.*, 2006a; Rocha *et al.*, 2006b; Tao *et al.*, 2008) and fortified wines (Câmara *et al.*, 2006a; Câmara *et al.*, 2006b, 2007).

Several SPME extraction phase geometries are available, such as fibre, thin film, tube, disk, vessel, stirrer, and suspended particles. From these, the fibre represents the highly used one, which consists of a syringe in which the needle has a base of fused silica coated with a thin layer of a stationary phase (Figure 11b) (Pawliszyn, 2000). Basically, the SPME sampling can be performed by immersion or direct extraction (IM-SPME), where the coated fibre is inserted into the sample and the analytes are transported directly from the sample to the extracting phase, or by headspace extraction (HS-SPME), where the analytes are extracted from the gas phase equilibrated with the sample.

The extraction efficiency and reproducibility of SPME technique are dependent on several experimental parameters, such as SPME fibre coating, temperature and time of extraction, sample amount, type and uniformity of the sample matrix, chemical composition of the sample, stirring effect, salt addition (salting-out effect), among others (Pawliszyn, 2000; Vas and Vékey, 2004; Weber *et al.*, 1999). Also, SPME fibre coatings may be used hundreds of times without showing any physical degradation. However, a regular control of the fibre performance should be carried out using a chemical standard, and between extractions, and the fibre should be thermally cleaned-up in order to avoid cross-over contaminations.

Several SPME stationary phases are available on the market, with different polarities, film thicknesses (Figure 11c), and types of interaction with the analyte (absorption and adsorption). Regarding sesquiterpenic determination, 5 types of SPME fibre coatings have been used (Table 1, Figure 11c): PDMS, PA, PDMS/DVB, CW/DVB, and DVB/CAR/PDMS. The PDMS/DVB, CW/DVB, and DVB/CAR/PDMS are produced using two and three different polymers, thus have a synergistic effect between adsorption and absorption phenomena. The mutually synergistic effect of adsorption and absorption of the stationary phases promotes a high retention capacity and, consequently, a higher sensitivity than fibres based on absorption only, such as PA and PDMS. Thus, DVB/CAR/PDMS has been highly used as it presents a wide range capacity of sorbing analytes with different physicochemical properties within a molecular weight ranging from 40 to 275 Da. According to Table 1, PA and, more recently, DVB/CAR/PDMS were the most commonly used SPME fibre coatings for sesquiterpenic compounds extraction and from these DVB/CAR/PDMS presented the higher extraction efficiency. As *V. vinifera* products exhibit sesquiterpenic components with different chemical structures, corresponding to different polarities (Figure 7), fibres containing two or more stationary phases are more efficient. In general, polar fibres should be used for polar analytes and non-polar fibres for non-polar analytes (Vas and Vékey, 2004). As PA is recommended for polar analytes, it not the most appropriate fibre to extract sesquiterpenic compounds. The studies reported using PA (Câmara *et al.*, 2006a; Câmara *et al.*, 2006b, 2007; Câmara *et al.*, 2004; May *et al.*, 2013; May and Wüst, 2012; Schmarr *et al.*, 2010) have been optimized toward the global volatile characterization of grapes, musts, or fortified wines, not specifically for sesquiterpenic compounds, which explains its selection.

The extraction temperature has also a significant effect on the efficiency of the SPME process, because it determines the vapour pressure of analytes and also their solubility on liquid matrices. For *V. vinifera* products, extraction temperatures ranging from 35 to 60 ºC are used (Table 1). For liquid samples, the compromise between the effect on volatility and solubility is determinant to establish the appropriate extraction temperature. Due to the low volatility of sesquiterpenic compounds at room temperature (boiling point: 250 to 280 ºC), higher extraction temperatures should increase their releasing to the headspace. For example, an extraction temperature of 40 ºC provides the higher extraction efficiency than 25 ºC for all the volatile components of Fernão-Pires table wines, including the sesquiterpenic ones (Rocha *et al.*, 2006b). However, extraction temperatures higher than 60 ºC are not recommended for the extraction of natural products, in order to prevent degradation of thermolabile molecules, such as sugars, among others.

Extraction times ranging from 10 min to 2 h have been applied to sesquiterpenic compounds extraction from *V. vinifera* products (Table 1). In order to promote higher transference of the analytes from the sample to the headspace, prior to the introduction of the SPME fibre coating, the sample can be thermostated (30 – 45 ºC) during 5 to 60 min (Table 1). Long extraction times are not convenient for routine laboratory assays, and a compromise between extraction time and maximum extraction efficiency should be established. Lower extraction times (20 min of extraction for wines) may be obtained when SPME is combined with high sensitive techniques, such as comprehensive two dimensional gas chromatography (Santos *et al.*, 2013), in comparison with one-dimensional gas chromatography (60 min), keeping constant all SPME experimental parameters (Câmara *et al.*, 2004).

For liquid samples, as juice, must, or wine, the effect of stirring and salt addition should also be considered. To increase extraction efficiency and facilitate rapid extraction, some level of agitation (200 to 1250 rpm, using a magnetic stirring bar – Table 1) is applied to transport analytes from the bulk of the solution to the vicinity of the fibre, reducing the effect caused by the depletion zone produced close to the fibre as a result of fluid shielding and decrease analytes diffusion coefficients (Lord and Pawliszyn, 2000). Besides stirring, the addition of salt is also required in order to improve the mass transport of analytes from the aqueous phase to the headspace and, therefore, to the SPME fibre coating, enhancing the extraction efficiency (Alizadeh *et al.*, 2007). NaCl with high purity (≥99%) is commonly used, ranging from 0.125 to 0.300 g mL^{-1} (Table 1). Basically, the addition of salt can change the properties of the boundary phase and decrease the solubility of the compounds in the aqueous phase (salting-out effect). The addition of salt promotes two processes (Lord and Pawliszyn, 2000). Initially, an increase in the extraction efficiency is observed due to the salting-out effect, whereby water molecules form hydration spheres around the ionic salt molecules, reducing the content of water available to dissolve the analyte molecules. This drives additional analytes into the headspace and, consequently, to the SPME fibre coating. At the saturation state, the analytes may participate in electrostatic interactions with the salt ions in solution, reducing their ability to move into the SPME fibre coating and thus decreasing the extraction efficiency (Lord and Pawliszyn, 2000; Melwanki and Fuh, 2008).

Stir bar sorptive extraction – SBSE

SBSE technique is rather straightforward and based on the same principles as SPME, being applied to solid, liquid, and gaseous matrices. In SBSE, a magnetic stir bar coated with a polymeric sorbent is placed in a flask containing the sample and stirred for a pre-determined period of time in order to promote the sorption of the sample analytes into the polymeric material (Baltussen *et al.*, 1999; Serôdio *et al.*, 2007). The stir bar can be put into contact with the liquid sample using either immersion or headspace sampling modes, by suspending the stir bar at the top of the flask, where the polymer is in static contact with the vapour phase from liquid or solid matrices, avoiding therefore possible contamination with non-volatile interferences, as exemplified in Figure 11d (Nogueira, 2012). After the extraction step, the stir bar needs to be removed, rinsed with distilled water for clean-up from any potential interferences (such as sugars or other undesirable sample constituents), dipped on a clean paper tissue to remove water, and submitted to the back-extraction process through liquid desorption (LD) using an appropriate solvent or by a thermal desorption unit (TD). However this later tool needs a specific unit for the back-out operation (up to 350 ºC), making this unit very expensive (Nogueira, 2012). SBSE with non-polar PDMS combined with TD or LD has been applied to grapes (Salinas *et al.*, 2004) and table, fortified and sparkling wines (Alves *et al.*, 2005; Coelho *et al.*, 2009; Coelho *et al.*, 2008) (Table 1).

Although the magnetic stir bar is the most used configuration (Figure 11d) there are also other SBSE configurations, such as a set of spheres coated with a sorbent stationary phase. PDMS, a non-polar coating, has turn the most used sorbent phase, especially for non-polar analytes (i.e. sesquiterpenic compounds), but for analysis that requires the extraction of the more polar analytes, it may represent a drawback. Thus, other strategies need to be performed, as for example multi-mode assays, derivatization procedures, other polymeric phases or alternative sorption-based approaches (Nogueira, 2012).

As in SPME, there are several parameters that influence the SBSE extraction process, namely the extraction time, stirring, salt addition, sample and stationary phase volumes, and analyte polarity, among others. Some of these variables affect the extraction kinetics (e.g. time and stirring), as well as the interaction among the analytes and the polymeric phase (e.g. analyte polarity) (Coelho *et al.*, 2008; Nogueira, 2012). For sesquiterpenic compounds determination, SBSE extraction times ranging from 10 min to 6h have been used, at temperatures from 20 to 30 ºC, and stirring from 500 to 800 rpm (Alves *et al.*, 2005; Coelho *et al.*, 2009; Coelho *et al.*, 2008; Salinas *et al.*, 2004), which was then followed by back-extraction with pentane for 15 min (Coelho *et al.*, 2009; Coelho *et al.*, 2008) or by desorption in a thermal unit (Alves *et al.*, 2005; Salinas *et al.*, 2004) (Table 1).

The advantage of SBSE over SPME is the relatively high content of polymeric sorbent phase. For instance, SBSE contains 24 to 126 μL of PDMS coating whereas SPME fibre coating comprises only up to 0.5 μL for a 100 μm film thickness. The lower phase ratio for SBSE between the sorbent phase and the sample medium allows the increase of its sensitivity and sorption capacity, especially of the non-polar compounds (as sesquiterpenic ones) on a factor of 50 to 250 times in comparison to SPME (Alves *et al.*, 2005; Marin *et al.*, 2005; Nogueira, 2012; Zalacain *et al.*, 2007). Although this higher sorption capacity of SBSE for the determination of trace analytes, this is not a determinant condition to select SBSE over SPME, in several cases, the higher recovery of the major analytes leads to overloaded chromatograms with several co-eluted peaks (Demyttenaere *et al.*, 2003), neglecting the trace analytes, including the sesquiterpenic ones.

The stir bar, as the SPME fibres, may also be used hundreds of times without showing any physical degradation of the PDMS coating material. However, before being reused, the stir bars should be cleaned-up with suitable solvents or through TD treatments in order to avoid any cross-over contamination (Nogueira, 2012). This technique is particularly interesting for liquid samples, as the implementation of the HS mode for solid sample analysis is not readily feasible.

I. 5.4. Gas chromatographic analysis

After extraction, the analytes are usually analysed using gas chromatographic techniques. One-dimensional gas chromatography (1D-GC) was widely used for sesquiterpenic compounds determination from *V. vinifera* related products (Álvarez *et al.*, 2011; Alves *et al.*, 2005; Bueno *et al.*, 2006; Câmara *et al.*, 2006a; Câmara *et al.*, 2006b, 2007; Câmara *et al.*, 2004; Coelho *et al.*, 2009; Coelho *et al.*, 2008; Coelho *et al.*, 2006; González-Álvarez *et al.*, 2012; Kalua and Boss, 2009, 2010; Keyzers and Boss, 2010; López-Vázquez *et al.*, 2010; Lukić *et al.*, 2010; May *et al.*, 2013; May and Wüst, 2012; Oliva *et al.*, 1999; Parker *et al.*, 2007; Perestrelo *et al.*, 2011; Perestrelo *et al.*, 2006; Piombino *et al.*, 2010; Rocha *et al.*, 2006a; Rocha *et al.*, 2000; Rocha *et al.*, 2006b; Ruberto *et al.*, 2008; Salinas *et al.*, 2004; Schreier *et al.*, 1976; Siebert *et al.*, 2008; Tao *et al.*, 2008; Versini *et al.*, 1994). Over the last decades, several improvements have been done on the development of chromatographic equipments and software allowing in-depth samples characterization, improving the limits of detection, chromatographic resolution, and reducing the time of analysis and data processing. Thus, more recently, a high sensitive and high throughput technique, the comprehensive two-dimensional gas chromatography (GC×GC), has also been applied for *V. vinifera* sesquiterpenic characterization (Robinson *et al.*, 2011; Schmarr *et al.*, 2010; Welke *et al.*, 2012). A global volatile profiling is established estimating the relative content of each analyte based on its chromatographic area. From these, few studies comprised quantitative analyses (Lukić *et al.*, 2010; Ruberto *et al.*, 2008; Siebert *et al.*, 2008). Actually, metabolite profiling using multivariate analysis has attracted the interest of several researchers as it is a fast, convenient, and effective tool that may be used to compare groups of samples and to extract significant differences between them.

I. 5.4.1. One-dimensional gas chromatography

Gas chromatography separation is based on the partition of the analytes between two immiscible phases, where a mobile phase (for example, helium, hydrogen, or nitrogen) is flowing through a stationary one (column). The elution of analytes in the column is according to both dispersion and specific interactions between analytes and column, allowing that compounds of different polarities to be spread over the total elution space. For the generic cases, the retention of analytes on each column may be classified according to the mechanism of retention (Marriott *et al.*, 2004).

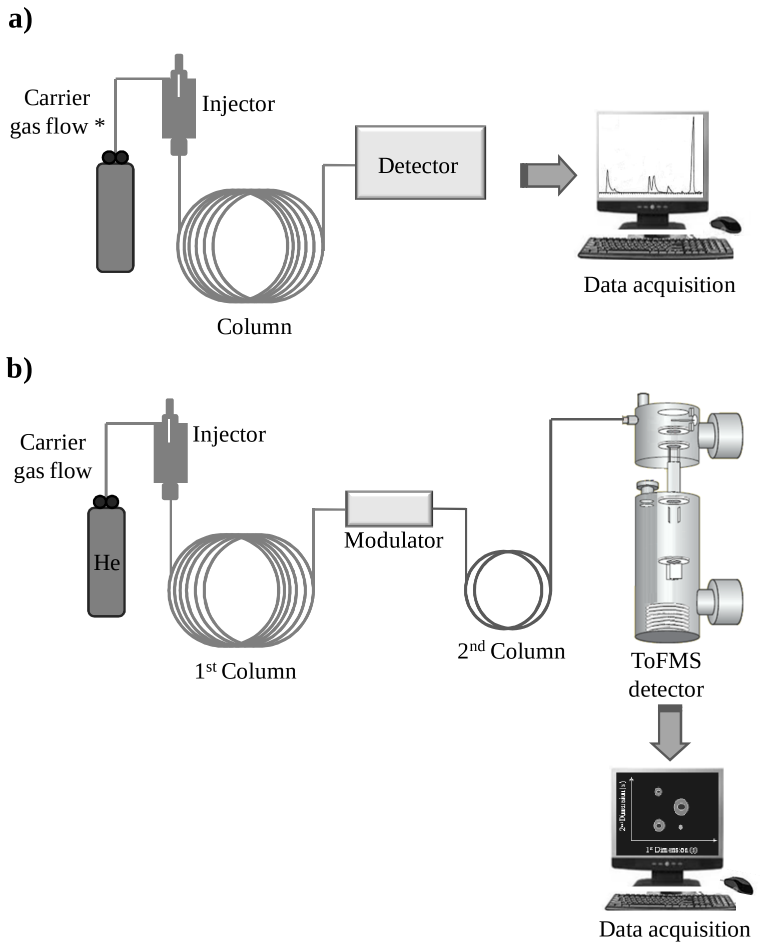


Figure 12. Schematic representation of a) one-dimensional gas chromatographic system (1D-GC), and b) comprehensive two-dimensional gas chromatographic system coupled to mass spectrometry with a high resolution time of flight analyzer (GC×GC-ToFMS). * Depending on the 1D-GC detector type, several carrier gases may be used, such as nitrogen, helium, or hydrogen, among others.

Figure 12a shows a schematic representation of one-dimensional gas chromatographic system (1D-GC). The chromatographic column is connected to an injection system and, on the other side, to a detector. Several studies conducted to perform the volatile profiling, including the sesquiterpenic compounds, used polar polyethylene glycol columns (Lukić *et al.*, 2010; Perestrelo *et al.*, 2011). However, non-polar columns such as those commonly composed with 5% phenyl polysilphenylene-siloxane or equivalents are recommended for sesquiterpenic compounds targeted GC analysis. The plot resultant of the registration of the detector response *versus* time of retention is the chromatogram (Figure 12a). Although gas chromatographs may be equipped with several types of detectors, flame ionization and mass spectrometry (MS) detectors are those commonly used. The flame ionization detectors (FID) are a quite cheaper option when compared to MS detectors and are widely used to targeted analyses for which the standards should be available, and the required limits of detection and quantification are not very low (≥ μg L^{-1}). The most reliable way to confirm the identification of each compound is based on authentic standard co-injection, which in several cases is economically prohibitive, and often unachievable in the time available for analysis, or because standards are not commercially available. Thus, the MS detectors and the commercial mass spectra databases represent a crucial tool to tentatively assess analytes identification. Currently, GC–MS equipments already have suitable and robust software algorithms that make easier the data processing (Dettmer *et al.*, 2007).

MS analysis may be operating in several acquisition or processing modes. Full-scan mode (scanning in a *m/z* range) is a current acquisition mode that may promote frequent co-elutions, specially for complex samples. To overcome this drawback, some strategies may be improved, namely the use of single ion monitoring mode (SIM – MS acquisition using *m/z* diagnostic ions), and ion extraction chromatography (IEC – data processing using specific *m/z* diagnostic ions) mode, that increase the specificity and sensitivity, thus minimizing the contribution of co-eluted compounds and increasing the peak area of the target analyte(s) (Coelho *et al.*, 2009; Rocha *et al.*, 2012; Tranchida *et al.*, 2013). IEC has the advantage to simultaneously obtaining improved data about the target analyte (i.e. sesquiterpenic compounds by using selected ions) plus data about the global volatile profile (full-scan acquisition data), that can be further explored for in-depth analysis of sample.

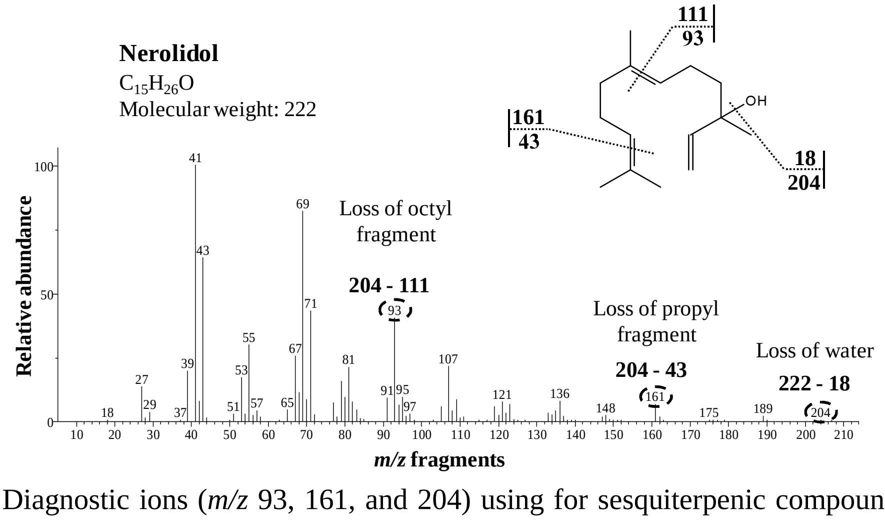


Figure 13. Diagnostic ions (*m/z* 93, 161, and 204) using for sesquiterpenic compounds detection, exemplified by the nerolidol mass spectrum.

The determination of sesquiterpenic compounds mass spectra allowed to define a set of *m/z* diagnostic ions: *m/z* 93, 161, and 204. The nerolidol mass spectrum is used in Figure 13 to illustrate the selected ions. The *m/z* ions 41, 69, and 43 correspond to the spectrum dominant peaks, however, as it is also common to MS fragmentation pattern of other frequent chemical families (i.e. alkanes, alkenes, aliphatic ketones, etc), these cannot be used as diagnostic ions of sesquiterpenic compounds. The ion at *m/z* 204 may be used to monitor the presence of oxygen-containing sesquiterpenic compounds. Also, the ion at *m/z* 93 allows the simultaneous detection of monoterpenic compounds (C_{10}) and C_{13} norisoprenoids, which is an advantage on the analysis of natural products due to the relevant and related properties of these metabolites. Further, C_{10} and C_{15} terpenic compounds and C_{13} norisoprenoids appeared on different positions on the chromatogram, thus with no co-elution.

Besides the co-injection of standards and the mass spectrum examination, other strategies may also be performed to improve identification confidence, as for example the retention indices (RI) values. RI may be computed using the van den Dool and Kratz equation (van den Dool and Kratz, 1963), after the injection of a *n*-alkanes series and sample, using the same GC column:

$$RI_X = C_N + 100[(t_{RX}-t_{RN}) / (t_{RN+1} - t_{RN})] \qquad \text{(van den Dool and Kratz, 1963)}$$

where RI_X refers to the retention index of chemical compound “X”; C_N is the number of carbons of *n*-alkane hydrocarbon eluting immediately before chemical compound “X”; t_{RN} and t_{RN+1} are retention times of the reference *n*-alkane hydrocarbons eluting immediately before and after chemical compound “X”; t_{RX} is the retention time of compound “X”.

The experimentally determined RIs can be compared with those reported in the literature or in the freely available open source RI libraries for chromatographic columns similar to the column used in the experimental analysis. While retention times vary with the individual chromatographic system conditions (i.e. column length and diameter, film thickness, carrier gas velocity, and pressure), the derived retention indices are quite independent of these parameters and allow comparing values measured by different analytical laboratories under varying conditions (Zellner *et al.*, 2008). To compute the RI, the retention time of a specific analyte is normalized with the retention times of adjacent eluting *n*-alkanes, thus RI is ruled by the number of carbons in the molecule. The sesquiterpenic RI values may range from ≈ 1300 to 1800 (Jalali *et al.*, 2013). That may be explained by the wide range of C_{15} structures of this chemical family (linear, cyclic, hydrocarbon type, and/or oxygen-containing structures).

Despite 1D-GC processes are widely used, providing rewarding analytical results, the complex nature of natural products requires extended GC runs. Furthermore, in-depth analysis of the chromatograms frequently indicates that some peaks are the result of two or more co-eluting analytes. This is due to the fact that the complexity of the natural products exceeds the capacity of one single separation system, and reliable MS identification is not possible (Rocha *et al.*, 2007a). Thus, in the last decades, 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). Multidimensional gas chromatography, such as comprehensive two-dimensional gas chromatography appeared to be a reliable alternative.

I. 5.4.2. Comprehensive two-dimensional gas chromatography

Multidimensional gas chromatography (MDGC) allows advanced characterization of complex samples due to its separation efficiency, which is unlikely to be accomplished by 1D-GC. MDGC analysis can be defined as ‘‘the process of selecting a (limited) region or zone of eluted compounds issuing from the end of one GC column, and subsequently subjecting the zone to a further GC displacement’’ (Marriott *et al.*, 2012). These approaches have become particularly known for enantioselective analysis, being applied in the determination of monoterpenic compounds in *V. vinifera* foliage (Hampel *et al.*, 2005), grapes (Luan *et al.*, 2004; Luan *et al.*, 2006), and wines (Marriott *et al.*, 2012), and in the determination of sesquiterpenes in plants (König *et al.*, 1999), among others.

An alternative MDGC approach used for sesquiterpenic compounds determination on *V. vinifera* products was comprehensive two-dimensional gas chromatography (GC×GC). The GC×GC system has already been used for the detail characterization of sesquiterpenic components of Cabernet Sauvignon wines from Australia (Robinson *et al.*, 2011), Merlot wines from Brazil (Welke *et al.*, 2012), and Pinot Noir, Cabernet Sauvignon and Dornfelder table wines from Germany (Schmarr *et al.*, 2010). Basically, 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 as, for example, a non-polar (separation ruled by boiling points) and a polar (separation by polarity), connected in series through a special interface, known as modulator (Górecki *et al.*, 2006; Herrero *et al.*, 2009) (Figure 12b). For instance, if a cryomodulator is used, the interface cuts small (several seconds) portions of the ^{1}D (first dimension) eluate by cryofocusing and re-injects them into the second column (^{2}D). Each ^{1}D peak is modulated several times, largely preserving the ^{1}D separation. The ^{2}D is very short and narrow and, consequently, each modulated portion is “flash” separated before the next modulation (Figure 12b). Therefore, GC×GC offers substantial advantages over conventional 1D-GC, namely enhanced resolution, increased peak capacity, faster run times, enhanced mass selectivity and sensitivity, and improved limits of detection due to the focusing of the peak in the modulator (Jalali *et al.*, 2012; Seeley and Seeley, 2013; Song *et al.*, 2004; Souza *et al.*, 2009).

GC×GC may be combined with several types of detectors, namely FID, MS or higher sensitive detectors as time-of-flight mass spectrometry (ToFMS). ToFMS detectors bring several advantages, such as full mass spectra acquisition at trace level sensitivity and mass spectral continuity, which allows for deconvolution of spectra of co-eluted peaks (Górecki *et al.*, 2006). Narrow peaks with width at half height of 0.1 s or less are preferably recorded by using the high data acquisition speed of ToFMS, providing the sufficient data density required for GC×GC separations (Shellie *et al.*, 2001). The analysis of *V. vinifera* cv. Cabernet Sauvignon table wine by GC×GC-ToFMS allowed to identify 22 sesquiterpenic compounds. From these, 10 have never been reported in table wines or even in another *V. vinifera* variety, such as α-alaskene, β-bisabolene, α-curcumene, 4,5,9,10-dehydro-isolongifolene, α-panasinsen, β-sesquiphellandrene, β-vetivenene, cabreuva oxide D, α-bisabolol, and cubenol (Robinson *et al.*, 2011). However, a study performed by GC×GC-MS on table wines belonging to the same variety, only allowed the identification of farnesol and nerolidol (Schmarr *et al.*, 2010). Beyond the fact that SPME experimental parameters were different in both studies, and also the variability of the product should be considered, the different level of detectors sensitivity were determinant for the level of data obtained.

The structured 2D chromatographic space is perhaps the most important underlying property of GC×GC that gives rise to a number of novel capabilities of GC×GC compared with 1D-GC. Essentially, this relates the positions of peaks in the 2D chromatographic space to the chemical properties of the corresponding analytes and, most significantly, the chemically-related analytes have related spatial distributions in the 2D space. This approach simplifies the data obtained, reduces the time of analysis (Cardeal and Marriott, 2009; Coelho *et al.*, 2007; Silva *et al.*, 2010), and represents a helpful tool for more reliable identifications, especially for complex samples when standards are not available. Figure 14 elucidates the structured chromatogram principle.

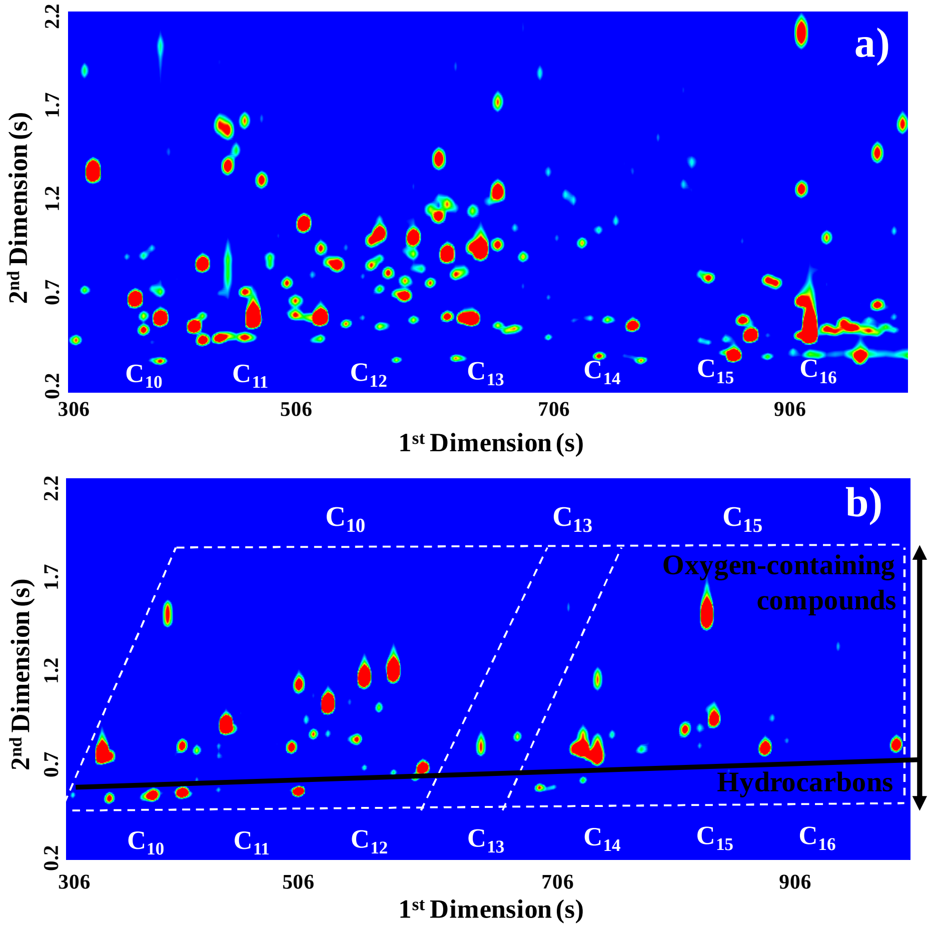


Figure 14. Blow-up of a part of GC×GC-ToFMS chromatogram contour plot obtained for *V. vinifera* grapes a) in full-scan acquisition mode and b) IEC mode of *m/z* 93, 161 and 204. The *n*-alkanes series (C_{10}–C_{16}) was superimposed on the contour plots. The clusters formed by structurally related compounds are indicated.

Chromatograms acquired in full-scan (Figure 14a) and IEC (Figure 14b - *m/z* 93, 161, and 204) modes are shown. IEC mode seems to be very useful to eliminate the majority of non-sesquiterpenic compounds, simplifying the data obtained, reducing the time of analysis, and also allowing the definition of specific 2D chromatographic space containing these compounds (^1t_R – 655 to 1005 s and ^2t_R – 0.5 to 1.7 s). According to Figure 14b, clusters are observed according to the ^{1}D (C_{10}, C_{13}, and C_{15}) ruled by volatility and ^{2}D (hydrocarbons *versus* oxygen-containing compounds) by polarity, as a non-polar thick-film ^{1}D column and a polar ^{2}D column are combined. Thus, two almost independent (orthogonal) separations are provided, allowing the resolution of many peaks that overlapped in the 1D chromatogram. The hydrocarbons are the least polar analytes, showing the lowest retention times for ^{1}D (^1t_R<0.7 s), while oxygen-containing ones (alcohols and ketones), with the highest polarity, present the highest ^{2}Dt_R values (^2t_R range from ca 0.7 to 1.7 s). Further, using this system, compounds with similar volatility may be separated according to their polarity. For instance, Figure 15 shows that propyl dodecanoate, α-calacorene, and 4,5,9,10-dehydro-isolongifolene, presenting similar volatility (^1t_R – 880 s), were separated on ^{2}D (^2t_R – 0.41, 0.49, and 0.60 s, respectively) due to their different polarities.

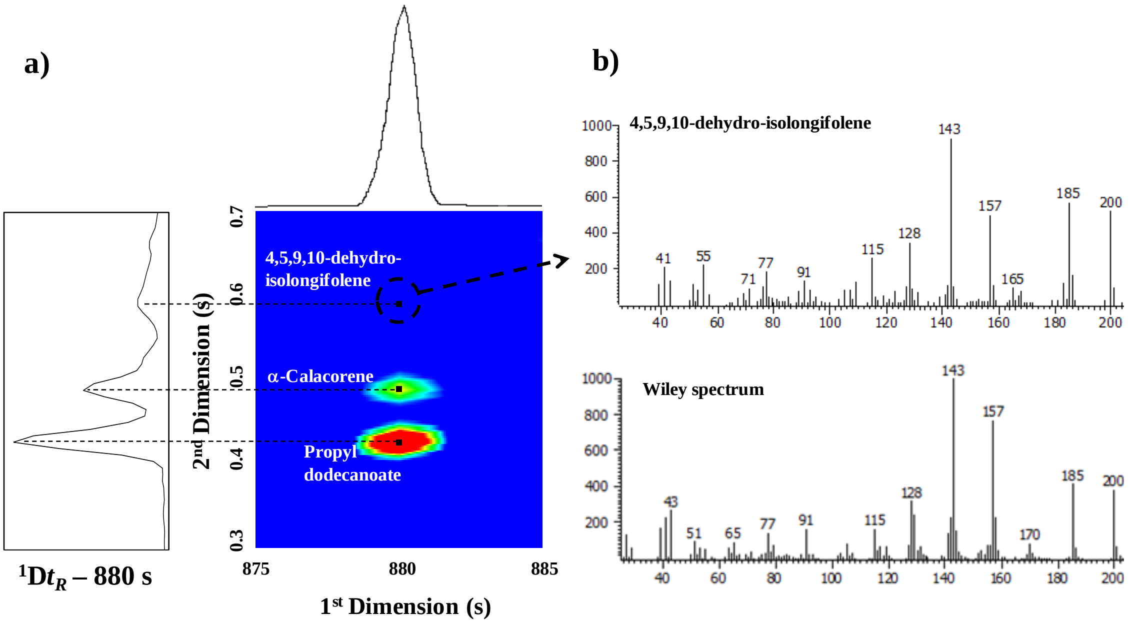


Figure 15. a) Blow-up of a part of total ion GC×GC chromatogram and corresponding contour plots obtained from a table wine, showing propyl dodecanoate, α-calacorene and 4,5,9,10-dehydro-isolongifolene, which are separated on the 2nd Dimension, according to their polarity properties. b) The 100 milliseconds-wide 4,5,9,10-dehydro-isolongifolene (trace wine sesquiterpenic compound) GC×GC peak is easily defined and identified at a mass spectral acquisition of 100 spectra/s, and its spectral quality allows its identification by comparison with mass spectrum of commercial databases (as Wiley database).

Sensitivity and spectral quality are two important issues, especially in the identification and quantification of trace components. During the cryofocusing process in the GC×GC modulator, the analytes fraction that elute from the ^{1}D column are significantly sharpened prior to being released into the ^{2}D column. As a consequence, narrow peaks are obtained (*ca*. 100 ms). However, the spectral quality allows comparison with commercial database, as observed for 4,5,9,10-dehydro-isolongifolene, a trace wine sesquiterpenic component (Figure 15). The integrated usage of several data that can be achieved from GC×GC-ToFMS analysis is an added-value in the analytes identification. For instance, the tentatively identification of 4,5,9,10-dehydro-isolongifolene may be achieved by combination of retention times on ^{1}D and ^{2}D (crucial to estimate its relative volatility and polarity if non-polar/polar set of columns is used), mass spectrum (and similarity with databases), and RI, as exemplified in Table 2.

Table 2. Example of the experimental data that can be achieved from GC×GC-ToFMS analysis.

Name	$^{1}t_R$, $^{2}t_R$^{a} **(s)**	**Formula**	**RI^{b} calc.**	**RI^{c} lit.**	*m/z*^{d}	**Similarity**	**Area**
α-Calacorene	880, 0.49	$C_{15}H_{20}$	1542	1542	157 : 999; 142 : 518; 141 : 354	932	1055387
4,5,9,10-Dehydro-isolongifolene	880, 0.60	$C_{15}H_{20}$	1543	1544	143 : 999; 185 : 756; 157 : 697	916	408414

[a] Retention times for first ($^{1}t_R$) and second ($^{2}t_R$) dimensions in seconds
[b] Retention Index obtained through the modulated chromatogram
[c] Retention Index reported in the literature for 5% phenyl polysilphenylene-siloxane or equivalents (Petronilho *et al.*, 2011; Setkova *et al.*, 2007)
[d] *m/z* : relative abundance

In summary, several pre-treatment procedures and extraction techniques are required to be performed to promote sesquiterpenic compounds enrichment before GC analysis, because these secondary metabolites are commonly present in trace amounts, in a free form or as a glycosidically-linked one. Comparatively to solvent extraction techniques (LLE, SLE and SDE) and SPE, microextraction techniques based on analytes sorptive/adsorptive capacity on polymeric phases (SBSE and SPME) can be a good example of saving preparation steps and time, and also disposal costs, reducing error sources. SPME is one of the most well-established extraction techniques for sesquiterpenic compounds. It integrates sampling, extraction, concentration, and sample introduction into a single solvent-free step, showing reliability in terms of the enrichment capacity, as well as great sensitivity and selectivity. As SPME coatings are mainly hydrophobic, they have high affinity for non-polar compounds such as the sesquiterpenic ones and have low affinity for the more abundant hydrophilic species, which put this technique in unique position to study these analytes.

The 1D-GC is the most used system, which is a cheaper option, having low consumables costs. It is responsible for solving several analytical problems. However, the study of complex matrices or even the research of trace analytes, such as the sesquiterpenic compounds, fully justifies the application of GC×GC chromatographs. Actually, all these equipments offer several tools to make easier the analysis, which includes mass spectra databases and highly developed software algorithms for data processing. GC×GC offers substantial advantages over conventional 1D-GC. However, the structured 2D chromatographic space is perhaps the most important underlying property of GC×GC compared with 1D-GC, as it represents a helpful tool that allows more reliable identifications, especially for complex samples such as natural products. Moreover, as the majority of the standards are not commercially available, it is not realistic to acquire hundreds of standards, and because the sesquiterpenic compounds present a similar mass spectra fragmentation pattern, the structured chromatogram is definitely a relevant add-value. Data processing and identification is a major challenge, particularly when large sample sets are analysed. Few methods have been developed for efficient treatment of large data sets produced by GC×GC-ToFMS, which have been implemented as a freely available open source software package. However, the GC×GC-ToFMS data processing is still undergoing progress, as the actual alternatives are not always able to meet the demands of terpenic determination from natural products, especially when they appear as minor components.

I. 6. Using multivariate analysis in sustainability context

To understand the grape variety oenological potential regarding the region characteristics, and mainly considering the uncontrollable environment conditions, the application of multivariate analysis in order to obtain fast and reliable information that helps the winemaker decision is crucial. Robust multivariate analysis including long term data may represent a key tool for the analysis of emerging policies, price sets, and to reduce the environmental impact on wine quality by implementing adequate winemaking technologies.

Over many years, research has been aimed at developing a simple model or method that could define and predict grape and wine quality. In fact, in the last years, different types of models constructed by application of different multivariate methods have been developed (Due *et al.*, 1993; Marais *et al.*, 2001; Santos *et al.*, 2012; Santos *et al.*, 2011; Urhausen *et al.*, 2011; Valdés-Gómez *et al.*, 2009). Multivariate analysis, machine learning and pattern recognition techniques play an important role in the assessment of the relationships that may occur between several factors such as climate, viticulture techniques, vineyard ecosystem, and grape composition, relevant for the definition of the wine quality. The general approach could include, in one hand, unsupervised studies of the major sources of variability, using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). On the other hand, the use of factors driven methods such as analysis of variance (ANOVA)-PCA, ANOVA-simultaneous component analysis (ASCA), Partial Least Squares-Discriminant Analysis (PLS-DA), and Canonical Correlation Analysis (CCA), could bring out important relationships among measured factors as well as to assess the influence of misleading factors that are pervasive in the analysis of environmental uncontrolled measures.

Some models to predict wine quality based on chemical, climatic and agricultural data are available in literature. A discussion about these examples should be important to highlight their relevance and drawbacks. A model to predict wine quality that correlates microclimatic data (temperature and radiation) with volatile component concentrations and wine sensory parameters of Sauvignon Blanc variety, was developed (Marais *et al.*, 2001). In this model, seventy-two independent data sets consisting of 3 harvests (1997 to 1999), 3 climatically different regions in South Africa, 2 canopy treatments (canopy is a function of different climatic and viticultural factors, which determines the effects of temperature and radiation), and 4 maturation stages, were developed. The microclimatic data within the 2 canopies were recorded as independent variables while the grape and wine measurements such as volatiles (monoterpenes and norisoprenoids) and sensory data (fruitiness and vegetative/asparagus/green pepper intensity) were recorded as dependent variables.

Pearson´s correlation coefficients were calculated between the above mentioned independent and dependent variables (Marais *et al.*, 2001). The model utilises above- and within-canopy radiation and can explain 68.8% of the variation in the cultivar-typical vegetative/asparagus/green pepper intensity of Sauvignon Blanc wine. Other selected example is a study carried out from 2005 to 2008 to calibrate and apply near infrared spectroscopy to assess the spatial behaviour of 3 grape varieties (Cabernet Sauvignon, Syrah and Merlot) quality parameters along the vineyards of Sao Joaquin Valley (USA), and promote differential mechanical harvesting, according to quality zone delineation (Santos *et al.*, 2012). The quality indicators (anthocyanin content, pH, titratable acidity and soluble solids) were subject to geo-spatial modelling, and calibrations were developed using PLS. Subsequently, the data set was utilized to delineate "within-field" grape quality zone and to determine the harvest time. The approach for field prediction of grape quality parameters and zone delineation allowed to distinguish two wines based on their different chemical composition, principally on anthocyanin content (Santos *et al.*, 2012).

The studies reported above and others available in the literature are restricted in space and in the number of parameters evaluated. Consequently, the prediction power of the models is very limited. In the context of sustainability, the ultimate goal of multivariate analysis should be to identify the natural factors, as vineyards and harvest conditions, and their interactions affecting grape composition and quality. In this PhD thesis, ASCA (analysis of variance-simultaneous component analysis) was employed for this purpose. ASCA is a merging of ANOVA and PCA that allows to remove the drawbacks of both methods, namely ANOVA cannot take into account the covariance between different variables and PCA does not take the experimental design into account, which means that the different contributions to the variation caused by the experimental design are confounded in the model (Jansen *et al.*, 2005; Smilde *et al.*, 2005). Thereby, a data analysis method is obtained that takes both the covariance between the multiple variables and the design of the experiment into account (Jansen et al., 2005). This method consists in partitioning the original data matrix into a set of matrices corresponding to the different factors of the experimental design and subsequently subjecting each of these matrices to a PCA. ANOVA-like model is constructed from the PCA models of all effects and interactions. Sub-matrices are defined to have orthogonal column spaces. Therefore, the column spaces of the sub-models fitted on these matrices are also mutually orthogonal.

This allows interpreting all sub-models independently of each other. Since loadings are calculated for each sub-model independently, contribution of the variables to every source of the variation in the data may be identified. For instance, partitioning of original data matrix was done in accordance with the design factors, namely factor “a” and factor “b”, and their interaction “ab”:

$X = X_a + X_b + X_{ab} + E$ (Jansen *et al.*, 2005),

where X_a is a matrix containing variation related to factor “a”, X_b is a matrix containing variation related to factor “b”, X_{ab} is a matrix containing the variation related to the interaction between factors “a” and “b”. After running PCA on each one of those matrices, the following ASCA model is obtained:

$X = T_aP_a' + T_bP_b' + T_{ab}Pa_b' + E$ (Smilde *et al.*, 2005),

where T and P are PCA scores and loadings, respectively, for each of the sub-models, and E is a residual error. Number of significant PCs for each sub-model was selected using cross-validation.

Significance of each factor was assessed using a permutation test (Meyners, 2001; Vis *et al.*, 2007; Westerhuis *et al.*, 2008). Permutation consists in changing randomly the order of the rows in the data set. As a result, in the permuted data set treatment, levels are assigned randomly to the measurements. Permutation test consists in testing the null hypothesis that a given effect is not significant and the respective ASCA sub-model describes noise using quality-of-fit criteria. Data were permuted 2000 times and the percentage of the variance explained by each sub-model in the total model was used as quality-of-fit criterion. Variance explained by each sub-model in the total model was calculated for each sub-model using the formula:

$$E_{a,tot}(\%) = \left(1 - \frac{\sum_m \sum_n (X - T_a\, P'_a)^2}{\sum_m \sum_n X^2}\right) \times 100\%$$

(Smilde *et al.*, 2005),

where $E_{a,tot}$ is the percentage of explained variance of the sub-model “a” in the total model, T_a and P_a are the scores and loadings for the sub-model “a”, X is an original data matrix, and “m” and “n” are number of variables and samples in the data set, respectively.

ASCA is particularly helpful for analysis of the effects in the designed data sets where large number of variables have been measured in a set of samples. This method was already applied to evaluate the effects of pressure (250–650 MPa) and pressure holding time (15–120 min) on phenolic content of Nero D'avola Syrah red wine. The results of ASCA indicated that both factors significantly ($p < 0.05$) affect the whole data set about wine quality parameters whereas their interaction was insignificant (Tao *et al.*, 2013). Furthermore, the effects of age and variety on the electronic tongue response and wine composition with respect to the organic acids, phenolics and furanic derivatives, by using HPLC, were evaluated. ASCA revealed that effects of age, grape variety and their interaction were significant ($p < 0.05$) for the HPLC data set and only the effect of age was significant for the electronic tongue data (Rudnitskaya *et al.*, 2010).

Chapter II - Grape sampling strategy

Grape sampling strategy

Overview

A three years sampling plan was performed to evaluate the effect of different vineyard parcels characteristics and harvest year conditions on the variety oenological potential. For this, 3 *V. vinifera* white varieties (Arinto, Bical, and Sauvignon Blanc) and 4 red varieties (Baga, Castelão, Touriga Nacional, and Sousão) were selected, each one collected during maturation, in 3 consecutive harvests (2010 to 2012), and obtained from vineyard parcels with different edaphoclimatic characteristics (3 parcels for each variety). Several physicochemical parameters were used to evaluate each variety oenological potential during maturation: berry weight, pH, titratable acidity, sugar and phenolic contents, antiradical activity, and volatile composition (free fraction). Besides these parameters, at maturity, both free volatile fraction and the glycosidically-linked fraction were considered. This was performed to grape varieties obtained from Bairrada Appellation.

II. 1. Framework

In viticulture context, a sampling strategy comprises the establishment of several parameters, namely, the number of samples, the sampling locations, the time of sampling, the vineyard characteristics and the selection of grape varieties (Totaro *et al.*, 2013). As a complex network of variables are known to influence grapes composition, and thus, wine quality (Figure 2), in order to evaluate vineyard parcel characteristics and harvest year conditions effects on the variety oenological potential, a sampling strategy was defined, where several parameters were selected (Figure 16). Therefore, a 3 years sampling plan was performed considering a total of 19 parcels into two vineyards of Manuel dos Santos Campolargo Herdeiros company, in Bairrada Appellation. For each variety, three vineyard parcels with different edaphoclimatic conditions (soil type, altitude, rows orientation, and sunlight exposure) were selected and marked in order to collect samples from the same parcel during the 3 harvest years (from 2010 to 2012). Grapes were collected randomly throughout each parcel along their maturation. As vineyard parcels characteristics and harvest year conditions influenced the variety oenological potential, and thus wine quality (Figure 16), several physicochemical parameters were evaluated during maturity: berry weight, pH, titratable acidity, sugar and phenolic contents, antiradical activity, and volatile composition (free fraction). Special attention was devoted to grapes at maturity since the glycosidically-linked fraction was also considered. Seven *V. vinifera* varieties (Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional and Sousão) from Bairrada Appellation, were considered.

The present Chapter aims to give the details related to the sampling strategy implemented to evaluate the effects of parcel characteristics and harvest year conditions on each variety oenological potential.

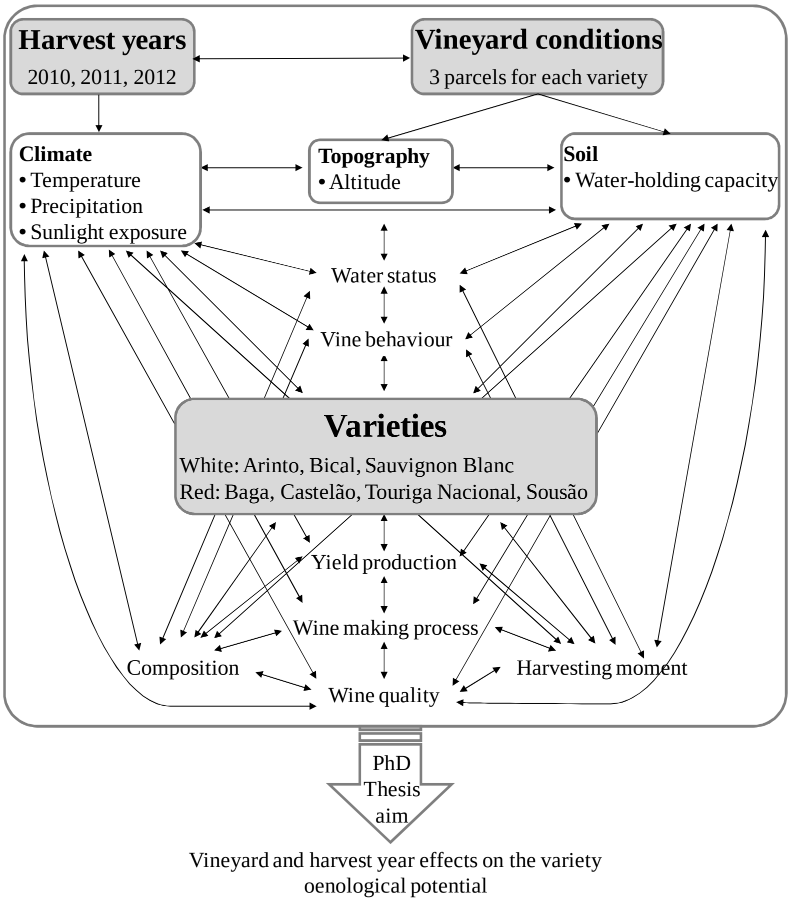


Figure 16. Influence of harvest year and vineyard parcel characteristics on each variety oenological potential.

II. 2. Varieties under study

From the 26 varieties recommended and authorized for controlled quality wines (CQW Bairrada) of Bairrada Appellation (Decreto-Lei nº 301/2003), 3 white varieties (Arinto, Bical, and Sauvignon Blanc) and 4 red ones (Baga, Castelão, Touriga Nacional, and Sousão) were selected. The selection of these varieties was made based on the representativeness of the varieties to Bairrada Appellation and also on the interests of Manuel dos Santos Campolargo Herdeiros company: these varieties are recommended for QWPSR of Bairrada, Baga is the most cultivated variety in this Appellation and represents 90% of the total red Bairrada vineyard. Also, Arinto and Bical varieties represent 20% (10% for each one) of the total white vineyard. Sauvignon Blanc is well appreciated in this Appellation due to its pleasant aroma sensory properties. On the other hand, Sousão is used as "teinturier" to blend with other varieties (poor in colour) to give colour to the resulting wines. Castelão and Touriga Nacional are well appreciated for their aroma characteristics.

The morphological aspects and particularities of the 7 *V. vinifera* varieties under study are described as following (adapted from www.cvbairrada.pt and www.vinetowinecircle.com):

Arinto

Morphologic aspects:

The leaves are very large and plane exhibiting salient and straight "teeth". Their bunch size is large, conical with several wings, and highly compacted, presenting a medium to large length peduncle. The berry is small to medium size, with elliptical shape, and a green-yellow colour.

Particularities:

This variety has the ability to adapt to different climates being widespread in most wine regions, from North to South of Portugal. It has a late maturation being resistant to year climatic conditions. The aroma is mildly intense showing essentially fruity (tree fruits) notes. The resulting wines have an interesting aroma development in its first year of life, preserving this aroma complexity for several years.

Bical

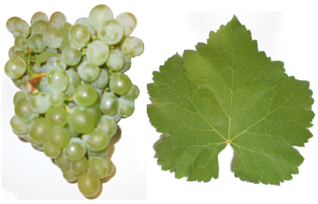
Morphologic aspects:

The leaves have a medium size, clear green colour and the sides slightly curve to down. Their bunch has a medium size and is low compacted, presenting a medium length peduncle. The berry is of medium size, with an elliptical shape, and a green-yellow colour with black points.

Particularities:

Bical is highly sensitive to year climatic conditions. This is a variety resistant to rot but particularly sensitive to oidium. Bical allowed obtaining wines with serene aromas being essentially fruity.

Sauvignon Blanc



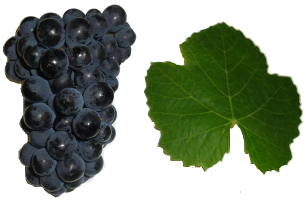
Morphologic aspects:

The leaves have a medium size and a clear green colour. Their bunch has a small/medium size and is low compacted. The berry is of small/medium size, with a round shape, and a green-yellow colour.

Particularities:

This variety presents a short cycle with an early maturation. Sauvignon Blanc adapts readily to all kinds of growing conditions. It presents exuberant aroma sensory properties. It is characterized as displaying vibrant citrus, tree and tropical fruit characters with some herbaceous notes.

Baga



Morphologic aspects:

The leaves have a medium size, with short convex teeth and medium to dark green colour. Their bunch has a small/medium size, conical shape, and is little to medium compacted, presenting medium length peduncle. The berry is of medium size, with a round shape, and a blue-dark colour.

Particularities:

This variety has a late maturation. Baga strongly reacts to year climatic conditions, thus the production results vary from one year to another. It is resistant to oidium, however maturation problems may occur with September rainfall originating grapes susceptible to berry rot. Baga adapts well to soils with clay in its texture, with high humidity, and requires good sunlight exposure for a long growth cycle. Baga wines have different alcohol and aroma characteristics, which are dependent on the year climatic conditions. Resulting Baga wines have a grenade colour, a fruity aroma, and have a good ageing potential.

Castelão

Morphologic aspects:

The leaves have a medium size, five lobes, irregular shape, medium to large convex teeth, and green colour with a slight wrinkling. Their bunch has a medium size, is conical-winged, and is medium compacted, presenting a short peduncle. The berry is of medium to large size, with a round shape, and a blue-dark colour.

Particularities:

Castelão has an early maturation when compared with the other red varieties. It is low sensitive to rot during maturation process, but sensitive during flowering which rot attacks the bunch peduncle. This red variety is well adapted to climate with maritime influence. Wines mainly produced from the Castelão grapes have a grenade colour and an intense and fruity aroma with notes of red/wild fruits. They have a good ageing potential.

Touriga Nacional

Morphologic aspects:

The leaves have a small size, five lobes with rectilinear short teeth, and green colour. Their bunch has a small size, with cylindrical to conical shape, and is moderately compacted, presenting a medium length peduncle. The berry is of medium size, with a slightly flattened shape, and a blue-dark colour.

Particularities:

This variety has a late maturation and requires good sunlight exposure for a long period. This is a variety that resists to different years climatic conditions, however it is very sensitive to excessive hot summers. This variety has intense colour and aroma giving it a high degree of complexity. Touriga Nacional presents fruity and floral aromas, being related with red and wild fruits and violets combined with some toasted notes. Also, Touriga Nacional originates wines full of colour. They have a good ageing potential.

Sousão

Morphologic aspects:

The leaves have a medium size, three to five lobes with a striate profile, and green colour. Their bunch has small to medium size, with cylindrical to conical shape and is compacted. The berry is of small/medium size, uniform, round shape, and with a blue-black colour.

Particularities:

Sousão presents a late maturation. This variety is resistant to wind being well adapted to vineyards at open spaces. Resulting wines have a ruby colour with weak aromas, and a light herbal taste. In Bairrada, this red variety is used as a teinturier. Sousão is commonly used to blend with varieties lacking colour but which have a good aroma potential.

II. 3. Vineyards characteristics

Several studies showed that vineyard characteristics influence grapes, and consequently, wine composition and quality. For instance, soil type is highly related to the water status through its water-holding capacity (Oliveira *et al.*, 2004; van Leeuwen *et al.*, 2004), while altitude are directly associated to the resulting temperature, humidity, vineyards surrounding vegetation height, among others (Jackson and Lombard, 1993) (Figure 16).

The soil in Bairrada Appellation has some heterogeneity in its texture, thus it is possible to find vineyard parcels in 3 soil types: i) clayey; ii) clay-calcareous; and iii) clay-sandy soils. Clayey soils are composed by microscopic size particles (< 0.002 mm). It has good water-holding capacity, volumetric wetness, and are poor drained. In opposition, sand particles are large (0.05 to 2 mm) with bigger spaces (macro pores) which facilitate rapid movement of air and water (Plaster, 2013). Thus, clay-sandy soils have lower water-holding capacity and dry out more quickly when compared with clayey soils. The clay-calcareous soils have particles with intermediate size between clay-sandy and clayey soils. Clay-calcareous soils can retain more water amount than clay-sandy and have better drainage capacity than clayey soils.

Besides differences in soil type, Bairrada Appellation also presents slight differences in altitude: from ca. 50 m to 90 m. Altitude is highly related with climatic conditions: lower altitudes are associated with higher temperatures and lower humidity, which influence grape maturation, and thus its composition. Also different sunlight exposures can be found in this Appellation (from West and also from both South and West). It is already known that grapes with higher sunlight exposure, during maturation, improves grape and thus wine composition, namely increasing the content of total phenolics (Dokoozlian and Kliewer, 1996; Spayd et al., 2002; Song et al., 2015) and increasing the level of both free and glycosylated terpenic compounds (Belancic et al., 1997; Reynolds and Wardle, 1989a; Reynolds et al., 1996a; Reynolds et al., 1996b).

In this PhD thesis, grapes from two vineyards belonging to Manuel dos Santos Campolargo Herdeiros company, at Bairrada Appellation, were considered: São Mateus (SM) and Vale de Azar (VA) (Figure 17, Table 3).

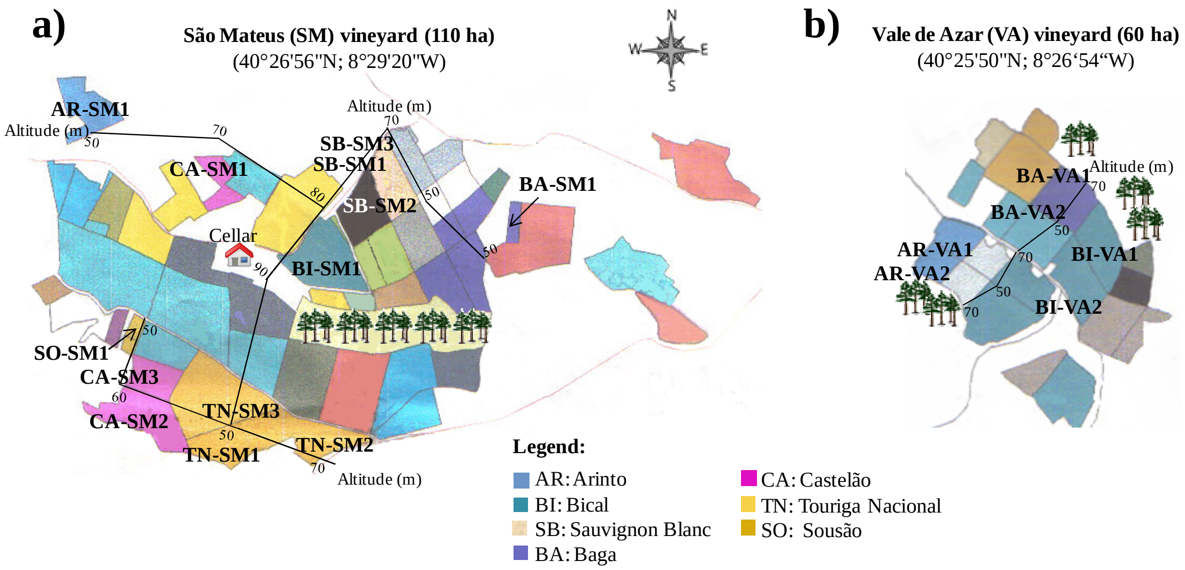


Figure 17. The vineyards of **a)** São Mateus (SM) and **b)** Vale de Azar (VA) are located in *Manuel dos Santos Campolargo Herdeiros* company, in Bairrada Appellation. Varieties (*Vitis vinifera* L cv. Arinto (AR), Bical (BI), Sauvignon Blanc (SB), Baga (BA), Castelão (CA), Touriga Nacional (TN) and Sousão (SO)), altitude (ca. 50 to 90 m), vineyard parcels (SM1 to SM3 and VA1 and VA2), are indicated.

Table 3. Main characteristics of the parcels of *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional and Sousão are identified.

Varieties	Vineyard parcels characteristics
White varieties	
Arinto	**AR-VA1** – clayey soil, 50 m of altitude, open space, and rows guided North-South direction, sunlight exposure from West
	AR-VA2 – clay-sandy soil, 70 m of altitude, near to pine trees, rows guided North-South direction, sunlight exposure from West
	AR-SM1 – clay-calcareous soil, 50 m of altitude, open space, rows guided East-West direction, sunlight from South and West
Bical	**BI-VA1** – clayey soil, 70 m of altitude, near to pine trees, rows guided North-South direction, sunlight from West
	BI-VA2 – clay-calcareous soil, 70 m of altitude, open space, rows guided North-South direction, sunlight from West
	BI-SM1 – clay-sandy soil, 90 m of altitude, open space, rows guided East-West direction, sunlight from South and West
Sauvignon Blanc	**SB-SM1** – clayey soil, 70 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	SB-SM2 – clay-calcareous soil, 50 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	SB-SM3 – clay-sandy soil, 70 m of altitude, open space, rows guided North-South direction, sunlight from South and West
Red varieties	
Baga	**BA-VA1** – clayey soil, 70 m of altitude, near to pine trees, rows guided North-South direction, sunlight from South and West
	BA-VA2 – clay-calcareous soil, 50 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	BA-SM1 – clay-sandy soil, 50 m of altitude, open space, rows guided North-South direction, sunlight from South and West
Castelão	**CA-SM1** – clay-calcareous soil, 70 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	CA-SM2 – clayey soil, 60 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	CA-SM3 – clay-sandy soil, 60 m of altitude, open space, rows guided North-South direction, sunlight from South and West
Touriga Nacional	**TN-SM1** – clayey soil, 50 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	TN-SM2 – clay-calcareous soil, 70 m of altitude, open space, rows guided North-South direction, sunlight from South and West
	TN-SM3 – clay-sandy soil, 50 m of altitude, open space, rows guided North-South direction, sunlight from South and West
Sousão	**SO-SM1** – clay-sandy soil, 50 m of altitude, open space, rows guided North-South direction, sunlight from South and West

São Mateus vineyard, with 110 ha, occupy the South and North side of a hill that starts in Mogofores (East) and goes to Paredes do Bairro (West), being at ca. 25 km from the Atlantic Ocean. This vineyard has a greater extension in the East to West direction and lower from North to South. The cellar of this company is located in São Mateus vineyard, at ca. 90 m of altitude (Figure 17a). At 3 km from the cellar is located the Vale de Azar vineyard. This vineyard is located in a valley and is smaller (60 ha) when compared to São Mateus vineyard. Vale de Azar vineyard shows a more homogeneous configuration but more extended from North to South direction (Figure 17b).

In these two vineyards are planted several parcels of different grape varieties. From the varieties under study, in São Mateus are planted 1 parcel of Arinto, 1 of Bical, 1 of Baga, 3 parcels of Sauvignon Blanc, 3 of Castelão, 3 of Touriga Nacional, and also 1 of Sousão. In São Mateus vineyard the soil texture of the different parcels is diverse with frequent alternation of soils typically clay-calcareous with clayey ones and also with clay-sandy soils. In Vale de Azar are planted 2 parcels of Arinto, 2 of Bical, and 2 of Baga (Figure 17), and the soil is essentially clayey and clay-calcareous, with only one parcel with clay-sandy soil (AR-VA2). Slightly differences in parcels altitudes were observed varying from ca. 50 to 90 m in São Mateus vineyard and ca. 50 to 70 m in Vale de Azar vineyard (Table 3, Figure 17). The cultivation of these varieties on each vineyard results from a continuous learning process of this company, over several decades, that allowed understanding how each variety interacts with the vineyard conditions. These varieties are already well adapted to the different conditions of each vineyard however this only result from the empirical knowledge and the objective influence of the different parcel characteristics in the composition of each variety is still unknown. Thus, it is essential to evaluate vineyard parcels conditions effect on each variety oenological potential.

According to Table 3, a set of 3 parcels with different characteristics was considered for each variety (with the exception of Sousão where only one parcel was considered), where the higher differences between them is soil type. Arinto parcels present slight differences in altitude (ca. 50 to 70 m) and one parcel is located in the top West of São Mateus vineyard (AR-SM1) and the other two are located in Vale de Azar vineyard (AR-VA1 and AR-VA2) and. AR-VA2 has clay-sandy soil and is near to pine trees while the other 2 parcels, AR-VA1 and AR-SM1, with clayey and clay-calcareous soils respectively, are in open spaces. However, no influence in sunlight on grapes from AR-

VA2 parcel due to pine trees shadow are expected, as these trees are at South of this parcel and the sunlight in Vale de Azar vineyard is essentially from West. Bical parcels are located in São Mateus (BI-SM1) and in Vale de Azar (BI-VA1 and BI-VA2) vineyards. These parcels have slight differences in altitude (ca. 70 to 90 m). BI-VA1 has clayey soil and is near to pine trees while the other 2 parcels, BI-VA2 and BI-SM1, with clay-calcareous and clay-sandy soils, respectively, are both in open spaces. The pine trees are at East of BI-VA1 parcel and thus may influence the sunlight on this parcel in the first hours of the morning. Sauvignon Blanc parcels are all located in São Mateus vineyard and are in open spaces, presenting differences in soils type and slight differences in altitude: SB-SM1 and SB-SM3 with clayey and clay-sandy soils are both at ca. 70 m, while SB-SM2 with clay-calcareous soil is at 50 m. One Baga parcel is located in São Mateus (BA-SM1) vineyard and two Baga parcels are located in Vale de Azar vineyard (BA-VA1 and BA-VA2). BA-VA1 has clayey soil, is at ca. 70 m, and is near to pine trees while the other 2 parcels, BA-VA2 and BA-SM1, with clay-calcareous and clay-sandy soils, respectively, are both at 50 m and in open spaces. Similar with Bical, the presence of pine trees at East may cause shadow early in the morning, influencing the sunlight on this parcel (BA-VA1). Castelão parcels are located in São Mateus vineyard, at ca. 60-70 m and in opens spaces. Thus the higher difference between them is related with soil type: CA-SM1, CA-SM2, and CA-SM3 have clay-calcareous, clayey and clay-sandy soils, respectively. The 3 parcels of Touriga Nacional are all located in open spaces in São Mateus vineyard, being the higher differences between them related with soil type and with slight differences in altitude: TN-SM2 parcel is at 70 m and has clay-calcareous soil, while TN-SM1 and TN-SM3 parcels are both at 50 m and have clayey and clay-sandy soils, respectively (Figure 17, Table 3). Sousão variety was only collected in one vineyard parcel (SO-SM1) with 14 years, characterized for presenting clay-sandy soil, at 50 m, and in an open space (Figure 17a, Table 3).

Different parcel rows orientations were also considered (Table 3). Globally, parcels were guided from North to South direction, with the exception of AR-SM1 parcel of Arinto and BI-SM1 parcel of Bical varieties, which rows were guided from East to West direction. In rows oriented North-South direction, the vineyard parcels tend to have higher sunlight exposed grapes when compared to East-West rows.

In addition to vineyard parcel characteristics, the grapes and wines quality also depends on several agricultural practices (Figure 2) which the winemakers can act on. This includes the use of several viticulture management practices, namely irrigation, and thinning (described as the suppression of leaves, flowers or clusters before full maturation) (Pastore *et al.*, 2011). Moreover, the addition of any bulk material (mulches) placed on the soil surface to control weeds and/or preserve moisture can also be used (Mundy and Agnew, 2001). Grapevine orientation in space through the trellising system needs to be adapted to the local climatic conditions in order to: i) optimize the utilization of sunlight and promote productivity, ii) adapt to the characteristics of the grape cultivar, iii) promote efficient and sustainable vineyard management practices, and iv) be economically feasible to establish and to maintain (Palliotti, 2012). In this PhD thesis, the influence of agricultural practices was not considered because similar agricultural conditions were used for all parcels under study: they were not irrigated, thinning was not performed, and also no kind of mulches was added to the soil. Furthermore, the bilateral cordon trellising system (two cordons are fastened to the top wire at 1.6 m and three to five-bud canes plus spurs totalling 20 buds left on each side) was used in all the parcels under study.

II. 4. Harvest climatic conditions (2010 to 2012): Global view

Harvest climatic conditions influence grapes development, and thus their composition (Jones *et al.*, 2005; Soar *et al.*, 2008). Among climate variables, temperature, sunlight exposure, and precipitation amount and the moment it occurs, have a great effect on physiological behaviour of the grapevine and on chemical changes in the berry during its formation and maturation (Holland and Smit, 2010; Jones and Davis, 2000), affecting grapes oenological potential (Figure 16). As uncontrollable climatic conditions varies from one year to another, in order to evaluate the harvest year effect on each variety oenological potential, this PhD thesis was conducted over the 3 consecutive harvest years. The climate of Bairrada Appellation is essentially Atlantic and it may present some Mediterranean characteristics. In a global way, precipitation amount of this Appellation attends its maximum during winter season, at November/December. The occurrence of precipitation in the spring is common until the middle of April or, rarely, until the first days of May. When excessive precipitation was observed, this may condition flowering period and thus maturation process, mainly on the early harvesting varieties. Furthermore, the abundance of rain in the second half of September may also occur, causing rotting whose extent depends on the varieties (adapted from http://www.cvbairrada.pt/).

For the 3 harvests under study were made all the efforts to obtain detailed information related to their climatic conditions however this information is not available for this region. However, according to grapes and wine producer involved in this PhD thesis, from the 3 harvests under study 2010 was considered an equilibrated year, with moderate temperature during spring and also summer, without excessive water stress due to moderate precipitation amount. These moderate temperatures and precipitation amounts were considered suitable for maturation process. On the other hand, 2011 was considered unusually warm, exhibiting a very hot and dry summer with some days of intense heat. The 2012 harvest year exhibited lower temperatures during maturation, being more rainy and fresh. All these different year climatic characteristics may modulate each variety characteristics.

II. 5. Grape sampling

Grape maturation is a very important period that influences the composition of the grapes and, consequently, the wine (Gómez *et al.*, 1995). Thus, the knowledge of the grape composition during maturation offers a means of evaluating the maturity state essentially to determine each grape variety characteristics and, consequently, the wine quality. For this, a sampling strategy was defined based on a previously reported work (Coelho *et al.*, 2006). Healthy-state grapes from the 7 *V. vinifera* varieties under study were weekly collected on the selected parcels, from July to October (Figure 18), from half-*véraison* to maturity and, if the grapes were available, also at post-maturity state. For each variety, ca. 1,000 g of grape berries (for each parcel and per each sampling moment) were picked randomly throughout the parcels, following a z-shaped pattern to avoid edge and centre effects, and taking into account the number of berries per bunch, and the balance between shadow and sun exposure at the different vineyard locations. In the post-maturity state, grapes in a starting rotting stage were observed in all parcels under study. In order to obtain random samples and avoid picking grapes from other parcels, every vine in the parcels was previously marked, and these were kept for the 3 harvest years. Samples were transported immediately under refrigeration (ca. 4 °C) to the laboratory to perform the classical physicochemical parameters determination: berry weight, pH, sugar content, and titratable acidity. Then, samples were stored at -20 °C until further analysis (phenolic content, antiradical activity and volatile composition determination). The sampling period was shown in Figure 18, which ranged from end of July to middle of October.

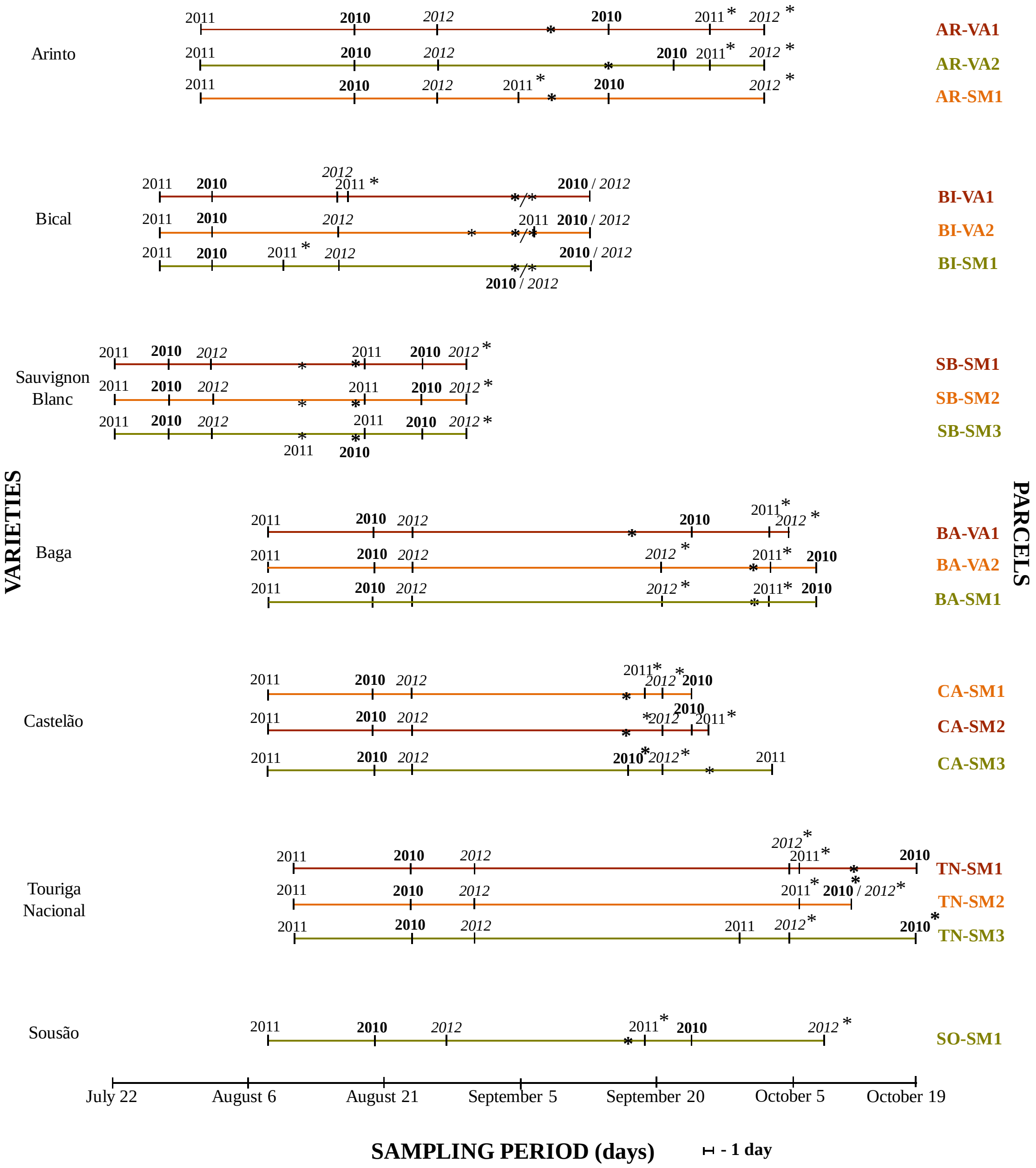


Figure 18. Sampling period performed for the 7 varieties under study, during maturation. For each variety the sampling period was organized for each parcel (SM1 to SM3, VA1 and VA2) where the 3 harvest years (2010 to 2012) were represented. The first point for each variety, indicate grapes collection at half-*véraison* and * refers to technologic maturity state. Different colours indicate the parcel soil type: red refers to clayey soil, orange to clay-calcareous soil, and green to clay-sandy soil.

Considering the white varieties under study, it can be observed that they have different maturation periods: for the 3 harvest years, the first sample collection performed at half-*véraison* on the 3 vineyards was made firstly for Sauvignon Blanc white variety, which has a precocious maturation, followed by Bical variety, and then by Arinto that exhibited a late maturation process. Besides, from the white varieties under study, Arinto also exhibited the longest maturation process (Figure 18). Regarding the red varieties, the first collection performed at half-*véraison*, started later than for the white varieties. From these, Castelão exhibited the smallest maturation process, while Touriga Nacional exhibited the longest one. For each variety, 2011 was the year where the half-*véraison* started earlier due to the higher temperatures of this harvest that allowed to accelerate grapes maturation process, while in 2012 this process started later since this was a cooler and fresh harvest.

II. 6. Parameters used to evaluate grapes oenological potential

During maturation several changes in grape varieties occur, namely berries become sweeter, less acidic, and they develop flavour, aroma and colour properties (Conde *et al.*, 2007). The development of these characteristics is essential to define grapes oenological potential, i.e. to estimate the possibility of their usage to produce wines with specific characteristics.

Figure 19 shows a scheme of the parameters selected to assess oenological potential of each variety. Briefly, i) berry weight is an indicator of berry development evolution during maturation, allowing to assess changes occurred in the berry, as for example berry dehydration; ii) sugar content is an indicator of the alcohol content in the wine (Conde *et al.*, 2007); iii) titratable acidity and pH allow to estimate acidity, and also in indirect way to estimate taste and microbial stability of the resulting wines (Boulton, 1980; Conde *et al.*, 2007); iv) total phenolic content determination allow to estimate colour and astringency of the wines (Conde *et al.*, 2007; Xia *et al.*, 2010); v) the knowledge of the volatile composition (including free and glycosidically-linked fractions) offers a means of evaluating the aroma potential (Ribéreau-Gayon *et al.*, 2000). Furthermore, attending to the interest of antiradical activity in human health, the determination of this parameter was also considered.

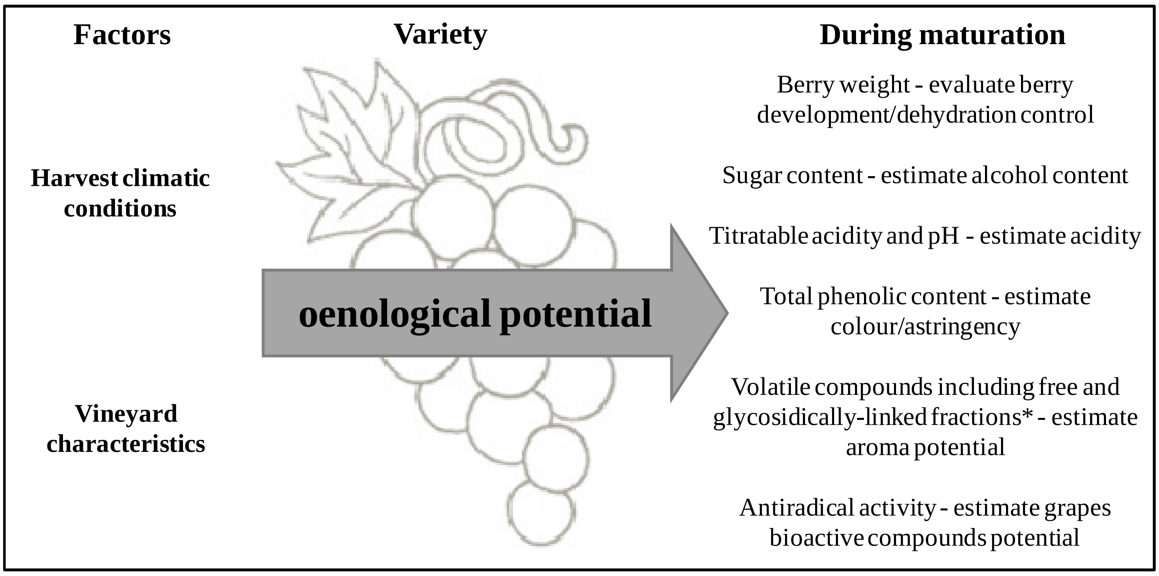


Figure 19. Parameters selected, during maturation, to evaluate harvest climatic and vineyard characteristics effects on grapes oenological potential. * Glycosidically-linked fraction was only determined at technologic maturity.

Chapter III - Evaluation of the impact of harvest and parcel characteristics, during maturation, on grapes oenological potential

Evaluation of the impact of harvest and parcel characteristics, during maturation, on grapes oenological potential

Overview

The evaluation of the impact of harvest year and vineyard parcel characteristics on each variety oenological potential was performed by using data obtained during maturation. Relevant physicochemical parameters that have impact on grapes composition and, consequently, on their oenological potential, were determined. For each variety, obtained from the different conditions under study, evaluation of berry development/ dehydration control, estimation of alcohol content in wine, acidity, colour/astringency, were evaluated in terms of berry weight, pH, titratable acidity, sugar content, and total phenolic content. Besides, antiradical activity determination was also considered as a parameter to valorise each variety oenological potential. Furthermore, the volatile composition of each variety was also determined.

Chapter III was divided in two main sections: Chapter III. 1 - Varieties general physicochemical parameters evaluation and Chapter III. 2 - Varieties volatile profile evaluation.

Chapter III. 1 - Varieties general physicochemical parameters evaluation

III. 1. 1. Framework

Grape maturation is a very important physiological period that starts at *véraison* and lasts when the maturity is reached, until fruit is fully mature. This period is intrinsically associated with vineyards environmental conditions, grape variety, among others, that influences grapes composition and, consequently wines composition and quality (Gómez *et al.*, 1995; Holland and Smit, 2010; Jones and Davis, 2000; Jones *et al.*, 2005).

Grapes maturation stage and also their oenological potential are evaluated by using several parameters. Commonly, winemakers use berry weight, pH, titratable acidity, and sugar content (Crippen and Morrison, 1986; del Llaudy *et al.*, 2008). Berry weight is used as an indicator of the berry development behaviour. During maturation, an increase in berry weight is typically observed, principally due to the increase in water content. Grape berries require a significant amount of water for growth and development, and water typically contributes to 70 to 80% of berry weight at maturity. Prior to *véraison*, most of the water required by the fruit is supplied by the xylem. However, shortly after *véraison* the xylem vessels entering the berry are blocked. With water flow via the xylem disrupted, the phloem becomes the primary supplier of water to the berry. Sugars, minerals, and micronutrients entering the fruit during maturation are also supplied by the phloem (Dokoozlian, 2000). If grapes were not harvested when maturity is established, berry weight starts to diminish and overripe grapes begin to appear. Besides, if during maturation the berry weight starts to decreased this may indicate the onset of dehydration (Ribéreau-Gayon *et al.*, 2000). Titratable acidity and pH are also important indicators of grapes oenological potential since they allow estimating acidity, and consequently taste and microbial stability of the resulting wines. The main organic acids found in mature berries are tartaric and malic acids, making up approximately 90% of total berry acidity. The concentration of these acids reaches their highest levels near *véraison* and then decline through the maturation period, remaining relatively constant near maturity. Once synthesized, tartaric acid is believed to be stable during berry maturation, being the decrease in its concentration attributed to a dilution effect, since water content increases in the berry. In contrast, malic acid is metabolised and used as an energy source during the maturation process. Thus, reduction in malic acid concentration after *véraison* may result from respiration and enzyme degradation, as well as dilution effects (Boulton, 1980; Conde *et al.*, 2007). The determination of pH is a measure of the hydrogen ion concentration in the berry. During maturation, the pH rises gradually and the amount of malic acid in the berry declines. During this period a decrease in free acid and tartaric acid: malic acid ratio was observed resulting in an increase of overall pH (Watson, 2003). Sugar content is also an indicator often used to assess grape maturity. As most of the sugar is fermented to ethanol during the vinification process, the determination of grapes sugar content allows to estimate the alcohol content in the wine (Conde *et al.*, 2007). Sugars accumulate rapidly in berries from *véraison* to maturity. Sucrose is produced in the leaves by photosynthesis and is transported to the berries through the phloem. Within the berries, sucrose is hydrolyzed to glucose and fructose, which are the primary sugars present at maturity. Glucose and fructose are the most abundant sugars, but several other sugars are present including sucrose, rhamnose, and two major pentose sugars, arabinose and xylose (Dokoozlian, 2000).

Phenolic compounds are secondary plant metabolites which total content varied with cultivar, soil type, climate, cultivation practices (Teixeira *et al.*, 2013). Total phenolic compound concentration increases during grapes development however, is followed by a slower accumulation during maturation. The procyanidinic tannins, derived from flavanol polymerization, attain a maximum concentration in the seeds before véraison. This then start to decrease to a lower and relatively stable value when the seeds are mature. At *véraison*, the skin tannin concentration is already high, sometimes corresponding to over half of the concentration at maturity. In white grapes, the concentrations of phenolic acids esterified by tartaric acid, flavan-3-ols and oligomeric procyanidins are high at the beginning of grapes development, and then diminish to low concentrations at maturity. In red varieties, the anthocyanins begin to accumulate in the skins about two weeks before the colour is visible. The concentration increases during maturation, but, as with tannins, it attains a maximum and generally diminishes at the time of maturity. The most abundant phenolic compounds in grapes mainly include tannins, anthocyanins, and flavonols. Considering the oenological potential of phenolic compounds since they contribute to colour, flavour, bitterness, and astringency of the wines, their total content was also evaluated. Besides, phenolic compounds are well known for their antiradical properties (Conde *et al.*, 2007; Xia *et al.*, 2010). Besides, attending to the importance of antiradical activity to human health and also to the fact that grapes are composed by several chemical compounds with this property (such as phenolics), in this PhD thesis this parameter was also considered to evaluate the oenological potential of the different varieties.

The present Chapter aims to evaluate the impact of harvest and vineyard parcel characteristics on each variety oenological potential by using data obtained during maturation. For this berry weight, pH, titratable acidity, sugar and phenolic contents, and antiradical activity, were determined.

III. 1. 2. Material and Methods

III. 1.2.1. Samples

Seven *V. vinifera* grape varieties, each one from 3 parcels, collected during maturation, were considered. The exception was Sousão which was only collected in one parcel. Depending on the variety, the parcel, and the harvest, the sampling moments varied from 3 to 9.

III. 1.2.2. Berry weight, pH, titratable acidity, and sugar content evaluation

The classical physicochemical parameters, as berry weight, pH, alcoholic degree, titratable acidity and sugar content were evaluated, during the sampling period, for the 7 *V. vinifera* varieties under study, in accordance with Regulation (EC) nº 2676/90.

Two hundred grape berries from each variety under study, each one obtained from 3 vineyard parcels, were randomly selected, weighted and used for the determination of pH, sugar content, and titratable acidity. After determining their weight, the other analyses were made by crushing the grape berries, and the obtained juice was separated from skins and seeds. The pH was measured using a pHmeter (micropH 2002, Crison, Barcelona, Spain). Sugar content was established through the determination of alcoholic degree measured using a refractometer (Fabre réfractomètres, Sarl Germain, France). The titratable acidity was measured by titrimetry using NaOH 0.1 M (Panreac, Barcelona, Spain) and Bromothymol blue as indicator. All the analyses were made in triplicate. Data was expressed as mean ± standard deviation (SD) and the reproducibility of the results was expressed as relative standard deviation given in percentage (RSD %). These experiments were performed at the company of Manuel dos Santos Campolargo Herdeiros.

III. 1. 2.3. Total phenolic content evaluation

Grapes total phenolic content was determined by Folin-Ciocalteu colorimetric method (Folin and Ciocalteu, 1927), with gallic acid as a standard. This method is based on the reaction between phenolic compounds of a sample and the redox Folin-Ciocalteu reagent, forming blue reaction products (Blainski *et al.*, 2013; Cicco and Lattanzio, 2011). Briefly, grape berries (ca 100 g for each variety) obtained from each sampling moment, from the different parcels, were crushed and centrifuged at 3000 rpm, during 10 minutes. Then, 0.125 mL of the resultant grape juice (diluted 5 times for white varieties and 10 times for the red ones) was mixed with 0.5 mL of distilled water and 0.125 mL of Folin-Ciocalteu reagent. After homogenization with a vortex, the sample was allowed to react during 5 min. Then, 1.250 mL of Na_2CO_3 (75 g L^{-1}) and 1.0 mL of distilled water were added. The mixture was homogenized in a vortex and letting react during 90 min at room temperature. The absorbance was measured at 760 nm in a spectrophotometer (6405 Jenway UV–Vis spectrophotometer, UK). The calibration curve of gallic acid in the concentration range of 10.0–200.0 mg L^{-1} was obtained in a similar manner to that described for the samples. Each sample was analyzed in triplicate and the total phenolic content was expressed as milligrams of gallic acid equivalents per liter (mg GAE L^{-1}). Data was expressed as mean ± SD (n=3). Results reproducibility was expressed as RSD %.

III. 1. 2.4. Antiradical activity evaluation

The antiradical activity of the grapes varieties, obtained from the vineyard parcels under study, was evaluated by $DPPH^{\bullet}$ radical (2,2-DiPhenyl-1-PicrylHydrazyl radical) scavenging assay. $DPPH^{\bullet}$ is a free radical capable of accept a hydrogen atom or an electron becoming a non-radical species very hardly oxidizable (Soares *et al.*, 1997). Because of the unpaired electron, the $DPPH^{\bullet}$ has a strong absorbance at 515 nm, having a purple colour; if this electron is paired the absorbance disappears, yielding a compound of pale yellow colour (Huang *et al.*, 2005).

The antiradical activity assay used was adapted from the $DPPH^{\bullet}$ method proposed by (Paixão *et al.*, 2007). Briefly, grape berries (ca 100 g for each variety under study) obtained from each sampling moment, from the different vineyards, were crushed and centrifuged at 3000 rpm during 10 minutes. Then, 0.1 mL of the obtained grape juice (without dilution for white varieties and diluted 5 times for the red ones), was added to 3.9 mL of 60 μM DPPH$^\bullet$ methanol solution. The reaction kinetic was read after 30 min at 515 nm on a UV/Visible light spectrophotometer (6405 Jenway UV/Vis spectrophotometer, UK) against blank (0.1 mL of distilled water), at room temperature. Percentage of the remaining DPPH$^\bullet$ (% DPPH$_{rem}$) was calculated in the following way:

$$\% \text{ DPPH}_{rem} = [(A_{sample} \text{ x } \% \text{ DPPH}_{rem} \text{ of the blank} / A_{blank}]$$

where, A_{sample} is the absorbance of each tested sample, A_{blank} is the absorbance of the blank and considering the % DPPH$_{rem}$ of the blank as 100%. The values of % DPPH$_{rem}$ were achieved from three independent assays performed in triplicate, for all experiments. Data are expressed as arithmetic mean ± SD (n = 3). The reproducibility of the results was expressed as RSD %.

III. 1. 3. Results and discussion

III. 1. 3.1. Berry weight, pH, titratable acidity, and sugar content evaluation

The obtained results for the varieties under study are shown in XY graphs, where “0” in the x axis refers to the first sampling moment performed at half-*véraison* for each variety, on the 3 parcels and for the 3 harvests. Detailed data regarding the evaluation of these parameters during maturation are given in Supplementary Tables S1 to S7 (supplementary information is given in a CD-R).

Arinto

The results obtained for Arinto variety (Figure 20) revealed that the values of the 4 parameters studied (berry weight, pH, titratable acidity and sugar content) were similar for grapes from the 3 parcels under study. The exceptions were observed in 2011 for the sugar content, which was inferior in grapes from AR-SM1 parcel (clay-calcareous soil, ca. 50 m of altitude, rows guided East-West direction), and in 2012 the pH values were lower in grapes from AR-VA2 (clay-sandy soil, ca. 70 m) and AR-SM1 parcels. Arinto grapes characteristics were also different from one harvest to another. In 2010 and 2012, technologic maturity was obtained 21/28 and 35 days after half-*véraison*, respectively, while in 2011 this was obtained latter (56 days after half-*véraison*) with the exception of grapes from AR-SM1 parcel (35 days after half-*véraison*). Arinto variety is known for its capacity to adapt and resist to different climates. The longer maturation period observed in 2011, a harvest year characterized as dry and warm (Chapter II) demonstrates the Arinto capacity to resist to year climatic conditions without berry dehydration or rot appearance, maintaining relatively constant the sugar and titratable acidity contents for a long period. Detailed data is given in supplementary Table S1 in a CD-R.

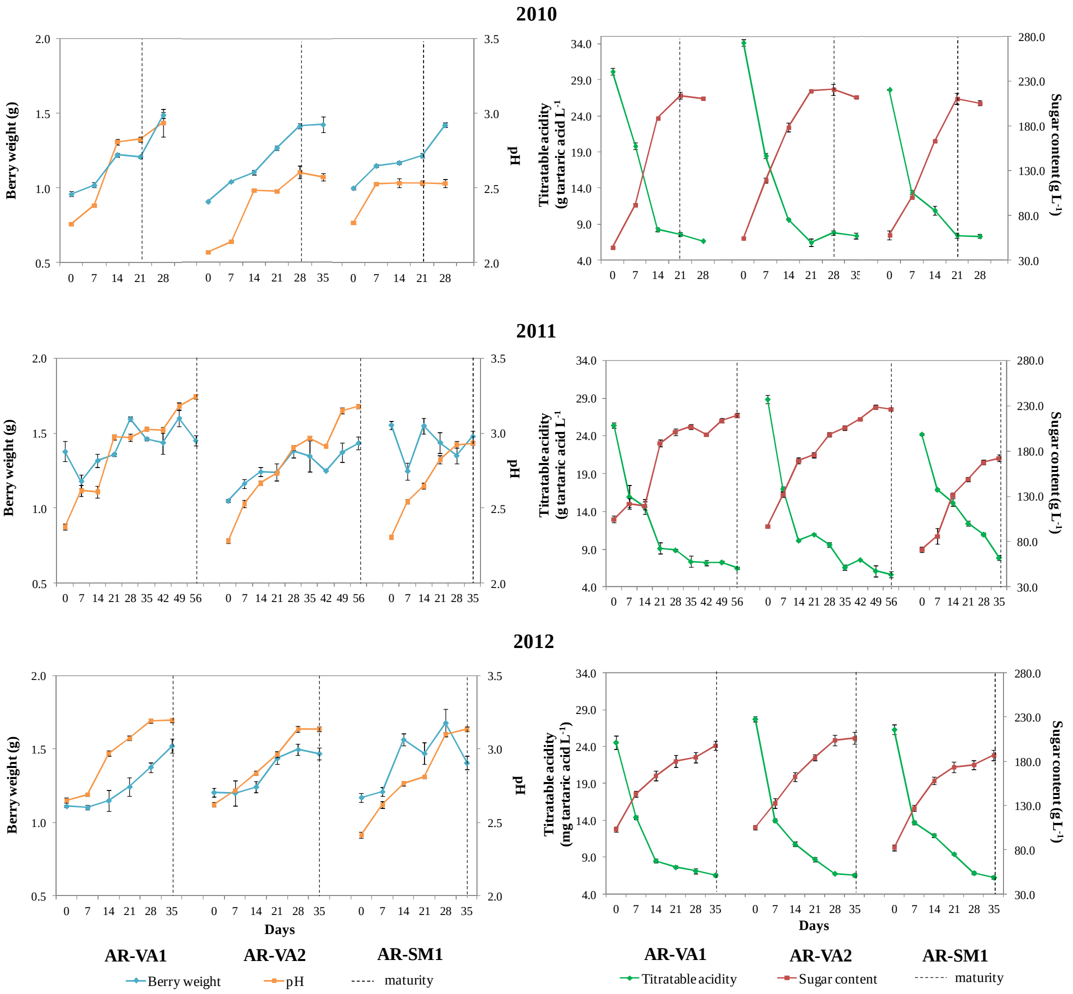


Figure 20. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Arinto, obtained during maturation, on the 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line.

Bical

Bical grapes characteristics in the different parcels are highly related with the harvest year conditions (Figure 21): in 2010, grapes from BI-SM1 parcel (clay-sandy soil, ca. 90 m) presented higher sugar content and lesser titratable acidity, while in 2012, the sugar content was lower in grapes from this parcel, while the acidity was similar for grapes from all parcels. Furthermore, in 2011, grapes from BI-VA2 (clay-calcareous soil, ca. 70 m) parcel presented lower titratable acidity (detailed data is given in supplementary Table S2 in a CD-R). Moreover, according to Figure 21 it was observed that a moderate climate without excessive heat and rain, as in 2010 harvest, provided a gradual maturation with grapes reaching technologic maturity at ca. 35 days after half-*véraison*. On the other hand, when climatic conditions were warmer and dry or fresh and rainy as observed in 2011 and 2012 harvests respectively, the maturation process was changed. For instance, hot temperatures and dry conditions of 2011 allowed grapes to rapidly accumulate sugars accelerating grapes maturation process (technologic maturity was obtained 14/21 days after half-*véraison*). Although in 2011, gapes from BI-VA2 parcel also accumulate sugars rapidly, they were collected 2 to 3 weeks later when compared with grapes from the other parcels. However berry weight starts to diminish at 21 days after half-*véraison* and than stabilized until technologic maturity. This suggests that some dehydration occurred probably caused by higher temperatures and lower precipitation amounts of 2011 harvest. The variability of the results observed for parcels conditions throughout the different harvest years demonstrated the high sensitivity attributed to this variety to the year climatic conditions (Chapter II).

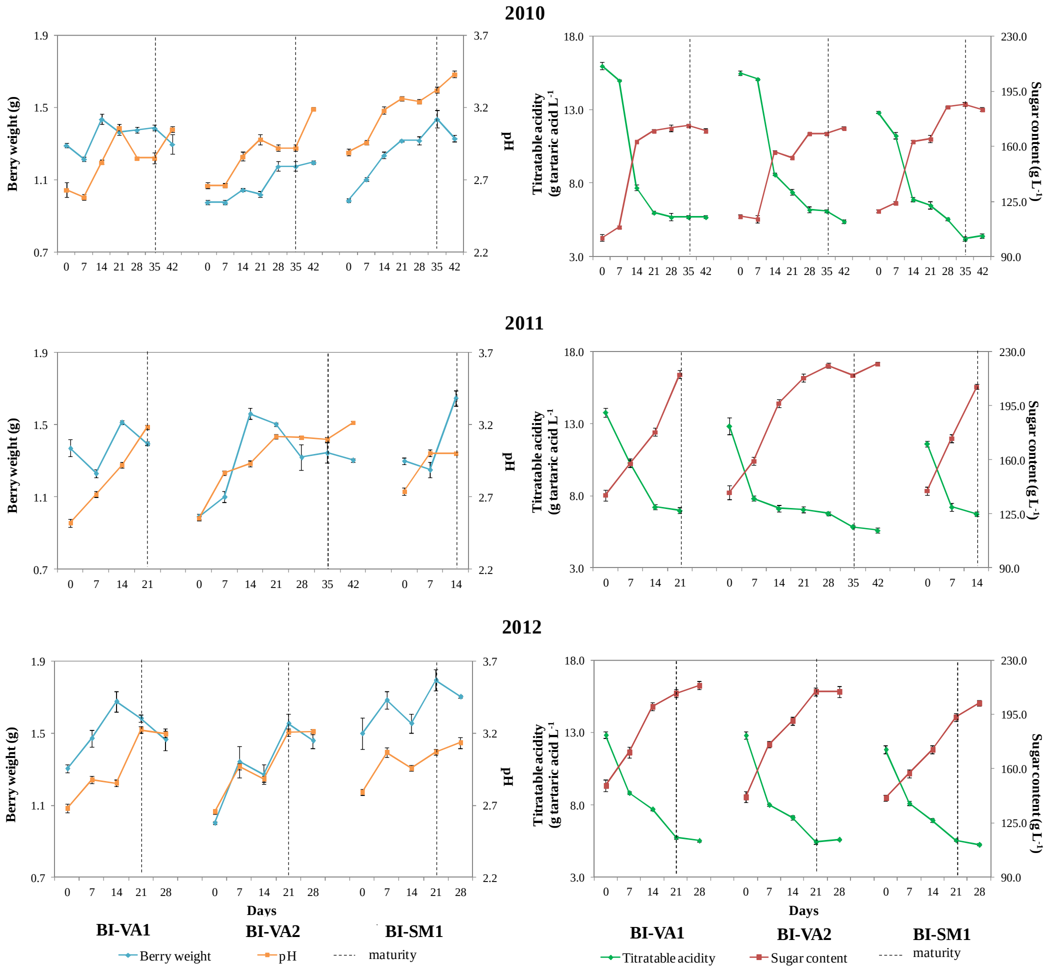


Figure 21. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Bical, obtained during maturation, on the 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line.

Sauvignon Blanc

For Sauvignon Blanc variety (Figure 22) similar pH and titratable acidity values were determined. However, differences in berry weight and also in sugar content were observed for grapes obtained from the 3 parcels: grapes from SB-SM2 (ca. 50 m, clay-calcareous soil) exhibited higher berry weight and lesser sugar content than grapes from the other parcels (SB-SM1 and SB-SM3, both at 70 m, with clayey and clay-sandy soils, respectively), being this particularly evident in grapes harvested in 2010 (detailed data is given in supplementary Table S3 in a CD-R). The characteristics of the 3 parcels of Sauvignon Blanc were very similar (Table 3, Figure 17), thus possible the soil type and the slight differences in altitude, may explain the different results observed. Parcels SB-SM1 and SB-SM3 were at high slope steep (ca. 70 m) while SB-SM2 (clay-calcareous soil, ca. 50 m) was at lower step, below the other 2 parcels. Thus, this parcel may accumulate higher water amount and thus higher humidity for a longer period. This higher amount of water may explain the higher berry weight and also may promote sugars dilution, which may explain the lower sugar content observed in grapes from this parcel. As can be seen in Figure 22, for the 3 harvests, Sauvignon Blanc grapes attended technologic maturity at 21/28 days after half-*véraison*. Sauvignon Blanc is known in Bairrada Appellation, for its short cycle and lower maturation process requiring few days to mature (Chapter II). Moreover, lower sugar content and higher acidity were determined in grapes from 2012 harvest, which was characterized as a fresh and rainy year. It was found that grape sugar content accumulation was not favoured in cool climates, while grapes acidity was elevated. Temperature determines the respiration rate, i.e. the combustion of tartaric and, especially, malic acid in grapes (hotter temperatures favoured malic acid respiration) (Ribéreau-Gayon *et al.*, 2000).

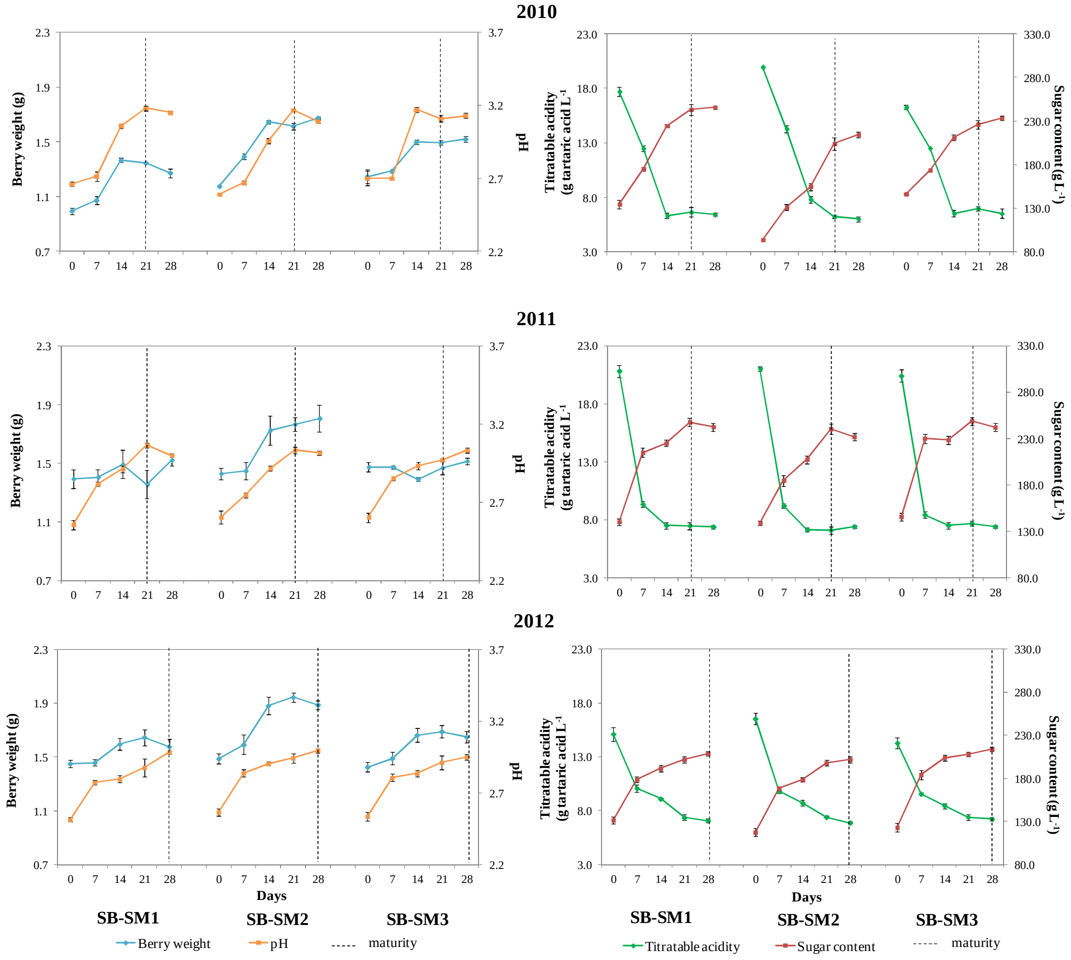


Figure 22. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Sauvignon Blanc, obtained during maturation, on the 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line.

Baga

For Baga variety, similar berry weight, pH and titratable acidity were determined for all parcels and harvests under study. However, differences can be noticed related with sugar content (Figure 23). Generally, grapes from BA-VA2 (ca. 50 m, clay-calcareous soil) followed by BA-VA1 (ca. 70 m, clayey soil) exhibited higher sugar content when compared with BA-SM1 parcel (ca. 50 m, clay-sandy soil). The exception was in 2012, where BA-VA2 grapes exhibited lower sugar content (detailed data is given in supplementary Table S4 in a CD-R). This suggests that grapes from soils with moderate (clay-calcareous soil of BA-VA2) to higher (clayey soil of BA-VA1) water-holding capacity seems to be related with higher sugar content of Baga grapes. Baga was also influenced by harvest climatic conditions: moderate temperatures and precipitation amounts of 2010 harvest, followed by hot temperatures of 2011, allowed to obtain grapes with higher sugar content and lower titratable acidity, while in 2012 the fresh and rainy climatic conditions allowed to obtain grapes with lower sugar amounts. It is known that the alcohol content in Baga wines is very variable depending on the harvest year climatic conditions (Chapter II). The results obtained corroborate these observations since the grapes sugar content was very dependent on the harvest year climatic conditions. Besides, Baga requires good sunlight for a long growth cycle. As 2012 was a cooler harvest, this may explain the lower sugar amount observed in grapes from this harvest. Temperature is essential for grapes development and for the resulting grapes sun exposure (Ribéreau-Gayon *et al.*, 2000).

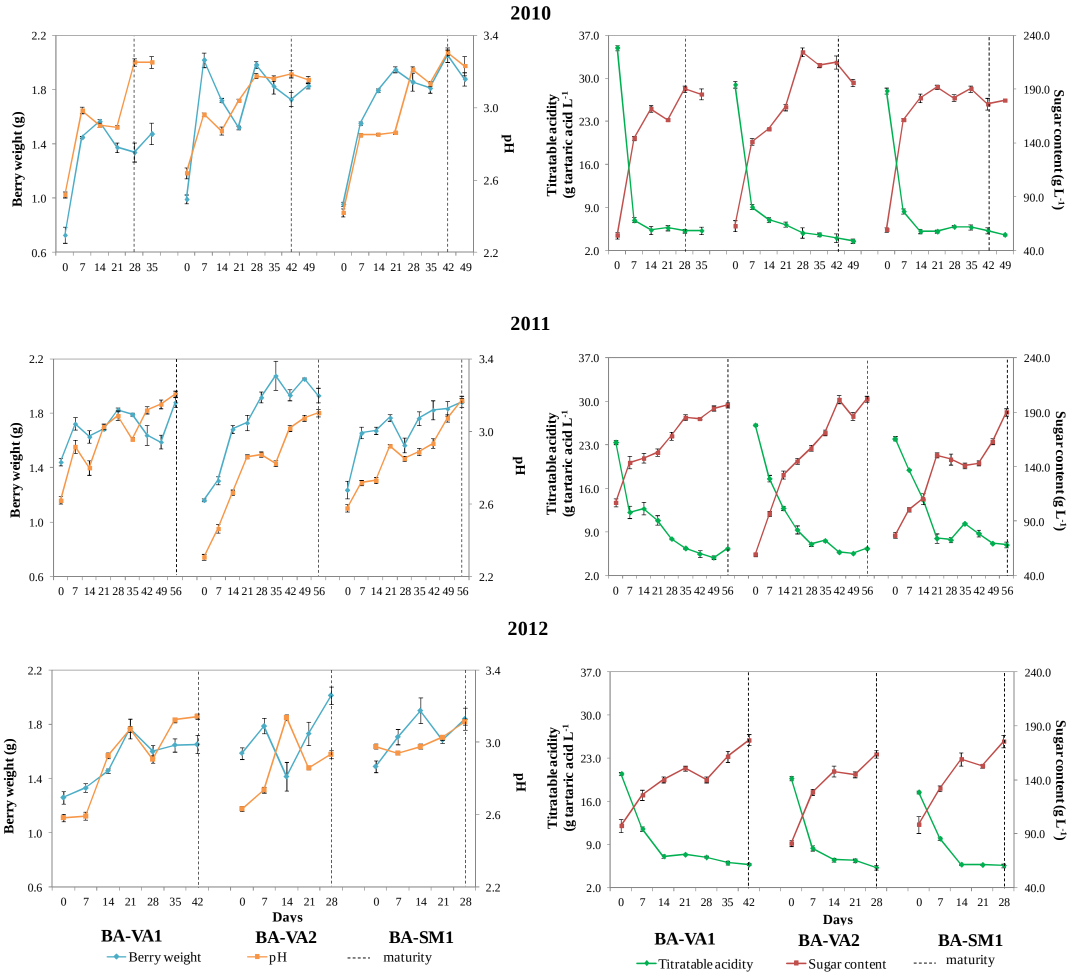


Figure 23. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Baga, obtained during maturation, on the 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line.

Castelão

Castelão variety collected in the different parcels and harvests under study exhibited similar berry weight, pH and titratable acidity for grapes obtained from CA-SM1 (ca. 70 m, clay-calcareous soil), CA-SM2 (ca. 60 m, clayey soil) and CA-SM3 (ca. 60 m, clay-sandy) parcels. Nevertheless, differences may be noticed related with sugar content (Figure 24). For Castelão grapes, sugar content had a tendency to decrease in the following order: grapes from CA-SM2, CA-SM1 and CA-SM3 parcels. The exception was in 2011, where CA-SM1 parcel presented lower sugar content than CA-SM3 (detailed data is given in supplementary Table S5 in a CD-R). As these parcels were located at an open space in São Mateus vineyard and rows were guided in the same direction (North-South) with only slight differences in altitude (ca. 60 to 70 m), these results suggest that the observed differences may be related with soil type: grapes from clay-calcareous (CA-SM1) and clayey (CA-SM2) soils, with higher water-holding capacity, seems to be related with higher sugar content of Castelão grapes than clay-sandy soils (CA-SM3), that had lower water-holding capacity. Besides the fact that Castelão variety is well adapted to climate with maritime influence, as in Bairrada Appellation (Chapter II), its grapes characteristics were different from one harvest to another (Figure 24): grapes from 2010 have a tendency to present higher sugar content and lower acidity when compared with 2011 and 2012.

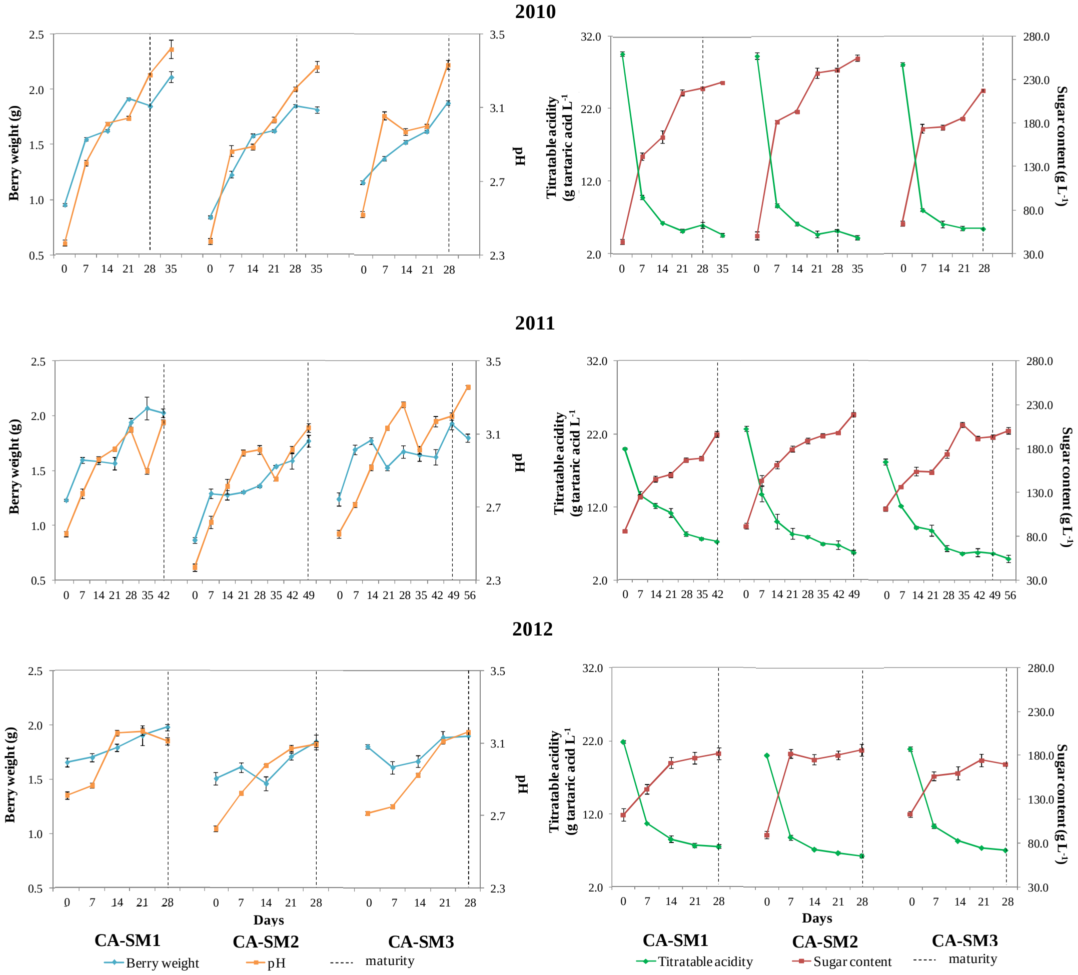


Figure 24. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Castelão, obtained during maturation, on the 3 parcels and 3 consecutive harvests. Maturity is indicated with a dash line.

Touriga Nacional

Grapes of Touriga Nacional variety presented similar berry weight, pH and also titratable acidity for all parcels under study (Figure 25). However, sugar content was quite different from one parcel to another. Generally, grapes from TN-SM2 (ca. 70 m, clay-calcareous soil) and TN-SM1 (ca. 50 m, clayey soil) had higher sugar content, when compared with TN-SM3 (ca. 50 m, clay-sandy soil). The exception was observed in 2010, where grapes from TN-SM1 exhibited lesser sugar content than grapes from TN-SM3 (detailed data is given in supplementary Table S6 in a CD-R). Similarly to the tendency observed for Castelão, soils with higher and moderate water-holding capacity, as clayey (TN-SM1) and clay-calcareous (TN-SM2) soils, seem to be related with higher sugar content than soils with lower water-holding capacity (clay-sandy soil of TN-SM3). Besides, also harvest year conditions influence Touriga Nacional grapes characteristics: lower sugar content and higher titratable acidity was observed for grapes from 2011 and 2012 harvest. During maturation, 2011 harvest was excessively hot while 2012 was cooler and rainy, when compared with moderate climatic conditions of 2010 harvest. Touriga Nacional variety has a late maturation and requires good sunlight exposure to mature. However, it is sensitive to excessive hot summers (Chapter II). Too much heat can delay physiological maturation and cause excessive dehydration, resulting in the appearance of overripe grapes, a characteristic that was observed in 2011 harvest for grapes from TN-SM3 parcel (Figure 25). On the other hand, lower temperature and higher precipitation amounts may dilute the sugar content and decrease malic acid respiration, accounting for the higher acidity of the berries (Bondada and Keller, 2012; van Leeuwen and Seguin, 2006). This may explain the reasons why Touriga Nacional grapes from 2012 harvest present lower sugar content and higher titratable acidity.

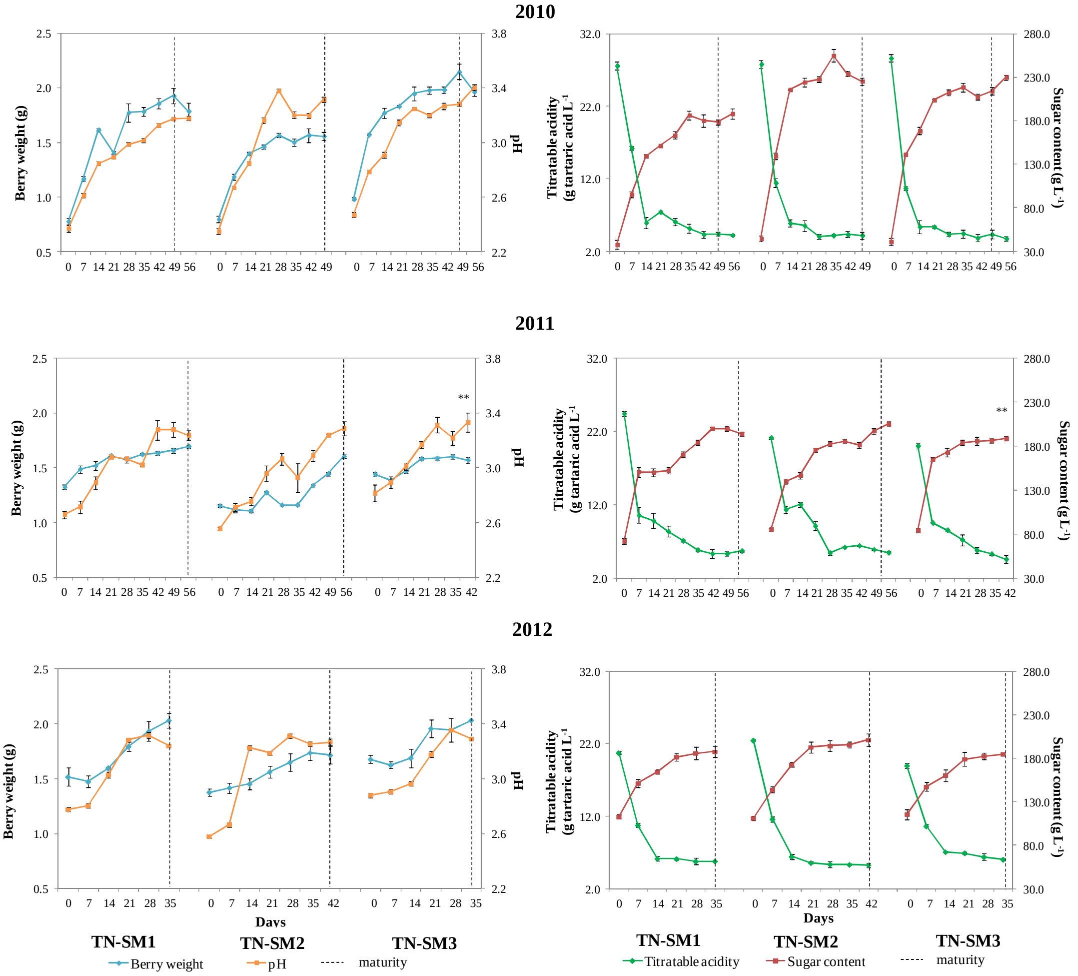


Figure 25. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Touriga Nacional, obtained during maturation, on the 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line. ** Overripe grapes.

Sousão

Sousão red variety was only evaluated during maturation in one parcel at São Mateus vineyard, for the 3 harvest years. According to Figure 26, grapes collected in SO-SM1 parcel (ca. 50 m, clay-sandy soil) exhibited similar berry weigh and pH for all harvests under study. Grapes collected in 2010 exhibited higher sugar content, while grapes from 2011 and 2012 exhibited higher titratable acidity and lower sugar content (detailed data is given in supplementary Table S7 in a CD-R). These results clearly shows that moderate temperatures and precipitation amounts observed in 2010 harvest allowed to obtain grapes with higher sugar content and lower acidity, when compared with fresh and rainy conditions of 2012 harvest and with hot and dry ones of 2011.

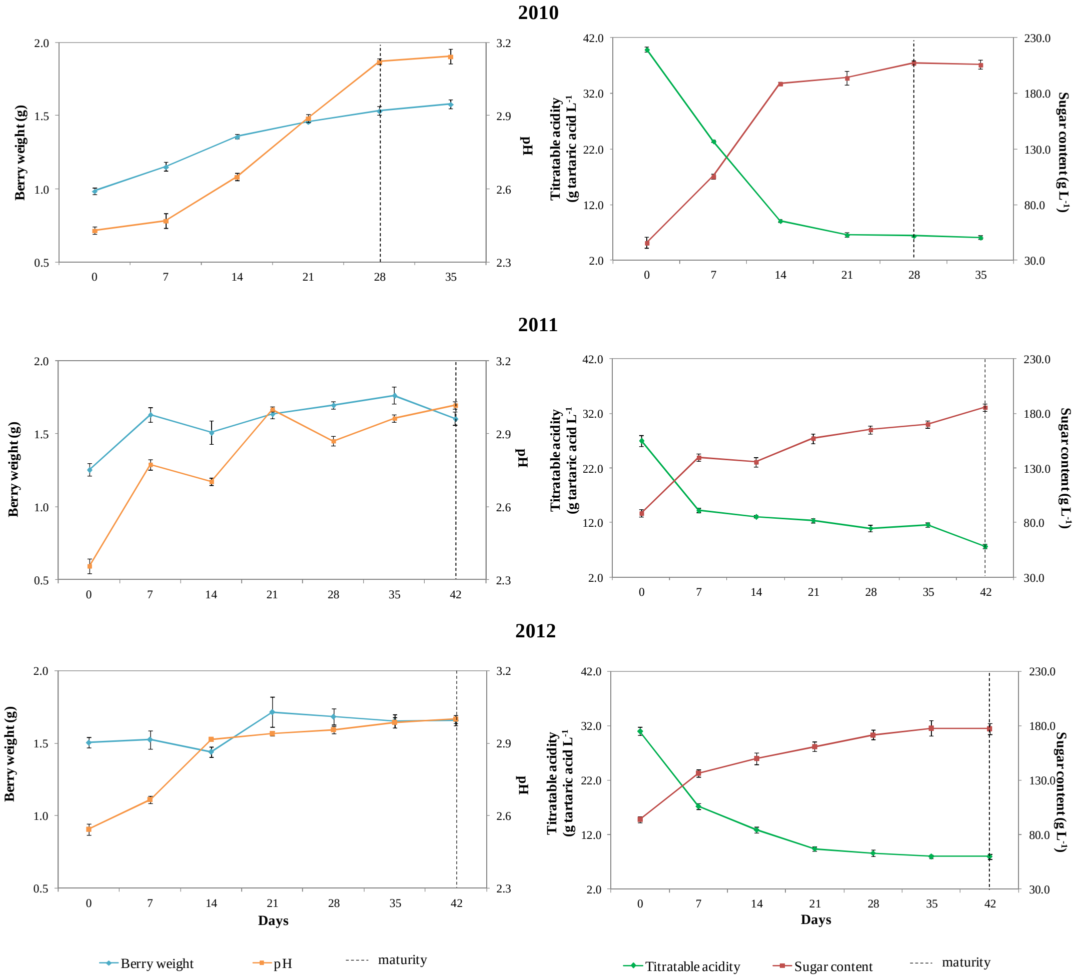


Figure 26. Berry weight, pH, sugar content and titratable acidity of *V. vinifera* cv. Sousão, obtained during maturation, on SO-SM1 parcel, form 3 consecutive harvests. Technologic maturity is indicated with a dash line.

III. 1. 3.2. Total phenolic content and antiradical activity evaluation

The results obtained for the total phenolic content and antiradical activity of the grape varieties under study, during maturation, were shown in bar graphics, where “0” in the x axis refers to the first sampling moment performed at half -*véraison* for each variety, in the 3 parcels and 3 harvests. Detailed data is given in Supplementary Tables S1 to S7 in a CD-R.

Arinto

According to the obtained results for Arinto variety, grapes obtained from AR-VA2 (ca. 70 m, clay-sandy soil, North-South direction) followed by grapes from AR-SM1 parcel (ca. 50 m, clay-calcareous soil, East-West direction) presented higher phenolic content and also higher antiradical activity, when compared with grapes from AR-VA1 parcel (ca. 50 m, clayey soil, North-South direction) (Figure 27). According to the main parcels characteristics, the different results observed may be related with parcels soil types. It is already shown that soils with higher water-holding capacity increase the oenological potential of Agiorgitiko red variety by promoting the concentration of anthocyanins and total phenolics in berries (Koundouras *et al.*, 2006). However, for Arinto white variety, soils with lower (clay-sandy soil - AR-VA2) and middle (clay-calcareous soil - AR-SM1) water-holding capacity than clayey soil seem to favour total phenolic content and also antiradical activity (Table 3, Figure 17). Besides, in 2010, during maturation, the phenolic content and also the antiradical activity were higher than in grapes collected in 2011 or even in 2012 (Figure 27). The moderate climatic conditions of 2010 harvest seem to favour grapes phenolic content as well as antiradical activity. A moderate climate, with adequate temperature and precipitation, was considered to provide ideal conditions for grapes maturation (Conde *et al.*, 2007). On the opposite, the higher temperatures of 2011 and the fresh and rainy conditions of 2012 diminished phenolic content and antiradical activity of Arinto grapes. This was in accordance with previous works that showed that high temperatures promote significant reductions on phenolic content of *V. vinifera* cv. Cabernet-Sauvignon (Goto-Yamamoto et al., 2009) and Merlot (Spayd et al., 2002) grapes. Also, high precipitation amounts promote the decrease in the concentration of phenolics content (Keller et al., 2006).

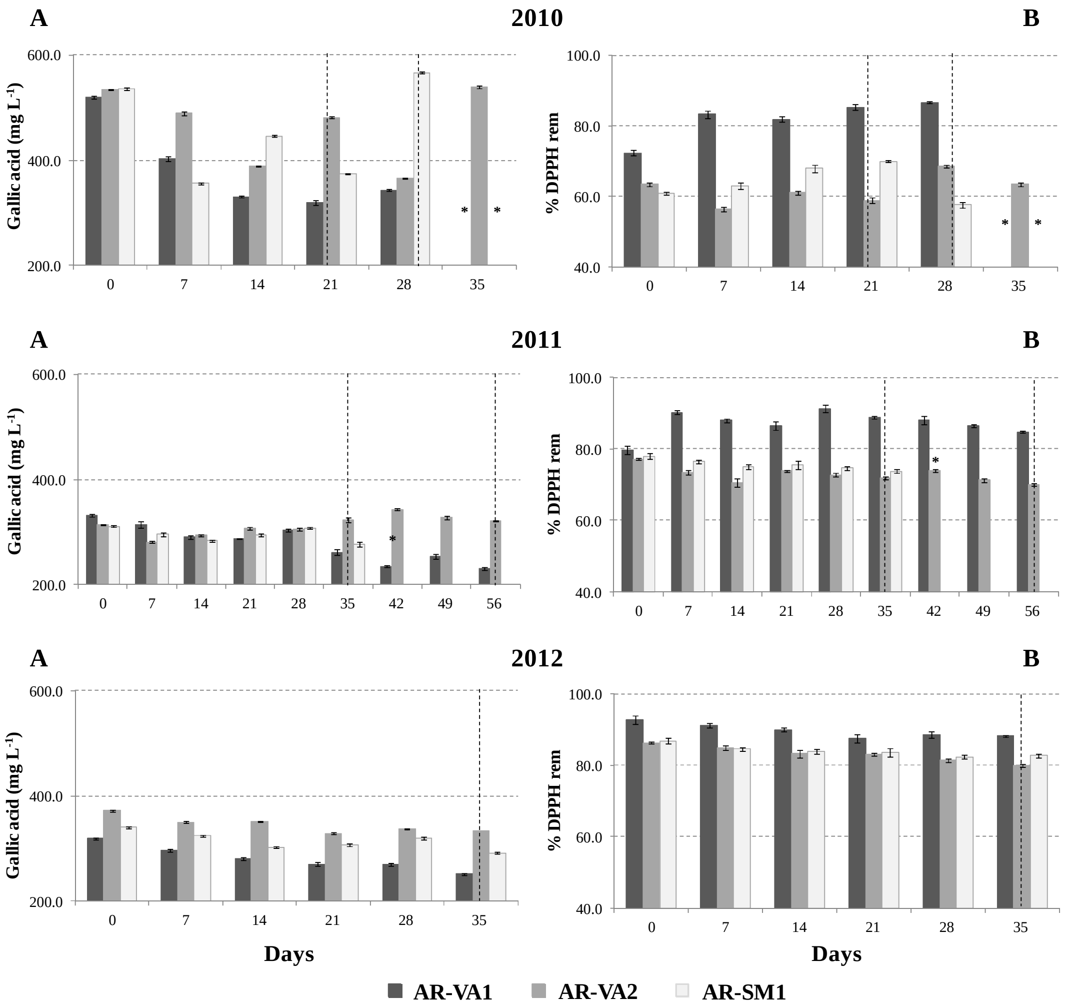


Figure 27. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Arinto, during maturation, in 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line. * Grapes not available.

Bical

Bical variety obtained from the 3 parcels under study revealed different total phenolic content and also different antiradical activity (Figure 28). According to the results, grapes obtained from BI-SM1 (ca. 90 m, clay-sandy soil, open space, East-West direction) and BI-VA2 (ca. 70 m, clay-calcareous soil, open space, North-South direction) parcels, followed by grapes from BI-VA1 (ca. 70 m, clayey soil, near to pine trees, North-South direction) presented higher phenolic content and also higher antiradical activity. Even in 2011, when Bical grapes from BI-VA1 and BI-SM1 parcels were collected earlier when compared to BI-VA2 grapes, this tendency was observed. As grapes from BI-SM1 (East-West direction) and BI-VA2 (North-South direction) parcels exhibited the higher phenolic content as well as higher antiradical activity, and these are at similar altitudes and both at open spaces, the different rows orientation of these vineyards appears to have no influence in Bical grapes phenolic content and antiradical activity. Thus, similarly with Arinto, the obtained results showed that clay-sandy and clay-calcareous soils seem to be related with higher phenolic content and also higher antiradical activity of Bical grapes. Besides, BI-VA1 parcel with clayey soil (higher water-holding capacity soil) is near to pine trees, which due to pines location at East of this parcel may reduce grapes sunlight in the first hours at the morning, which may reduce BI-VA1 grapes phenolic composition, and thus its oenological potential. Furthermore, similarly to Arinto variety, the higher phenolic content and also antiradical activity were found in 2010 harvest.

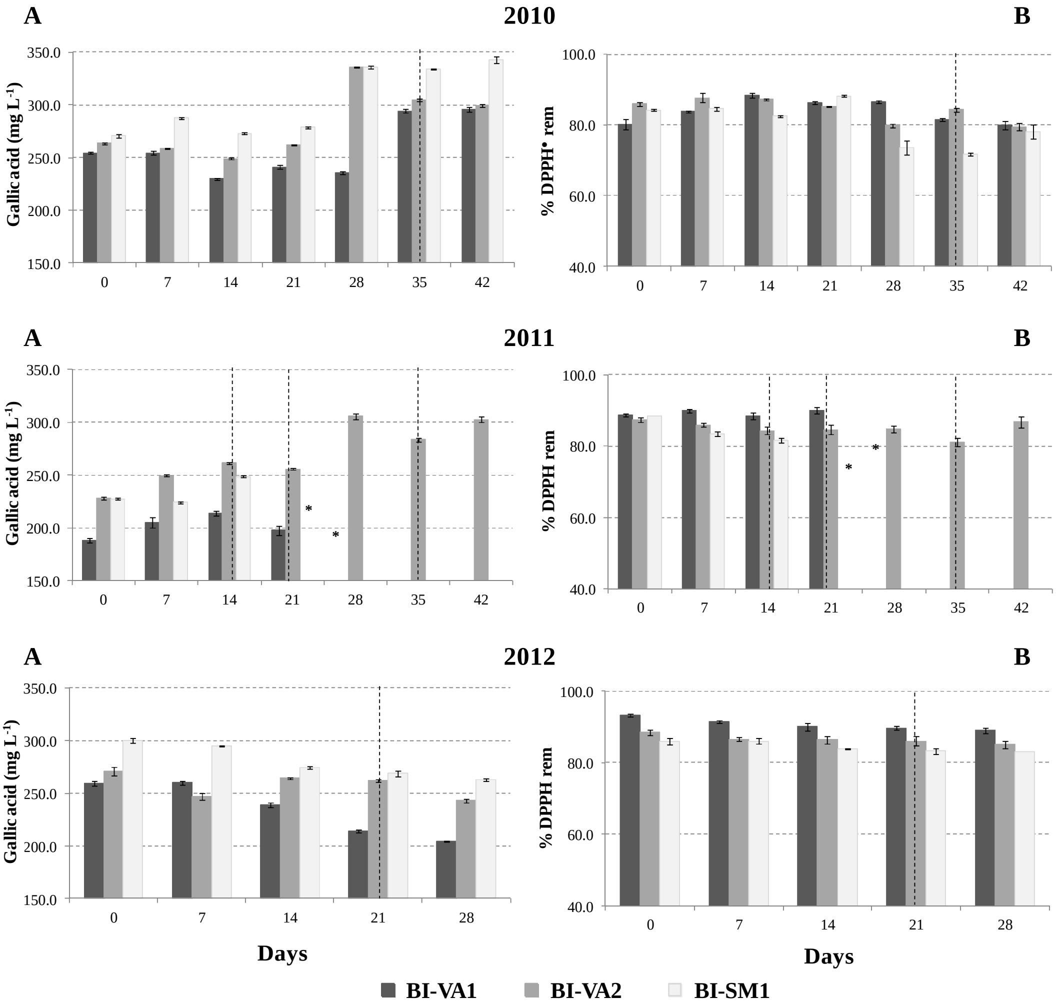


Figure 28. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Bical, during maturation, in 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line. * Grapes not available.

Sauvignon Blanc

For Sauvignon Blanc variety the determination of total phenolic content and antiradical activity during maturation was only evaluated for 2010 harvest (Figure 29). For Sauvignon Blanc variety, higher phenolic content and antiradical activity were obtained for SB-SM3 (ca. 70 m, clay-sandy soil) and SB-SM2 (ca. 50 m, clay-calcareous soil) grapes, while the opposite was obtained from SB-SM1 grapes (ca. 70 m, clayey soil). As the main characteristics of these 3 parcels are very similar (Table 3, Figure 17), the different results determined may be related with the soil texture: clay-sandy (SB-SM3) and clay-calcareous (SB-SM2) soils, with low and medium water-holding capacity, respectively, allowed obtaining grapes with higher phenolic and antiradical activity, while the opposite was determined for grapes form clayey soil (SB-SM1).

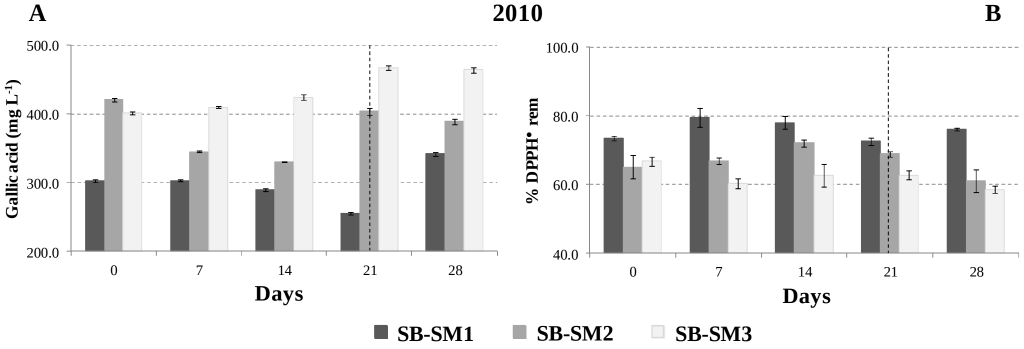


Figure 29. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Sauvignon Blanc, during maturation, in 3 parcels, in 2010. Technologic maturity is indicated with a dash line.

Baga

The total phenolic content and antiradical activity of Baga red variety was evaluated, during maturation, only in 2010 harvest (Figure 30). For this variety, higher phenolic content as well as higher antiradical activity were determined in grapes obtained from BA-VA2 (ca. 50 m, clay-calcareous soil) followed by grapes from BA-VA1 (ca. 70 m, clayey soil), when compared with grapes from BA-SM1 parcel (ca. 50 m, clay-sandy soil) parcel. Slightly differences in altitude and the same rows orientation were observed for all the 3 Baga parcels under study (Table 3, Figure 17). BA-VA1 parcel was near to pine trees, while the others were at open spaces. Similar with Bical, the presence of pine trees at East of BA-VA1 parcel may influence its sunlight early in the morning. However, as grapes from this parcel presented higher phenolic content and lower antiradical activity than open space parcel located in São Mateus vineyard (BA-SM1), this suggests that the main parcel characteristic that influences Baga grapes composition was the soil type: clayey and clay-calcareous soils allowed obtaining grapes with higher phenolic content and also higher antiradical activity. These soils have higher water-holding capacity than clay-sandy soil (BA-SM1). It is already shown that soils with higher water-holding capacity increased the oenological potential of other red grape variety (*V. vinifera* cv. Agiorgitiko) by promoting the concentration of anthocyanins and total phenolics in the berries (Koundouras *et al.*, 2006).

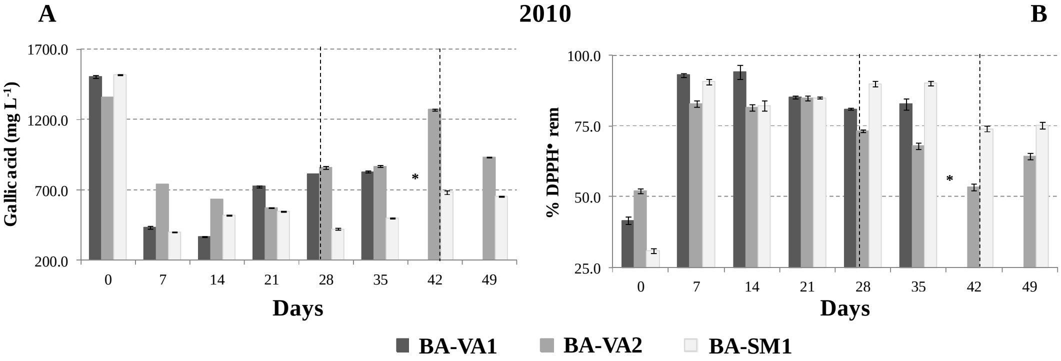


Figure 30. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Baga, during maturation, in 3 parcels, in 2010. Technologic maturity is indicated with a dash line.* Grapes not available.

Castelão

Regarding the results obtained for *V. vinifera* cv. Castelão different phenolic content and antiradical activity were obtained for the 3 parcels (Figure 31). Castelão grapes from CA-SM1 (ca. 70 m, clay-calcareous soil) and CA-SM2 (ca. 60 m, clayey soil) parcels presented higher phenolic content as well as higher antiradical activity, than grapes from CA-SM3 (ca. 60 m, clay-sandy soil). According to the parcels characteristics (Figure 17, Table 3) the different phenolic content and antiradical activity observed between grapes from the 3 parcels can be explained by the different soil type of these parcels: grapes from clay-sandy soil of CA-SM3 parcel, with lesser water-holding capacity, had lesser phenolic content and antiradical activity than grapes from clay-calcareous and clayey soils. Furthermore, in 2010 harvest, grapes from all parcels under study presented higher phenolic content and also higher antiradical activity when compared with the other 2 harvest years.

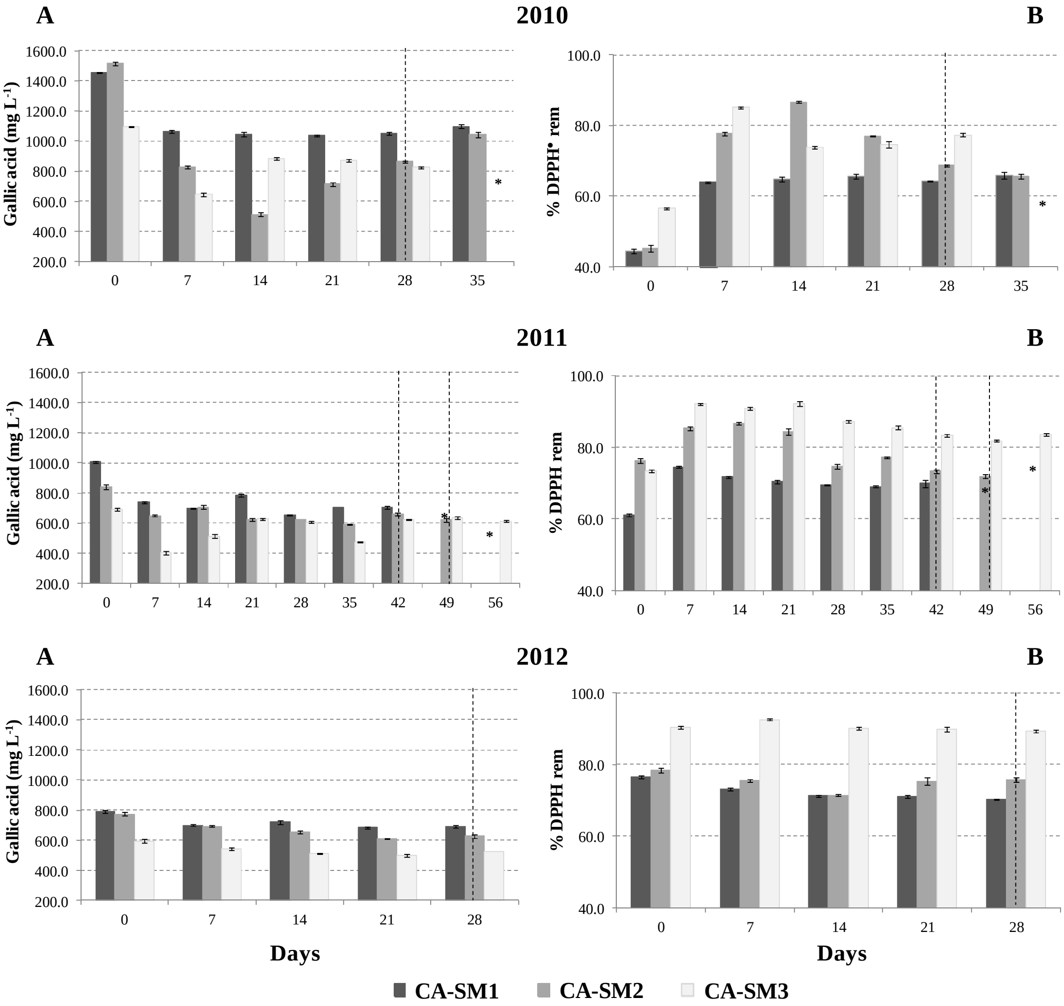


Figure 31. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Castelão, during maturation, in 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line. * Grapes not available.

Touriga Nacional

Touriga Nacional variety was also evaluated in terms of their total phenolic content and antiradical activity, for grapes from 3 parcels and 3 harvests (Figure 32). According to the obtained results grapes from TN-SM2 parcel (ca. 70 m, clay-calcareous soil), followed by grapes from TN-SM1 (ca. 50 m, clayey soil) and TN-SM3 (ca. 50 m, clay-sandy soil) parcels, presented higher phenolic content as well as higher antiradical activity. The 3 parcels of Touriga Nacional variety have different soil types and also slightly differences in altitudes (Figure 17, Table 3) which may explain the different grapes characteristics determined for Touriga Nacional parcels: TN-SM2 parcel presents clay-calcareous soil and is at ca. 70 m which seems to be related with higher total phenolic content and antiradical activity. Furthermore, moderate climatic conditions of 2010 harvest seem to be related with higher phenolic content and antiradical activity of Touriga Nacional grapes. Also, 2011 climatic conditions caused berries dehydration with overripe grapes formation in TN-SM3, a parcel with clay-sandy soil thus with lower water-holding capacity and with higher drainage when compared with the other parcels (Figure 32).

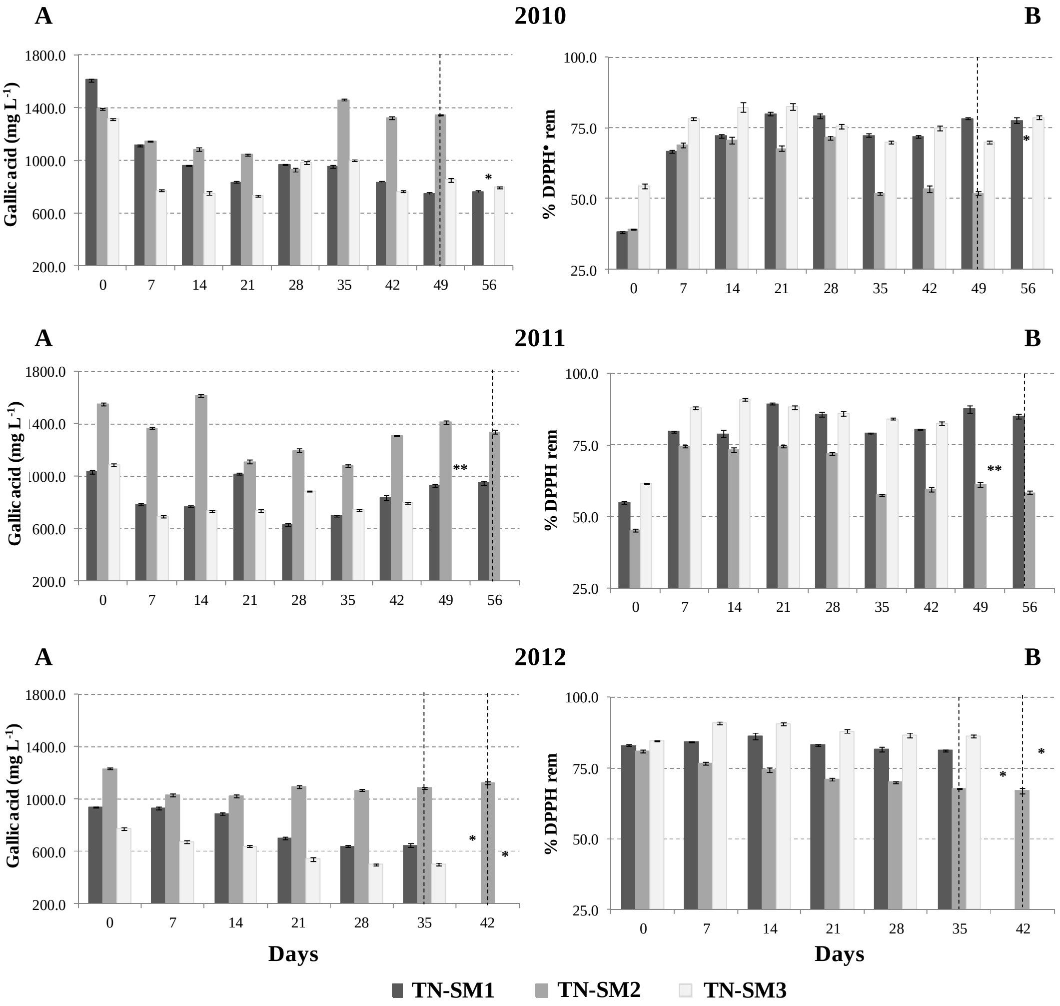


Figure 32. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Touriga Nacional, during maturation, in 3 parcels and 3 consecutive harvests. Technologic maturity is indicated with a dash line. * Grapes not available ** Overripe grapes

Sousão

For Sousão variety only grapes from SO-SM1 parcel (ca. 50 m, clay-sandy soil), collected in 2010 harvest were considered (Figure 33). During maturation, phenolic content and antiradical activity were similar, with a slight decrease in day 14 after half-*véraison*. The phenolic content and also the antiradical activity of this red variety were similar or even higher when compared with the other red varieties under study. This shows its huge oenological potential related with wines colour potential.

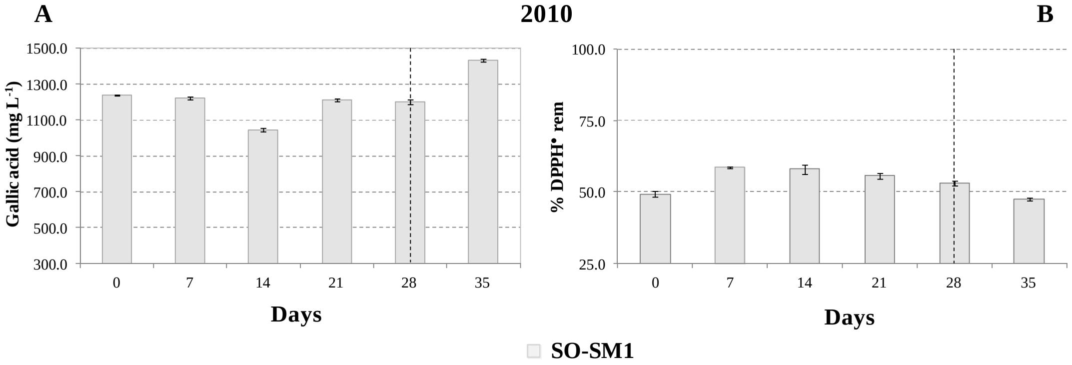


Figure 33. Total phenolic content (A) and antiradical activity (B) evaluation of *Vitis vinifera* L. cv. Sousão, during maturation, in SO-SM1 parcel, in 2010. Technologic maturity is indicated with a dash line.

Once determined the general physicochemical parameters for each variety in the different conditions under study, according to the overview of Chapter III, the following sub-chapter (Chapter III. 2) refers to the determination of volatile composition (free fraction) of each grape variety.

Chapter III. 2 - Varieties volatile profile evaluation

III. 2. 1. Framework

Grapes undergo many changes during the maturation process which involve a number of physical and chemical modifications, including changes in weight, sugar, acidity, colour and aroma (volatile composition). The volatile composition of grapes is one of the most important factors to determine grapes, and thus, wine aroma quality, which is determinant for consumer acceptance. However, the type and concentration of volatile compounds is dependent of several natural factors such as harvest climatic conditions, soil type, vineyard altitude, as well as agricultural practices, among other factors. The knowledge of the volatile composition offers a means of evaluating the aroma potential of each variety (Ribéreau-Gayon *et al.*, 2000). There are several volatile and semi-volatile compounds already reported in grapes from different varieties, representing different chemical families, namely alcohols, aldehydes, ketones, norisoprenoids, and terpenic compounds (González-Marco *et al.*, 2008; Polášková *et al.*, 2008). Form these, terpenic compounds (mono-, sesqui-, and diterpenic ones), norisoprenoids, aromatic alcohols, and C_6 alcohols and aldehydes, were considered in this PhD thesis, due to their importance to the grapes aroma properties. This will allow determining the variety volatile characteristics in the different vineyard parcels, for the 3 harvests.

Although a number of volatile compounds contributing to wine quality are produced by the yeasts during fermentation, or are derived from precursors during the wine aging, a good many are already present in the grapes (González-Barreiro *et al.*, 2015; Jackson, 2014). These compounds present in grapes are responsible for the varietal aroma of the wines and are biosynthesized during grapes maturation. The term ‘varietal aroma’ should not, however, be taken to imply that each variety has specific volatile compounds. In fact, the same volatile compounds are found in different varieties. Thus, the individual aroma properties of wines made from each grape variety is due to the infinitely varied combinations and concentrations of the various compounds (Ribéreau-Gayon *et al.*, 2006). The terpenic compounds and norisoprenoids are important chemical families associated to the varietal aromas.

Terpenic compounds belong to secondary plant constituents whose biosynthesis begins with the production of mevalonic acid from glucose by acetyl-coenzyme A (CoA) pathway. All terpenic compounds are built from isopentenyl pyrophosphate (IPP), a C_5 isoprenic-base unit, produced from mevalonic acid. This IPP unit is isomerised into dimethylallyl pyrophosphate (DMAPP). These two units play an active role in terpenic compounds synthesis. By the action of prenyltransferases, DMAPP molecule condensates with varying numbers of IPP units to form geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP). Then, terpene synthases convert GPP, FPP, and GGPP to mono-, sesqui- and diterpenic compounds, respectively. These, after losing the pyrophosphate residue, the synthetic pathways can form either acyclic or cyclic terpenic compounds (Ribéreau-Gayon *et al.*, 2000). Mono- and sesquiterpenic compounds, formed from two and three isoprene units, respectively, are the most commonly determined terpenic compounds in grapes and wines. From these, linalool, α-terpineol, nerol, geraniol, citronellol, and nerolidol are the most common ones. Although terpenic compounds are present at trace amounts, their sensory thresholds are rather low (few hundred micrograms per litre), thus these compounds can therefore contribute significantly to the aroma potential of the varieties. These are greatly related with fruity, citric, and floral aromas (Ribéreau-Gayon *et al.*, 2000; Ribéreau-Gayon *et al.*, 2006).

Norisoprenoids are a diverse group of aroma compounds derived from grape carotenoids. The oxidative degradation of carotenoids, terpenes with 40 carbons (tetraterpenes) produces derivatives with 9, 10, 11 or 13 carbon atoms. Among these compounds, the 13 carbon atoms derivatives (C_{13} norisoprenoids) are the most commonly found in grapes. Although they are only present at trace levels, sensory thresholds for most norisoprenoids are very low (e.g. 0.09 μg/L for β-ionone and 0.05 μg/L for β-damascenone), thus these compounds can therefore contribute significantly to the aroma potential of the varieties, essentially with flowery, fruity, and sweety aromas (Ribéreau-Gayon *et al.*, 2006).

The aromatic alcohols, as benzyl alcohol and 2-phenylethanol, are commonly determined in grapes. Although they are synthesized in grapes in small amounts, they are mainly produced during fermentation process by yeasts. Besides the fact that a portion of these compounds can derive from glycosidic hydrolysis, a greater proportion is formed from grape-derived aldehydes, in the metabolism of the amino acid phenylalanine. Aromatic alcohols are an important chemical family that contributes with pleasant aromas to the wines, principally with flowery and sweet aromas (Jackson, 2014; Moreno-Arribas and Polo, 2009).

Besides the varietal aromas, also pre-fermentative ones are found in grapes. These aromas result from several mechanical or technological operations (transport, crushing, maceration and clarification) performed before the beginning of the fermentation process. Six-carbon alcohols and aldehydes are mainly related with the pre-fermentative aroma (González-Barreiro *et al.*, 2015). The C_6 aldehydes (like hexanal, 2- and 3-hexenal) are formed by enzymatic oxidation of fatty acids in the grapes, namely linolenic and linoleic acids, by the activity of lipoxygenase enzymes, following mechanical or technological processes. Briefly, an acylhydrolase hydrolyses the fatty acids from membrane lipids. Next, the lipoxigenase catalyzes the fixation of oxygen on these C_{18} unsaturated fatty acids. This enzyme preferentially forms hydroperoxides in C_{13} from linoleic and linolenic acids which are then cleaved into C_6 aldehydes. Some of these aldehydes are then reduced to their corresponding alcohols by the action of alcohol dehydrogenase of the grapes (Bakker and Clarke, 2011; Crouzet, 1986). These compounds are related with herbaceous aromas, which are commonly regarded as negative quality of the wine aroma, although consumers appreciate a certain herbaceous notes in some wines (Welke *et al.*, 2012).

The present Chapter aims to evaluate the impact of harvest and vineyard parcel characteristics on each variety volatile composition by using data obtained during maturation. For this, varietal (terpenic compounds, norisoprenoids, and aromatic alcohols) and pre-fermentative (C_6 alcohols and aldehydes) aroma compounds were selected. These chemical families were selected because the varietal volatile composition offers means of evaluating the aroma potential of each variety and the herbaceous odours of C_6 alcohols and aldehydes are associated as negative quality parameters, however in some wines these notes are appreciated by the consumers.

III. 2. 2. Material and Methods

III. 2.1. Samples

Seven *V. vinifera* grape varieties, each one from 3 parcels, collected during maturation, were considered. The exception was Sousão which was only collected in one parcel. Depending on the variety, the parcel, and the harvest, the sampling moments varies from 3 to 9.

III. 2. 2.2. Determination of grapes volatile profiles by HS-SPME/GC×GC-ToFMS

HS-SPME procedure

The HS-SPME experimental parameters were adopted from a previously study using grapes (Perestrelo *et al.*, 2011). The SPME holder for manual sampling and fibre were purchased from Supelco (Aldrich, Bellefonte, PA, USA). The SPME device included a fused silica fibre coating partially cross-linked with 50/30 μm DVB/CAR/PDMS). This coating (molecular weight ranging from 40 to 275 Da) combines the absorption properties of the liquid polymer with the adsorption properties of porous particles, which contains macro (>500 Å), meso (20–500 Å) and microporous (2–20 Å) and has bipolar properties. The mutually synergetic effect of adsorption and absorption of the stationary phase promotes a high retention capacity and, consequently, higher sensitivity than fibres based only on absorption, which seems to be adequate for the analysis of complex matrix such as grapes. Prior to use, the SPME fibre was conditioned at 270 °C for 60 min in the GC injector, according to the manufacturer's recommendations. For HS-SPME assay, 4 g of each variety (from each sampling moment) were crushed manually and inserted into a 20 mL glass vial. After the addition of 5 mL of ultra-pure water, 2 g of sodium chloride (NaCl) and stirring (0.5 × 0.1 mm bar) at 400 rpm, which corresponds to a volume ratio of 0.5 of the liquid phase relatively to the headspace volume (1/β), the vial was capped with a PTFE septum and an aluminium cap (Chromacol, Hertfordshire, UK). The vial was placed in a thermostated bath adjusted to 60.0 ± 0.1 °C for 5min, and then the SPME fibre was inserted in the headspace for 20 min. All measurements were performed in triplicate.

GC×GC-ToFMS analysis

The analysis of volatile composition of the 7 *V. vinifera* under study were carried out based on a previously reported work (Perestrelo *et al.*, 2010) that uses a LECO Pegasus 4D (LECO, St. Joseph, MI, USA) GC×GC-ToFMS system consisted of an Agilent GC 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE), with a dual stage jet cryogenic modulator (licensed from Zoex) and a secondary oven, and mass spectrometer equipped with a high resolution ToF analyzer. After the extraction/concentration step, the SPME coating fibre was manually introduced into the GC×GC-ToFMS injection port at 250 ºC and kept for 3 min for desorption. The injection port was lined with a 0.75 mm I.D. splitless glass liner. Splitless injections were used (30 s). An Equity-5 column (30 m x 0.32 mm I.D., 0.25 µm film thickness, Supelco, Inc., Bellefonte, PA, USA) was used as first-dimension (^{1}D) column and a DB-FFAP (0.79 m x 0.25 mm I.D., 0.25 µm film thickness, J&W Scientific Inc., Folsom, CA, USA) was used as a second-dimension (^{2}D) column. The carrier gas was helium at a constant flow rate of 2.50 ml/min. The primary oven temperature program was: initial temperature 40 ºC (hold 1 min), and then raised to 230 ºC (10 ºC min^{-1}) (hold 2 min). The secondary oven temperature program was 30 ºC offset above the primary oven. The MS transfer line and the MS source temperatures were 250 ºC. The modulation time was 6 s; the modulator temperature was kept at 20 ºC offset (above secondary oven). A 6 s modulation time with a 20 ºC secondary oven temperature offset was chosen to be a suitable compromise as it maintained the 1D separation, maximized the 2D resolution, and avoided the wrap-around effect (i.e. when the elution time of a pulsed solute exceeds the modulation period) for compounds that were late to elute from the ^{2}D. Ideally, all peaks must be detected before the subsequent re-injection and, hence, ^2t_R must be equal or less than the modulation period (Dallüge *et al.*, 2003; Mondello *et al.*, 2008). The ToFMS was operated at a spectrum storage rate of 100 spectra/s. The mass spectrometer was operated in the EI mode at 70 eV using a range of *m/z* 35-350 and the detector voltage was -1434 V. Total ion chromatograms (TIC) were processed using the automated data processing software ChromaTOF (LECO) at signal-to-noise threshold of 100.

Contour plots were used to evaluate the separation general quality and for manual peak identification. For identification purposes, the mass spectrum and retention times (^{1}D and ^{2}D) of the analytes were compared with standards, when available. Also, the mass spectrum of each compound detected was compared to those in mass spectral libraries, which included an in-house library of standards and two commercial databases (Wiley 275 and US National Institute of Science and Technology (NIST) V. 2.0 – Mainlib and Replib). The identification was also supported by the experimentally determined retention index (RI) values that were compared, when available, with the values reported in the bibliography for chromatographic columns similar to the one used in the present PhD thesis for one dimensional GC with 5%-phenyl-methylpolysiloxane GC column or equivalent (Adams, 1995, 2000; Adams *et al.*, 2005; Alissandrakis *et al.*, 2007; Araujo *et al.*, 2003; Baccouri *et al.*, 2007; Beaulieu and Grimm, 2001; Buchin, 2002; Carrapiso *et al.*, 2002; Cho *et al.*, 2007; Couladis *et al.*, 2001; D'Arcy *et al.*, 1997; Dickschat *et al.*, 2005; El-Sayed *et al.*, 2005; Engel *et al.*, 2002; Engel and Ratel, 2007; Ferhat *et al.*, 2007; Flach *et al.*, 2004; Flamini *et al.*, 2002; Hazzit *et al.*, 2006; Hognadottir and Rouseff, 2003; Isidorov *et al.*, 2003; Jalali-Heravi *et al.*, 2006; Kim *et al.*, 2006; Kobaisy *et al.*, 2002; Lalel *et al.*, 2003a; Marongiu *et al.*, 2006; Mevy *et al.*, 2006; Mondello and Costa, 2006; Pino *et al.*, 2003; Pino *et al.*, 2005; Radulović *et al.*, 2012; Robinson, 2006; Sarikurkcu *et al.*, 2008; Saroglou *et al.*, 2006; Setzer *et al.*, 2006; Vasta *et al.*, 2007; Vujisic *et al.*, 2006; Zhao *et al.*, 2006; Zhao *et al.*, 2008) and for comprehensive GC×GC system with Rxi-5SilMS and VF-5MS for the ^{1}D column (Robinson *et al.*, 2011; Weldegergis *et al.*, 2011) or with the same column set used in this PhD thesis - Equity-5 ^{1}D column and a DB-FFAP for ^{2}D column (Jalali *et al.*, 2012; Jalali *et al.*, 2013; Petronilho *et al.*, 2011; Petronilho *et al.*, 2013; Rocha *et al.*, 2012; Rocha *et al.*, 2007a; Rocha *et al.*, 2013; Silva *et al.*, 2010). RI values were determined using a C_8-C_{20} *n*-alkanes series (the solvent *n*-hexane was used as C_6 standard) and calculated according to the van den Dool and Kratz equation (van den Dool and Kratz, 1963). The majority (> 85%) of the identified compounds presented similarity matches ≥ 850 (850/1000). The DTIC (Deconvoluted Total Ion Current) GC×GC area data were used as an approach to estimate the relative content of each volatile component in the grape varieties under study, and were expressed as arbitrary units (a. u.). Three independent replicates were analysed for each variety at the different conditions under study. Reproducibility was expressed as relative standard deviation (RSD). Detail data was given in supplementary information in a CD-R.

III. 2. 3. Results and Discussion

The volatile composition of each variety under study was analyzed by GC×GC-ToFMS. The obtained GC×GC-ToFMS total ion chromatogram contour plot (data not shown) exhibited several hundreds of peaks. As several hundreds of chromatograms were obtained for the different varieties and conditions under study, only one illustrative figure of these chromatograms were shown (Figure 34). Figure 34 shows a blow-up of part of the chromatogram contour plot obtained for Arinto variety (for AR-VA2 parcel), indicating the chromatographic spaces corresponding to varietal (aromatic alcohols, mono (C_{10}) and sesquiterpenic compounds (C_{15}) and C_{13} norisoprenoids) and pre-fermentative (C_6 alcohols and aldehydes) volatile compounds determined at half-*véraison* and at technologic maturity. This figure clearly shows the different volatile composition of grapes collected at half-*véraison* when compared to technologic maturity state.

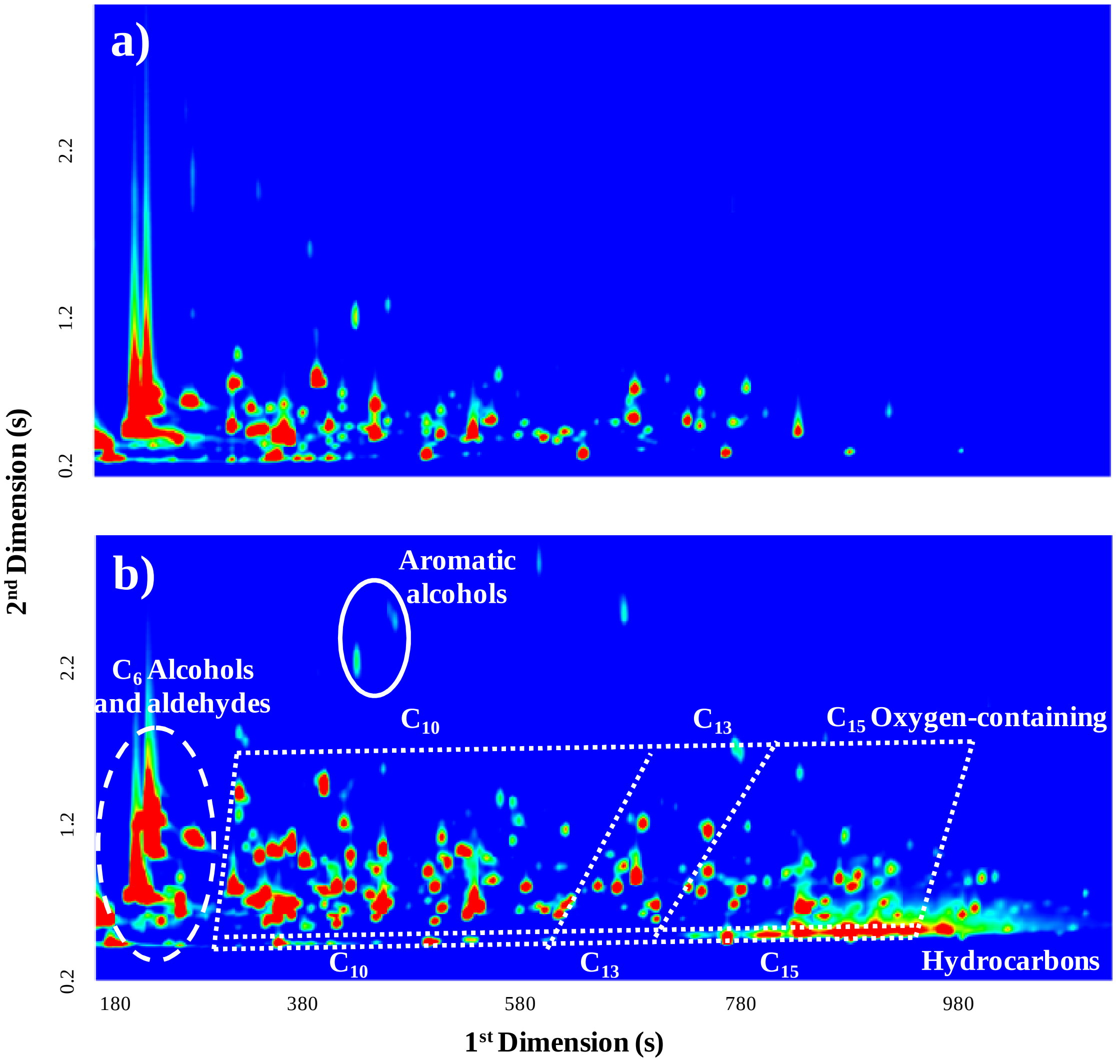


Figure 34. Blow-up of a part of GC×GC-ToFMS chromatogram contour plot obtained for Arinto variety in full-scan acquisition mode for a) grapes collected at half-*véraison* and b) grapes collected at technologic maturity. The chromatographic spaces corresponding to C_6 alcohols and aldehydes, aromatic alcohols, mono (C_{10}) and sesquiterpenic compounds (C_{15}) and C_{13} norisoprenoids were highlighted.

The GC×GC analysis was performed on a system comprising a non-polar thick-film ^{1}D (first-dimension) column and a ^{2}D (second-dimension) column containing a thin-film polar stationary phase. This column combination provided two almost independent separations (orthogonality). On the non-polar column, analytes were separated according to their vapour pressure/volatility, and on the polar column, analytes were separated according to their polarity. Consequently, compounds with similar vapour pressures had similar retention times in the ^{1}D and compounds with similar polarities had similar retention times in the ^{2}D. Thus, it was expected that structurally related compounds exhibited similar elution order, i.e. they eluted within a cluster in a GC×GC plane (Rocha *et al.*, 2007). Therefore, it was possible to relate their chemical structures with their chromatographic position (Marriott *et al.*, 2004). According to the ^{1}D, the GC×GC-ToFMS extracted ion chromatogram contour plots (Figure 34) shows that the compounds were organized into four groups: the C_6 alcohols and aldehydes, the C_{13} norisoprenoids, and the mono (C_{10}) and sesquiterpenic components (C_{15}). However, according to the ^{2}D, the GC×GC–ToFMS extracted ion chromatogram contour plot shows that the compounds were organized into five groups: the C_6 alcohols and aldehydes, the aromatic alcohols, the C_{13} norisoprenoids, the hydrocarbons (C_{10} and C_{15}) placed in the lower retention times (lower polarity) and the terpene oxygen-containing (C_{10} and C_{15}) placed in the higher retention times (higher polarity). Thus, a classification based on the presence of ordered structures in the GC×GC chromatogram of structurally related compounds was observed (Figure 34). The peak finding routine based on deconvolution method allowed to identify a total of 95 varietal and pre-fermentative compounds, in the 7 *V. vinifera* varieties and conditions studied, which were identified based on comparison of their mass spectra to mass spectra of high purity chemical standards, reference commercial and in-house MS databases, and by comparison of the RIs calculated (RI_{calc}) with the values reported in the literature (RI_{lit}) for the columns used (or equivalents) (Table 4). Table 4 summarizes the information related with the varietal and pre-fermentative volatile compounds determined by GC×GC-ToFMS analysis in the 7 *V. vinifera* varieties, obtained from the different parcels of São Mateus and Vale de Azar vineyards under study, during 3 consecutive harvests, grouped by chemical families: 7 C_6 compounds (alcohols and aldehydes), 3 aromatic alcohols, 17 norisoprenoids (1 C_9 and 16 C_{13} norisoprenoids), and 68 terpenic compounds (47 mono-, 20 sesqui-, and 1 diterpenic compounds).

Table 4. Volatile components determined in *Vitis vinifera* L. cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional, during maturation, for the parcels and harvests under study.

					$RI_{lit.}$^{c}		Ref. $RI_{lit.}$^{d}		Varieties						
^{1}Dtr(s),^{2}Dtr(s)^a	Compound	CAS number	Formula	$RI_{calc.}$^{b}	1D-GC	GC×GC	1D-GC	GC×GC	Arinto	Bical	Sauvignon Blanc	Baga	Castelão	Touriga Nacional	Sousão
C_6 compounds															
194, 0.640	Hexanal	66-25-1	$C_6H_{12}O$	801	800	800	(Adams, 1995)	(Rocha *et al.*, 2012)	x	x	x	x	x	x	x
206, 0.630	3-Hexenal	6789-80-6	$C_6H_{10}O$	805	803	_	(Carrapiso *et al.*, 2002)	_	x	x	x	x	x	x	x
230, 0.651	2-Hexenal	6728-26-3	$C_6H_{10}O$	851	854	855	(Adams, 2000)	(Rocha *et al.*, 2013)	x	x	x	x	x	x	x
242, 0.630	3-Hexen-1-ol	928-96-1	$C_6H_{12}O$	858	857	861	(Adams, 2000)	(Rocha *et al.*, 2013)	x	x	x	x	x	x	x
248, 1.076	2-Hexen-1-ol	928-95-0	$C_6H_{12}O$	864	862	_	(Baccouri *et al.*, 2007)	_	x	x	x	x	x	x	x
266, 0.903	1-Hexanol	111-27-3	$C_6H_{14}O$	876	865	877	(Cho *et al.*, 2007)	(Rocha *et al.*, 2013)	x	x	x	x	x	x	x
296, 0.930	2,4-Hexadienal	142-83-6	C_6H8O	914	912	_	(Engel and Ratel, 2007)	_	x	x	x	x	x	x	x
Aromatic alcohols															
420, 3.014	Benzyl Alcohol	100-51-6	C_7H_8O	1048	1052	1044	(Dickschat *et al.*, 2005)	(Robinson *et al.*, 2011)	x	x	x	x	x	x	x
446, 1.426	α,α-Dimethyl Benzyl alcohol	617-94-7	$C_9H_{12}O$	1089	1102	_	(Dickschat *et al.*, 2005)	_	x	x	x	x	x	x	
470, 1.960	2-Phenylethanol	60-12-8	$C_8H_{10}O$	1115	1129	1107	(Dickschat *et al.*, 2005)	(Weldegergis *et al.*, 2011)	x	x	x	x	x	x	x
C_9 Norisoprenoid															
506, 0.761	Norinone	38651-65-9	$C_9H_{14}O$	1142	1142	1183	(Zeng *et al.*, 2007)	(Rocha *et al.*, 2007a)				x	x	x	
Monoterpenic compounds															
314, 0.440	α-Pinene	80-56-8	$C_{10}H_{16}$	938	939	941	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
338, 0.480	Dehydroxylinalooloxide	13679-86-2	$C_{10}H_{18}O$	972	971	_	(Pino *et al.*, 2003)	_	x				x	x	x
344, 0.457	β-Pinene *	18172-67-3	$C_{10}H_{16}$	988	989	987	(Buchin, 2002)	(Jalali et al., 2012)	x	x	x	x	x	x	x
356, 0.570	β-Myrcene	123-35-3	$C_{10}H_{16}$	1001	991	1008	(Adams, 1995)	(Jalali *et al.*, 2012)	x		x	x			
362, 0.520	3-Carene	13466-78-9	$C_{10}H_{16}$	1007	1004	1020	(Engel *et al.*, 2002)	(Jalali *et al.*, 2012)			x			x	
368, 0.790	α-Phellandrene	99-83-2	$C_{10}H_{16}$	1013	1010	_	(Jalali-Heravi *et al.*, 2006)	_			x				
392, 0.405	*m*-Cymene	535-77-3	$C_{10}H_{14}$	1025	1020	1027	(Araujo *et al.*, 2003)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x

398, 0.476	Limonene *	138-86-3	$C_{10}H_{16}$	1028	1029	1035	(Adams *et al.*, 2005)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
404, 0.476	1,8-Cineole	470-82-6	$C_{10}H_{18}O$	1034	1032	1039	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
416, 0.560	β-Ocimene	3779-61-1	$C_{10}H_{16}$	1045	1050	1043	(Adams, 2000)	(Jalali *et al.*, 2012)	x		x	x		x	
428, 0.678	Linalool oxide (isomer)		$C_{10}H_{18}O_2$	1071	1071	1078	(Saroglou *et al.*, 2006)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
434, 0.727	Dihydromyrcenol	53219-21-9	$C_{10}H_{20}O$	1073	1073	1076	(Vasta *et al.*, 2007)	(Rocha *et al.*, 2007a)	x	x	x	x	x	x	x
440, 0.560	α-Terpinolene	586-62-9	$C_{10}H_{16}$	1076	1088	1097	(Adams, 2000)	(Jalali *et al.*, 2012)	x		x	x	x	x	
440, 0.790	Linalool oxide (isomer)		$C_{10}H_{18}O_2$	1076	1087	1097	(Saroglou *et al.*, 2006)	(Jalali *et al.*, 2012)			x		x	x	
446, 0.700	Dihydrolinalool	78-69-3	$C_{10}H_{22}O$	1088	1097	_	(Pino *et al.*, 2005)	_	x	x	x	x	x	x	
452, 0.746	Linalool *	78-70-6	$C_{10}H_{18}O$	1096	1098	1108	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
464, 0.600	Rose oxide (isomer)		$C_{10}H_{18}O$	1107	1111	1117	(Adams, 1995)	(Jalali *et al.*, 2012)	x		x	x	x	x	
464, 0.844	Fenchol	22627-95-8	$C_{10}H_{18}O$	1108	1112	1118	(Couladis *et al.*, 2001)	(Jalali *et al.*, 2012)	x	x	x	x	x		x
470, 0.646	Hotrienol	53834-70-1	$C_{10}H_{16}O$	1113	1114	1122	(D'Arcy *et al.*, 1997)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
470, 0.780	Camphenal	4501-58-0	$C_{10}H_{14}O$	1114	1125	_	(Adams, 2000)	_			x	x	x		
476, 0.770	Rose oxide (isomer)		$C_{10}H_{18}O$	1118	1127	1130	(Adams, 1995)	(Jalali *et al.*, 2012)	x						
482, 0.890	1-Terpineol	586-82-3	$C_{10}H_{18}O$	1120	1127	_	(Kim *et al.*, 2006)	_			x	x			
488, 0.690	Cosmene	460-01-5	$C_{10}H_{14}$	1122	1134	_	(Flamini *et al.*, 2002)	_	x		x				
494, 1.050	Pinocarveol	547-61-5	$C_{10}H_{16}O$	1130	1139	1142	(Adams, 2000)	(Jalali *et al.*, 2012)		x			x		
500 , 0.970	β-Terpineol	138-87-4	$C_{10}H_{18}O$	1136	1137	1150	(Isidorov *et al.*, 2003)	(Jalali *et al.*, 2012)			x			x	
506, 1.190	Pinocarvone	34-41-3	$C_{10}H_{14}O$	1140	1144	1164	(Boskovic *et al.*, 2005)						x		x
512, 0.635	Nerol oxide	1786-08-9	$C_{10}H_{16}O$	1151	1151	1172	(Saroglou *et al.*, 2006)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
518, 0.834	Ocimenol	5986-38-9	$C_{10}H_{18}O$	1166	1155	1179	(Zhao *et al.*, 2008)	(Jalali *et al.*, 2012)	x	x	x		x		x
518, 1.200	*m/z* 68, 94, 79, 109 (alcohol)		$C_{10}H_{18}O_2$	1167	_	_	_	_			x		x	x	
524, 0.860	Borneol	507-70-0	$C_{10}H_{18}O$	1169	1165	1172	(Adams, 1995)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
530, 0.884	*p*-Mentha-1,5-dien-8-ol	1686-20-0	$C_{10}H_{16}O$	1171	1170	1172	(Adams *et al.*, 2005)	(Jalali *et al.*, 2012)			x	x	x		
530, 0.984	Menthol *	1490-04-6	$C_{10}H_{20}O$	1175	1170	_	(Flach *et al.*, 2004)	_	x	x	x	x	x	x	x
536, 0.715	Terpinen-4-ol	562-74-3	$C_{10}H_{18}O$	1183	1177	1181	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
536, 1.269	*p*-Cymen-8-ol	1197-01-9	$C_{10}H_{14}O$	1184	1183	1203	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
542, 0.835	α-Terpineol *	98-55-5	$C_{10}H_{18}O$	1195	1189	1206	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
548, 0.850	Dihydrocarvone	7764-50-3	$C_{10}H_{16}O$	1197	1193	1211	(Adams, 1995)	(Jalali *et al.*, 2012)				x			
554, 0.900	Safranal	116-26-7	$C_{10}H_{14}O$	1199	1197	_	(Saroglou *et al.*, 2006)	_			x				

560, 0.850	Verbenone	80-57-9	$C_{10}H_{14}O$	1214	1204	1214	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
566, 0.703	p-Menth-1-en-9-al	29548-14-9	C10H16O	1217	1217	1219	(Pino *et al.*, 2003)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
572, 1.340	2-Hydroxycineole	92999-78-5	$C_{10}H_{18}O_2$	1219	1228	1237	(Setzer *et al.*, 2006)	(Petronilho *et al.*, 2013)			x		x		
578, 0.700	*m/z* 93, 121, 119, 136 (alcohol)		$C_{10}H_{16}O$	1224	_	_	_	_					x		
584, 0.873	Geraniol (isomer) *		$C_{10}H_{18}O$	1235	1224	1235	(Vujisic *et al.*, 2006)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
584, 0.943	β-Citronellol *	106-22-9	$C_{10}H_{20}O$	1237	1233	1237	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
590, 0.737	Geraniol (isomer) *		$C_{10}H_{18}O$	1244	1237	1242	(Ferhat *et al.*, 2007)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	
596, 0.976	Citral (isomer)		$C_{10}H_{16}O$	1247	1240	1245	(Beaulieu and Grimm, 2001)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
602, 0.815	Carvone *	99-49-0	$C_{10}H_{14}O$	1251	1253	1245	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	x	x
626, 0.775	Citral (isomer)		$C_{10}H_{16}O$	1274	1270	1287	(Beaulieu and Grimm, 2001)	(Rocha *et al.*, 2007a)	x	x	x	x	x	x	
C_{13} Norisoprenoids															
566, 0.532	*m/z* 159, 91, 131 (hydrocarbon)		$C_{13}H_{18}$	1216	_	_	_	_	x	x	x	x	x	x	x
602, 0.660	α-Ionene	475-03-6	$C_{13}H_{19}$	1250	1266	1261	(El-Sayed *et al.*, 2005)	(Robinson *et al.*, 2011)	x			x			
620, 0.595	Vitispirane	65416-59-3	$C_{13}H_{20}O$	1286	1281	1287	(Pino *et al.*, 2003)	(Robinson *et al.*, 2011)	x	x	x	x	x	x	x
632, 0.517	Theaspirane (isomer)		$C_{13}H_{22}O$	1302	1313	1305	(Sarikurkcu *et al.*, 2008)	(Robinson *et al.*, 2011)	x	x	x	x	x		
644, 0.528	Theaspirane (isomer)		$C_{13}H_{22}O$	1323	1331	1322	(Sarikurkcu *et al.*, 2008)	(Robinson *et al.*, 2011)	x	x	x	x	x	x	
668, 0.790	TDN (1,2-Dihydro-1,1,6-trimethyl- naphthalene)	30364-38-6	$C_{13}H_{16}$	1357	1355	1361	(Alissandrakis *et al.*, 2007)	(Robinson et al., 2011)	x		x				
674, 0.681	β-Damascenone (isomer)		$C_{13}H_{18}O$	1369	1359	1364	(Kobaisy *et al.*, 2002)	(Robinson *et al.*, 2011)	x	x	x	x	x	x	x
680, 0.840	*m/z* 142, 157, 115 (ketone)		$C_{13}H_{18}O$	1371	_	_	_	_	x			x	x		
700, 0.702	β-Damascenone (isomer)		$C_{13}H_{18}O$	1383	1381	1385	(Kobaisy *et al.*, 2002)	(Robinson *et al.*, 2011)	x	x	x	x	x	x	x
724, 0.750	Hydroxydihydroedulan		$C_{13}H_{22}O_2$	1446	1446	_	(Radulović *et al.*, 2012)	_	x	x					
736, 0.648	Geranylacetone *	3796-70-1	$C_{13}H_{22}O$	1455	1449	1454	(Adams *et al.*, 2005)	(Rocha *et al.*, 2013)	x	x	x	x	x	x	x
742, 0.850	5,6-Epoxy-β-ionone	23267-57-4	$C_{13}H_{20}O_2$	1463	1473	_	(Mevy *et al.*, 2006)	_	x	x	x	x	x	x	
760, 0.868	3,4-Dehydro-β-ionone	1203-08-3	$C_{13}H_{18}O$	1474	1485	1483	(Zhao *et al.*, 2006)	(Robinson *et al.*, 2011)	x	x	x		x	x	
778, 0.635	α-Iso-methyl ionone	127-51-5	$C_{14}H_{22}O$	1485	1471	_	(Mondello and Costa, 2006)	_	x	x	x	x	x	x	
784, 0.717	β-Ionone *	79-77-6	$C_{13}H_{20}O$	1488	1485	1494	(Adams, 1995)	(Silva *et al.*, 2010)	x	x	x	x	x	x	
900, 0.894	Methyl dihydrojasmonate	24851-98-7	$C_{13}H_{22}O_3$	1661	1650	_	(Mondello and Costa, 2006)	_	x	x	x	x	x	x	x
Sesquiterpenic compounds															

650, 0.521	δ-Elemene	20307-84-0	$C_{15}H_{24}$	1329	1330	1330	(Jantan *et al.*, 2003)	(Jalali *et al.*, 2013)				x	x	x	
656, 0.583	Longipinene epoxide	142792-93-6	$C_{15}H_{24}$	1337	1334	_	(Marongiu *et al.*, 2006)	_	x	x	x				
680, 0.469	α-Copaene	3856-25-5	$C_{15}H_{24}$	1371	1376	1375	(Adams, 1995)	(Jalali *et al.*, 2013)		x	x		x	x	
686, 0.510	β-Bourbonene	5208-59-3	$C_{15}H_{24}$	1379	1381	1379	(Saroglou *et al.*, 2006)	(Jalali *et al.*, 2013)				x		x	
712, 0.508	Longifolene	475-20-7	$C_{15}H_{24}$	1414	1416	1395	(Adams *et al.*, 2005)	(Jalali *et al.*, 2013)	x	x	x				
718, 0.481	β-Caryophyllene	87-44-5	$C_{15}H_{24}$	1418	1418	1417	(Adams *et al.*, 2005)	(Jalali *et al.*, 2013)				x			
724, 0.541	α-Humulene	6753-98-6	$C_{15}H_{24}$	1445	1455	1450	(Adams *et al.*, 2005)	(Jalali *et al.*, 2013)			x		x		
756, 0.630	Aromadendrene	489-39-4	$C_{15}H_{24}$	1477	1484	1478	(Robinson, 2006)	(Petronilho *et al.*, 2013)	x	x	x	x	x	x	x
762, 0.450	α-Muurolene	31983-22-9	$C_{15}H_{24}$	1485	1499	1490	(Adams, 2000)	(Jalali *et al.*, 2013)			x		x		
790, 0.660	α-Farnesene	502-61-4	$C_{15}H_{24}$	1501	1508	1505	(Adams, 2000)	(Petronilho *et al.*, 2011)	x	x	x		x	x	x
796, 0.525	γ-Cadinene	39029-41-9	$C_{15}H_{24}$	1504	1513	1511	(Adams, 1995)	(Jalali *et al.*, 2013)			x	x			
808, 0.630	Calamenene	483-77-2	$C_{15}H_{22}$	1514	1521	1520	(Adams, 2000)	(Jalali *et al.*, 2013)	x	x	x		x	x	x
826, 0.629	α-Calacorene	21391-99-1	$C_{15}H_{20}$	1555	1542	1554	(Adams, 2000)	(Jalali *et al.*, 2013)		x	x	x	x	x	
832, 0.880	Nerolidol	7212-44-4	$C_{15}H_{26}O$	1560	1564	1568	(Adams, 1995)	(Jalali *et al.*, 2013)	x	x	x	x	x	x	
844, 0.810	Epiglobulol	88728-58-9	$C_{15}H_{26}O$	1579	1582	_	(Robinson, 2006)	_	x						
850, 0.751	Globulol	489-41-8	$C_{15}H_{26}O$	1594	1598	1592	(Robinson, 2006)	(Petronilho *et al.*, 2013b)		x				x	
862, 0.726	Caryophyllene oxide	1139-30-6	$C_{15}H_{24}O$	1605	1606	1601	(Hognadottir and Rouseff, 2003)	(Jalali *et al.*, 2013)			x		x	x	
886, 0.690	β-Eudesmol	77-53-2	$C_{15}H_{26}O$	1642	1649	1642	(Adams, 1995)	(Jalali *et al.*, 2013)	x	x	x	x	x	x	
912, 0.654	*m/z* 119, 91, 191, 109 (alcohol)		$C_{15}H_{26}O$	1675	_	_	_	_	x	x	x		x	x	x
942, 0.820	Farnesal	502-67-0	$C_{15}H_{24}O$	1731	1730	1724	(Adams, *et al.*, 2005)	(Jalali *et al.*, 2013)			x				
Diterpenic compound															
1116, 0.929	Phytol	596-84-9	$C_{20}H_{34}O$	2021	2022	2022	(Hazzit *et al.*, 2006)	(Petronilho *et al.*, 2013b)		x		x		x	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] RI_{calc}: retention index obtained through the modulated chromatogram.

[c] RI_{lit}: retention index reported in the literature for 5% phenyl polysilphenylene-siloxane GC column or equivalents, reported for 1D-GC and GC×GC.

[d] Ref. RI_{lit}: references found in the literature for 5% phenyl polysilphenylene-siloxane GC column or equivalents, reported for 1D-GC and GC×GC.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

Notation **x** indicates that the compound was determined in the corresponding variety. Detailed data related to the volatile components determined for each grape variety under study, during maturation, for 3 parcels and 3 consecutive harvests, was given in Supplementary Tables S8 to S14.

For straight through and rapid interpretation of the volatile composition determined for the different varieties, each one obtained from 3 parcels and 3 harvests, a graphical representation of the total GC×GC peak area (a.u.) of each compound was performed, organized by chemical families (from Figure 35 to Figure 41). For each XY graph “0” in the x axis refers to the first sampling moment performed at half-*véraison*. Besides, the same y-axis scale was kept for the 3 harvests, in order to obtain a rapid visual access of each variety volatile composition, allowing the comparison of the similarities and differences between the different parcels and harvests under study. Detailed data was given in Supplementary Tables S8 to S14 in a CD-R.

Arinto

According to Figure 35, the content of Arinto varietal volatile components increased in both parcels and harvests under study, in the first or second weeks after half-*véraison* and then tends to stabilize, as observed for terpenic compounds, or continues to increase until technologic maturity (the case of aromatic alcohols). The exception was observed for C_{13} norisoprenoids that increased in the first week after half-*véraison* and then a continuous decrease was observed. This could be explained by the fact that between *véraison* and technologic maturity occur the glycosylation of norisoprenoids, leading to a decrease of these compounds in the free form (Baumes *et al.*, 2002). Besides the variability on the composition of pre-fermentative compounds, these compounds tend to increase until technologic maturity. However, a maximum was reached in 2010 at day 7 for AR-VA1 grapes and in 2011 at day 35 for AR-VA1 and AR-VA, decreasing in the following weeks to values that approached those observed at half-*véraison* (Figure 35).

Despite the observed trend through maturation, several differences between grapes volatile composition from the 3 parcels were observed (Figure 35): similarly to the tendency observed for Arinto phenolic content and antiradical activity, the total GC×GC peak area of varietal compounds (mono- and sesquiterpenic compounds and also aromatic alcohols) tends to be higher in grapes from AR-VA2 (clay-sandy soil, ca. 70 m, near to pine trees, rows guided North-South direction) followed by AR-SM1 (clay-calcareous soil, ca. 50 m of altitude, open space, rows guided East-West direction) parcels than in AR-VA1 (clayey soil, ca. 50 m, open space, rows guided North-South direction). The areas of C_{13} norisoprenoids and C_6 compounds were similar in grapes from both parcels.

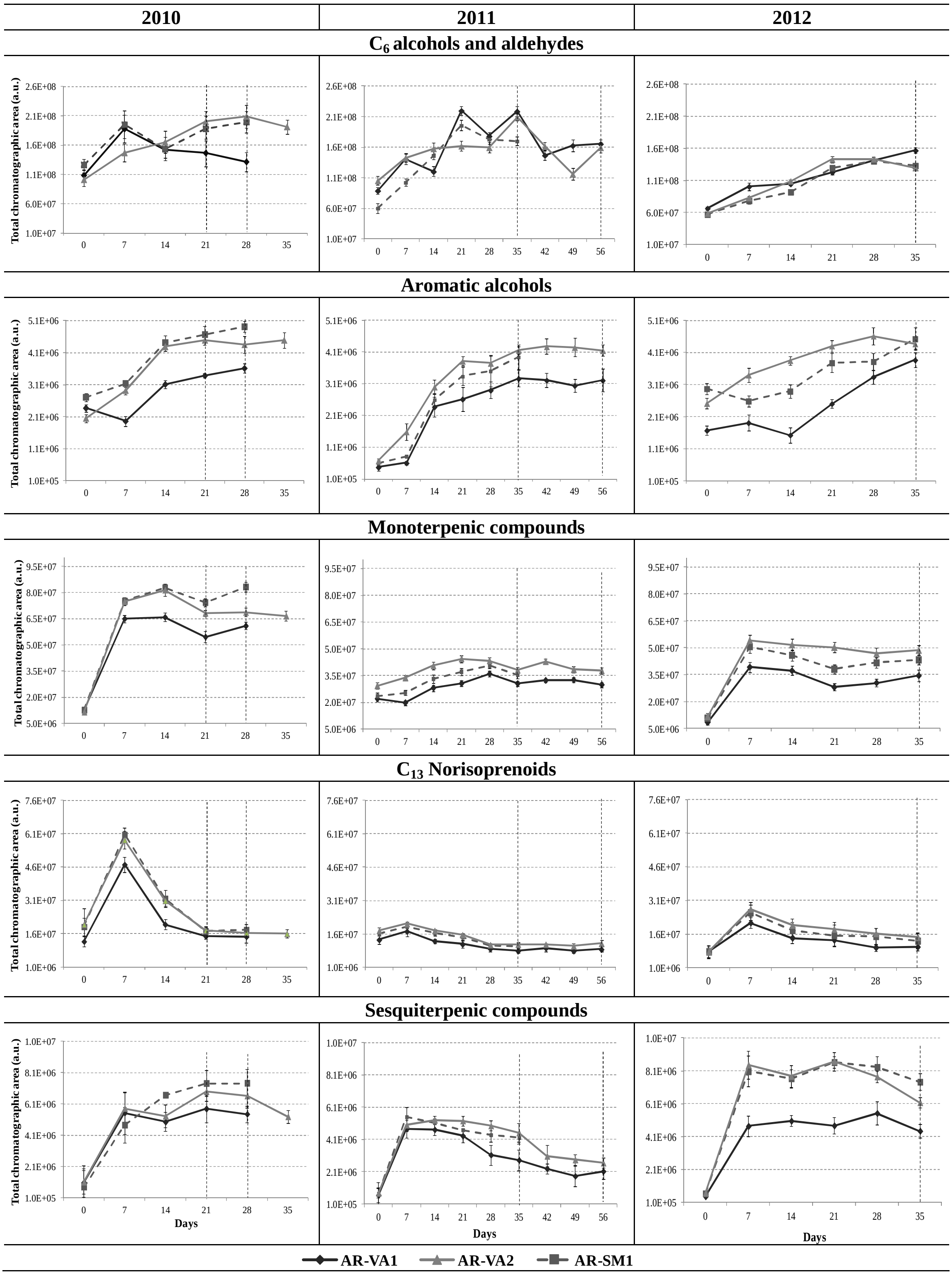


Figure 35. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Arinto maturation, obtained from AR-VA1, AR-VA2, and AR-SM1 parcels, along 3 harvests (2010-2012). Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S8 in a CD-R.

As AR-VA1 parcel (clayey soil) was at an open space and at ca. 50 m, similarly to AR-SM1, and the rows orientations were similar to AR-VA2 (North-South direction), the lower level of volatile components of grapes from AR-VA1 parcel can be explained by the different soil types (Figure 35): clayey soil (AR-VA1) with higher water-holding capacity, seems to be related with lower grapes varietal volatile content when compared to the other soils parcels (clay-calcareous and clay-sandy). This was in accordance with a recent study performed with sparkling wines obtained from Fernão-Pires white variety, from Bairrada Appellation, where wines produced from the clay-calcareous and sandy soils presented the highest content of varietal compounds (Coelho *et al.*, 2009). These results showed that middle to lower water-holding capacity soils seem to favour Arinto volatile composition.

Arinto grapes volatile composition was also different from one harvest to another (Figure 35): in 2011 the GC×GC peak areas of terpenic compounds and C_{13} norisoprenoids were lower when compared to the other harvests, increasing from 2011 to 2010. The content of C_6 compounds and aromatic alcohols were similar between the 3 harvests. The warm and dry conditions of 2011 harvest and the fresh and rainy ones of 2012 seem to be related with lower varietal volatile content of Arinto grapes, while the moderate climatic conditions of 2010 harvest seems to favour grapes volatile composition. It was already shown that vines that experience hot and dry climates (Ribéreau-Gayon *et al.*, 2006) or higher precipitation amounts (Jackson and Lombard, 1993) cause grapes aromatic quality losses.

Arinto variety is known to resist to different year climatic condition (Chapter II). Although sugar content and titratable acidity were kept constant during a long period in 2011 harvest, without rot appearance or berry dehydration (Figure 20) resisting to warm conditions of this year, the hot temperatures may influence sesquiterpenic composition of Arinto grapes, allowing sesquiterpenic content to slightly decrease from day 35 until technologic maturity (Figure 35). These compounds provide a wide spectrum of aromas, mostly perceived as very pleasant and may contribute positively to the aroma perception of the final wines.

Bical

Figure 36 shows the total GC×GC peak area of varietal- and pre-fermentative-related volatile compounds, during the maturation process of Bical. For the parcels and harvests under study, the content of volatile components increased in the first week after half-*véraison* and then tends to stabilize (monoterpenic compounds), or continues to increase until technologic maturity (aromatic alcohols and sesquiterpenic compounds). Similarly with Arinto, C_{13} norisoprenoids increased in the first week after half-*véraison* and then a continuous decrease was observed (norisoprenoids glycosylation).

Several differences between grapes volatile composition from the 3 parcels were observed (Figure 36): with the exception of C_6 compounds, which content was very similar between grapes from the 3 parcels, the volatile composition was, during maturation, always lower in BI-VA1 parcel than in BI-SM1 and BI-VA2. This trend was followed by terpenic compounds, principally the monoterpenic ones, which were the major contributing group for the total area of Bical varietal volatile compounds. This was in accordance with phenolic content and antiradical activity of Bical grapes from the 3 parcels (Figure 28). According to the main characteristics of Bical parcels (Figure 17, Table 3), grapes from clay-calcareous (BI-VA2) and clay-sandy (BI-SM1) soils with middle and lower water-holding capacity, exhibited the higher volatile content comparatively to grapes from clayey soil (Figure 36). Thus, higher water-holding capacity soil seems to be related with lower Bical volatile content. There was also pine trees located at East of BI-VA1 parcel, reducing grapes sunlight exposure in the first hours at the morning, while the other two parcels are at open spaces (Figure 17, Table 3). This may modulate BI-VA1 grapes volatile composition, since lower sun exposed berries inhibited the synthesis and accumulation of terpenic compounds and C_{13} norisoprenoids (Reynolds and Wardle, 1989b; Skinkis, 2010; Zhang *et al.*, 2014).

The harvest climatic conditions also influence Bical grapes composition: in 2011, the GC×GC peak areas were lower, principally regarding the varietal components, followed by grapes from 2012, and higher in grapes from 2010. Warm conditions of 2011 harvest accelerate grapes maturation process, principally for grapes from BI-SM1 and BI-VA1, allowing grapes to achieve technologic maturity earlier comparing with the other harvests. However, this is not followed with a rapidly accumulation of volatiles.

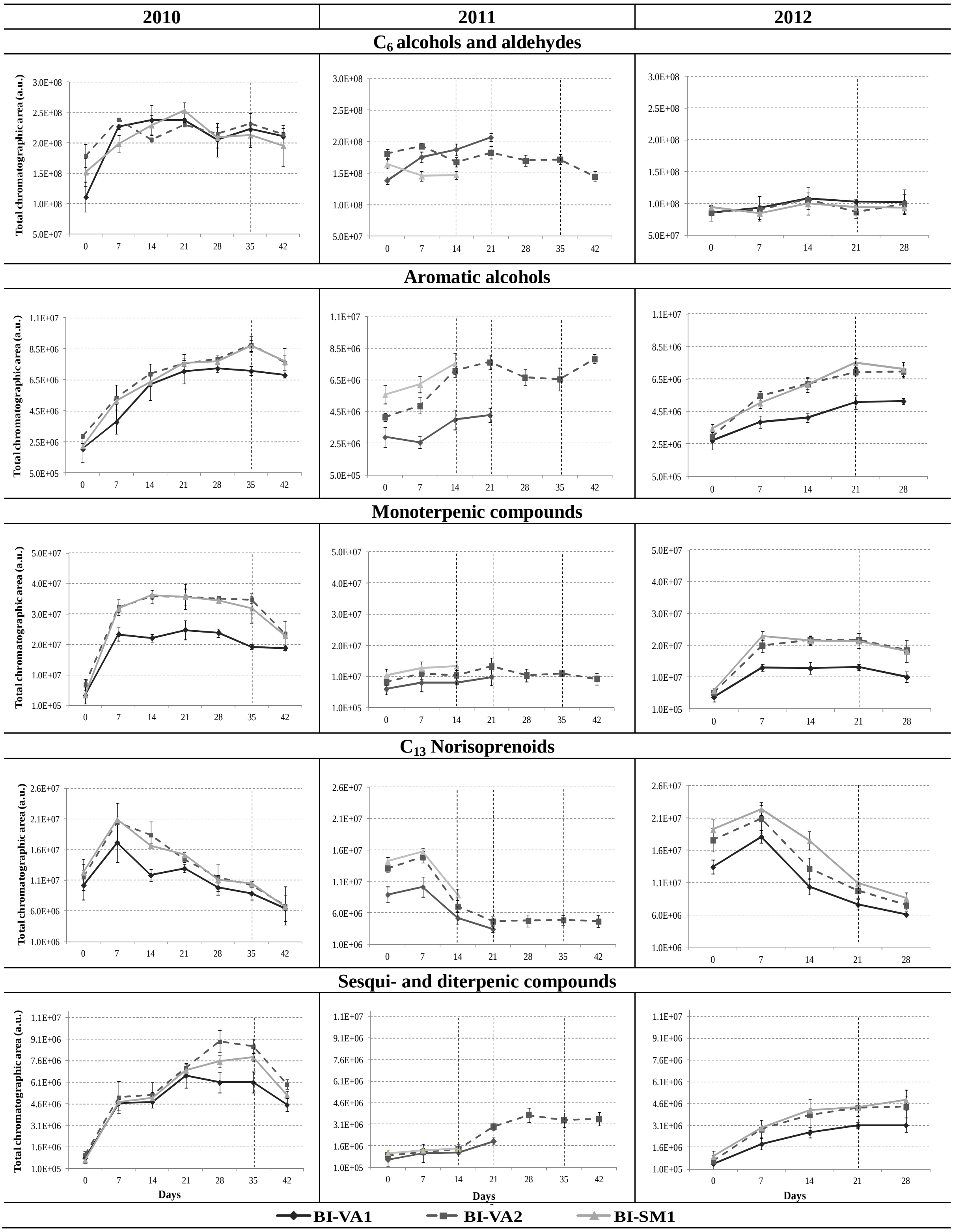
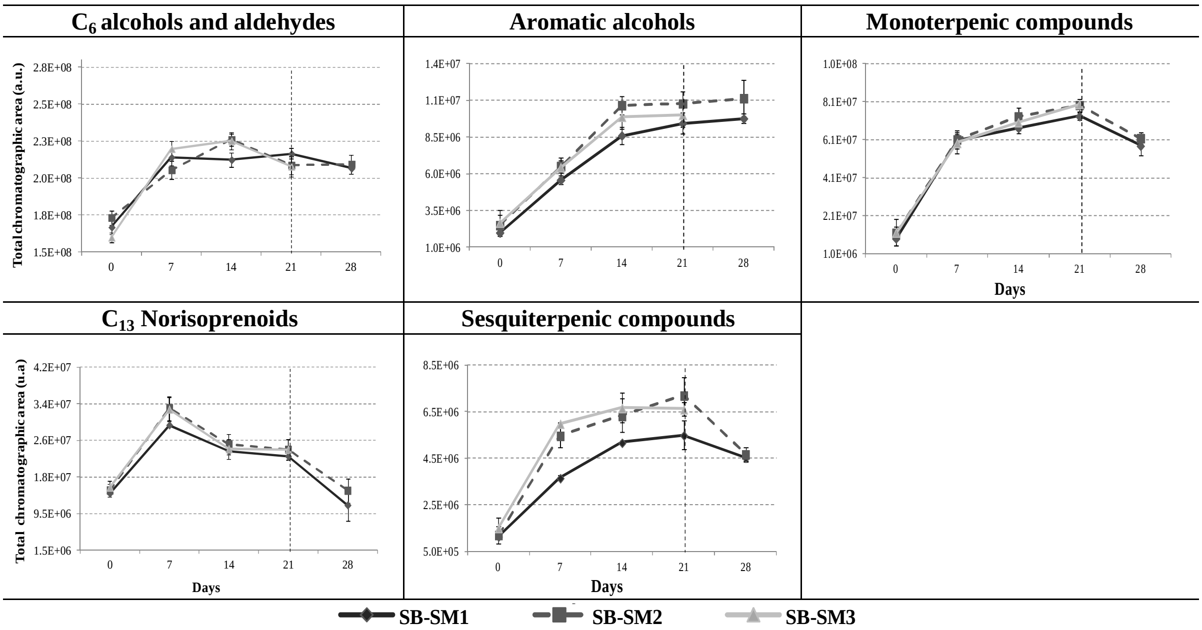


Figure 36. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Bical maturation, obtained from BI-VA1, BI-VA2, and BI-SM1 parcels, along 3 harvests (2010-2012). Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S9 in a CD-R.

Sauvignon Blanc

For the particular case of Sauvignon Blanc variety, the evaluation of the volatile composition for grapes from the 3 parcels during maturation was only performed in 2010 (Figure 37). Similar volatile composition was observed for Sauvignon Blanc grapes from the 3 parcels under study with only slightly differences related with sesquiterpenic content (Figure 37). The main characteristics of these parcels were very similar: they were at an open space and the rows were guided from North to South direction, presenting slightly differences in altitudes and different soils type (Table 3). Contrarily to the trend observed for the determination of Sauvignon Blanc phenolic content and antiradical activity (Figure 29), which contents were higher for grapes from SB-SM3 (ca. 70 m, clay-sandy soil) and SB-SM2 (ca. 50 m, clay-calcareous soil), these results showed that the 3 parcels conditions seem to have no influence on the volatile composition of Sauvignon Blanc grapes.


C_6 alcohols and aldehydes

Aromatic alcohols

Monoterpenic compounds

C_{13} Norisoprenoids

Sesquiterpenic compounds

Figure 37. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Sauvignon Blanc maturation, obtained from SB-SM1, SB-SM2, and SB-SM3 parcels, along 2010 harvest. Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S10 in a CD-R.

Baga

Figure 38 shows the total GC×GC peak area of the volatile compounds of Baga grapes, obtained from BA-VA1, BA-VA2, and BA-SM1 parcels, for 2010 harvest. Despite the observed variability during maturation, different grapes volatile compositions were determined: grapes from BA-VA2 followed by BA-VA1 parcels tend to exhibit higher varietal volatile content, mainly on mono- and sesquiterpenic compounds, when compared to grapes from BA-SM1. This was in accordance with phenolic content and antiradical activity determined in Baga grapes, since BA-SM1 grapes exhibited also lower phenolic content and antiradical activity when compared with the other parcels (Figure 30). As BA-SM1 parcel (clay-sandy soil) was at an open space, at ca. 50 m, and the rows orientation was from North to South direction, similarly with BA-VA2 conditions, the lower level of volatile components of grapes from BA-SM1 parcel can be explained by the different soils type (Table 3): clay-sandy soil of BA-SM1 seems to be related with lower grapes varietal volatile content when compared to the other parcels. The obtained results allowed inferring that clay-calcareous and clayey soils, with middle to higher water-holding capacity than clay-sandy soil, seem to favour varietal volatile composition of Baga grapes.

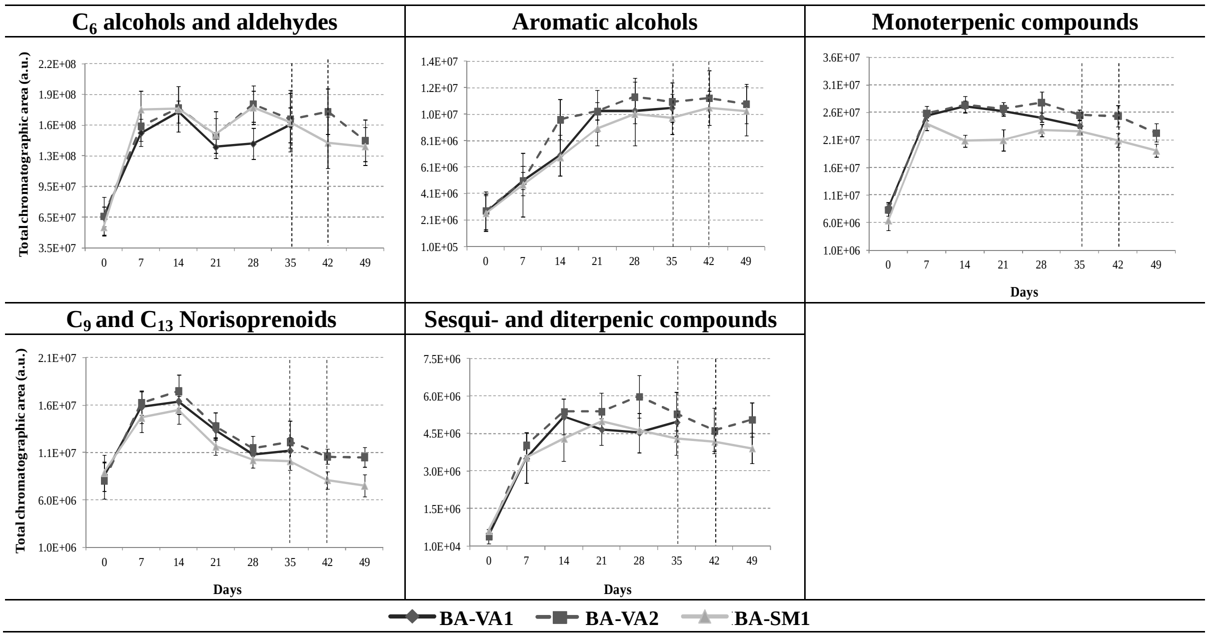


Figure 38. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Baga maturation, obtained from BA-VA1, BA-VA2, and BA-SM1 parcels, along 2010 harvest. Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S11 in a CD-R.

Castelão

The evaluation of the volatile composition of Castelão variety, during maturation, for gapes from the 3 parcels and 3 harvests under study, was shown in Figure 39. Besides the fact that the GC×GC areas of C_6 alcohols and aldehydes were similar for grapes from the 3 parcels, it was determined that grapes from CA-SM1 and CA-SM2 parcels tend to exhibit higher volatile content, especially on monoterpenic compounds and C_{13} norisoprenoids, when compared to grapes from CA-SM3. This was similar to the trend observed for Castelão phenolic content and antiradical activity (Figure 31). The 3 Castelão parcels under study were located at an open space in São Mateus vineyard and the rows were guided from North to South direction presenting similar altitudes (ca. 60-70 m). Thus, the higher differences between them were related with the soil type (Figure 17,Table 3): the higher volatile content was found for grapes from CA-SM2, with clay-calcareous soil, followed by grapes from CA-SM1 with clayey and CA-SM3 with clay-sandy soils. Thus, middle followed by higher water-holding capacity soils of CA-SM2 and CA-SM1 parcels respectively, seem to be related with higher varietal volatile content. This trend was also followed by the results determined for Castelão grapes phenolic content and antiradical activity (Figure 31).

Harvest climatic conditions also influence Castelão grapes volatile composition. Considering the 3 harvests under study, differences can be noticed (Figure 39): with the exception of C_6 alcohols and aldehydes, in 2011 harvest, the GC×GC peak areas of varietal volatile compounds were lower when compared with 2010 and also with 2012 harvests. Furthermore, the maturation process of CA-SM1 and CA-SM3 grapes was longer in 2011, however the hot temperatures have not changed the varietal volatile content of Castelão grapes, maintaining it constant until technologic maturity (Figure 39).

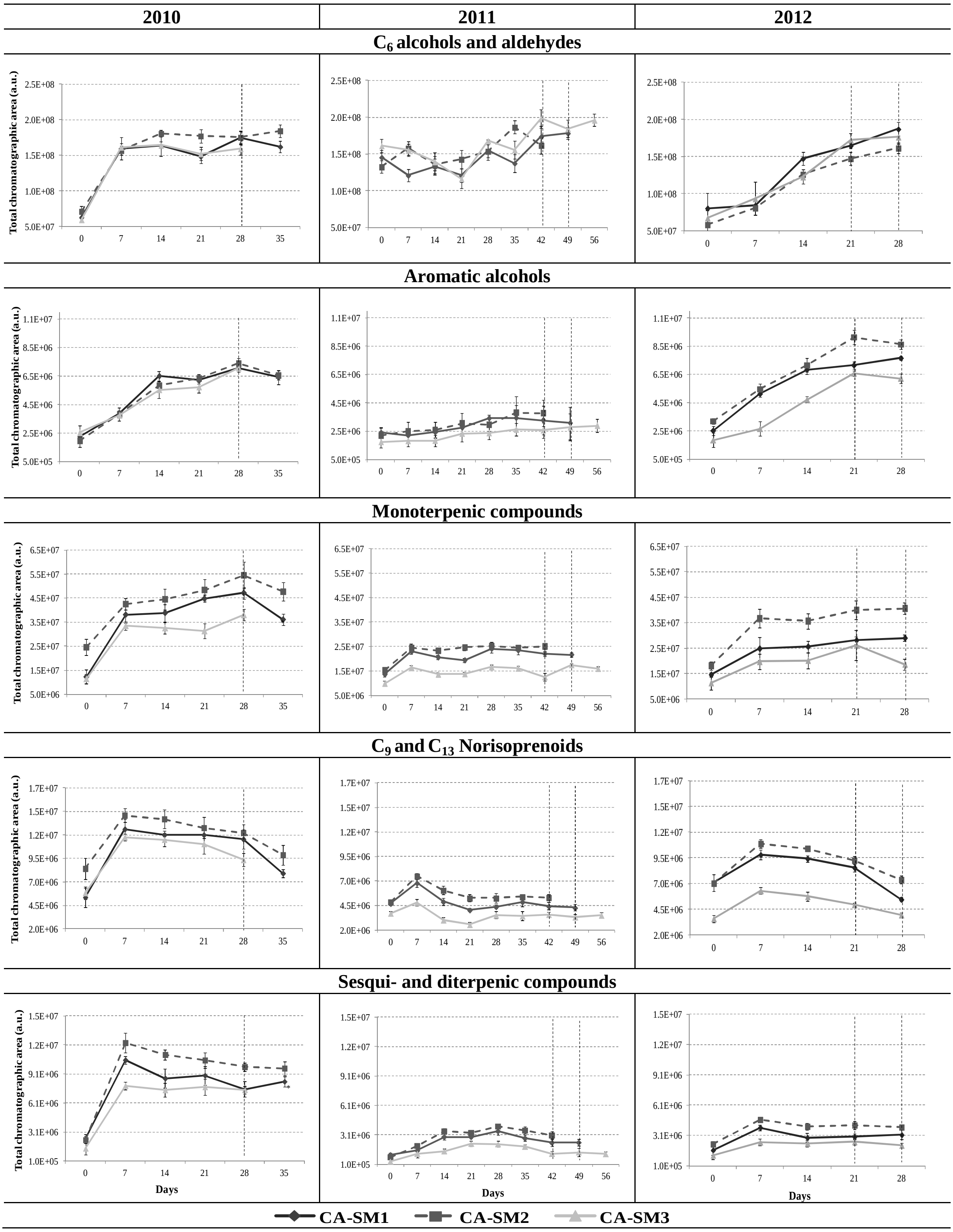


Figure 39. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Castelão maturation, obtained from CA-SM1, CA-SM2, and CA-SM3 parcels, along 3 harvests (2010-2012). Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S12 in a CD-R.

Touriga Nacional

Figure 40 shows the volatile composition obtained, during maturation, for Touriga Nacional variety, at 3 parcels and 3 harvests (2010 to 2012). The total GC×GC peak area of terpenic compounds and norisoprenoids was, during maturation, higher in grapes from TN-SM2 than in TN-SM1, followed by TN-SM3. On the contrary, aromatic alcohols (with the exception of 2012 harvest) and C_6 alcohols and aldehydes were similar in both parcels. Touriga Nacional parcels was at an open space of São Mateus vineyard, and the rows were guided from North to South, presenting differences in soil type and also slightly ones in altitude (Figure 17, Table 3): the higher volatile content was found in grapes form a parcel with clay-calcareous soil and at ca. 70 m (TN-SM2), followed by grapes from parcels both at ca. 50 m - TN-SM1 (clayey soil) and TN-SM3 (clay-sandy soil).

The influence of harvest climatic conditions were not as evident as observed for the other varieties under study (Figure 40). However, it can be seen that grapes from 2010 seem to have a little higher volatile content, principally in norisoprenoids and sesqui- and diterpenic compounds, when compared with 2011 and 2012. As the volatile content between grapes from 2011 and 2012 harvests was very similar, this shows that this is a variety that resists to different harvests climatic conditions, maintaining similar its volatile content. However, the excessive hot and dry summer of 2011 harvest, combined with a soil with higher drainage and lower water-holding capacity (TN-SM3), allowed to obtained overripe grapes.

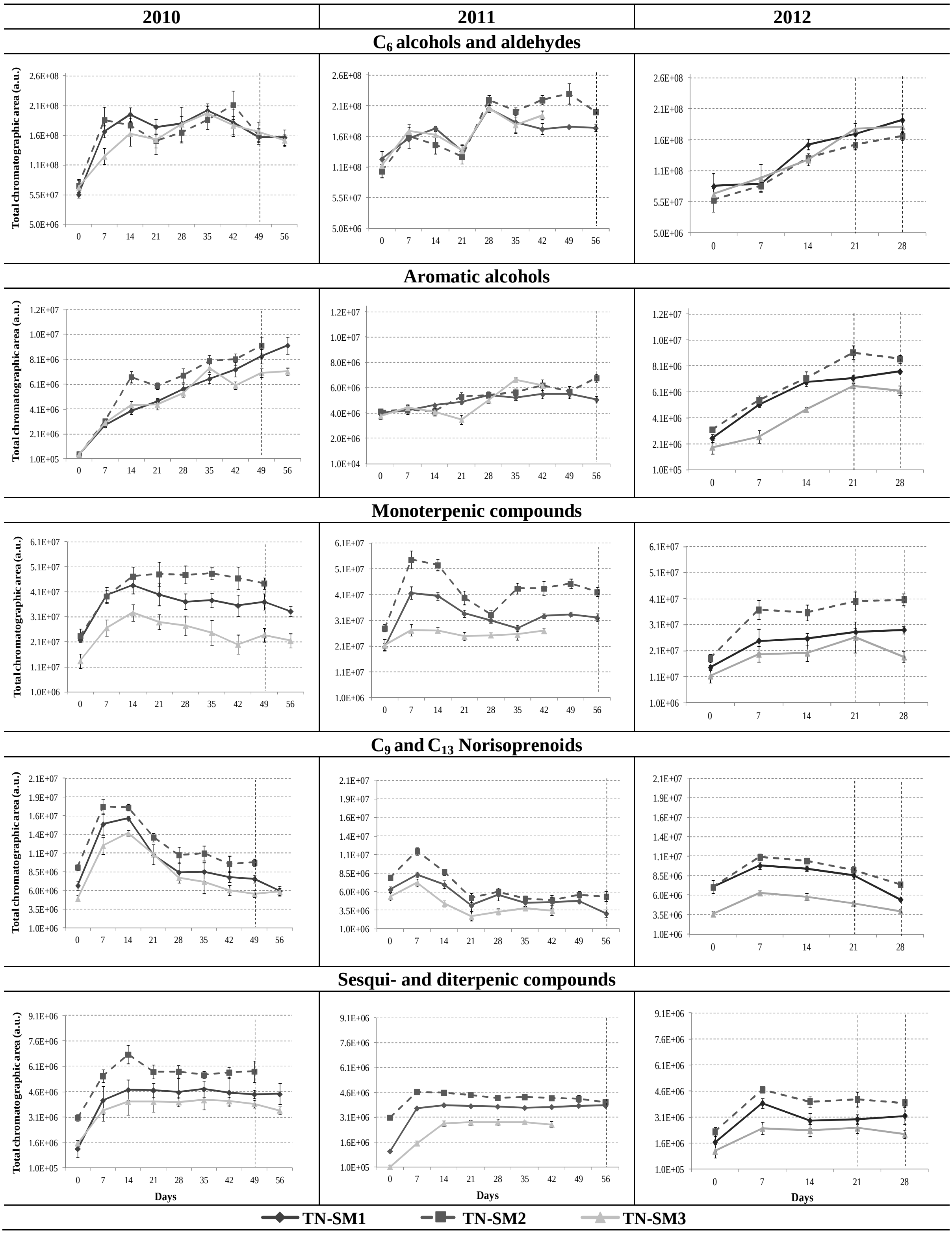
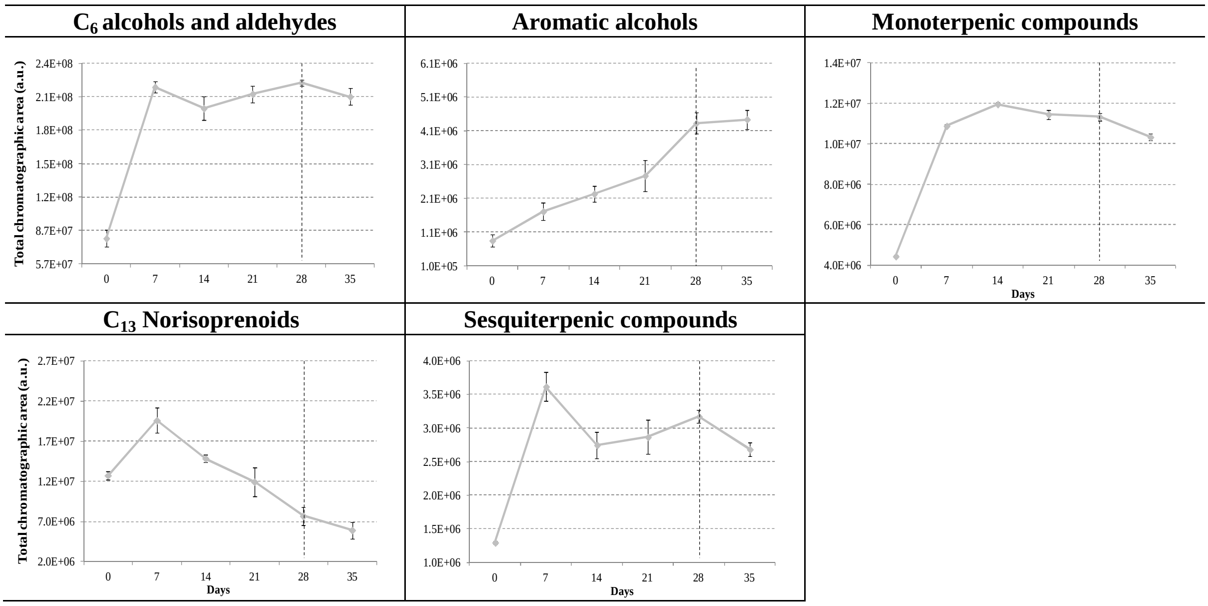


Figure 40. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Touriga Nacional maturation, obtained from TN-SM1, TN-SM2, and TN-SM3 parcels, along 3 harvests (2010-2012). Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S13 in a CD-R.

Sousão

Figure 41 shows the total GC×GC peak areas of the chemical families determined in Sousão variety obtained from SO-SM1 parcel (sandy soil, ca. 50 m, open space, rows guided from North-South direction), during maturation, at 2010 harvest. According to this figure, the volatile content increased in the first week of analysis and then tends to stabilize, as observed for C_6 alcohols and aldehydes and monoterpenic compounds, or continues to increase until technologic maturity, which was the case of aromatic alcohols. The exception was observed for C_{13} norisoprenoids that increased in the first week of analysis, and then a continuous decrease was observed, which could be explained by the glycosylation of norisoprenoids (Baumes *et al.*, 2002). The sesquiterpenic compounds attended their maximum at the first week of analysis and then slightly decreased and stabilized until technologic maturity.


C_6 alcohols and aldehydes

Aromatic alcohols

Monoterpenic compounds

C_{13} Norisoprenoids

Sesquiterpenic compounds

Figure 41. Varietal and pre-fermentative volatile components evolution, organized by chemical families, during Sousão maturation, obtained from SO-SM1 parcel, along 2010 harvest. Technologic maturity is indicated with a dash line. The areas are expressed as arbitrary units (a.u.). Detail data was given in Supplementary Table S14 in a CD-R.

III. Concluding remarks

The results obtained for each parameter under study reveals the unique character of each variety. According to parcels characteristics it was found that, berry weight, pH, titratable acidity and sugar content were similar for the 3 parcels of Arinto, followed by Sauvignon Blanc, but were variable for Bical. Besides, Arinto, Bical and Sauvignon Blanc white varieties from parcels with clay-sandy and clay-calcareous soils have higher phenolic content and antiradical activity. However, Arinto and Bical from these parcels exhibited also higher volatile content, while for Sauvignon Blanc grapes the volatile content was similar between the 3 parcels. This suggests that parcels conditions seem to have low influence in aroma potential of Sauvignon Blanc grapes. For Baga, Castelão and Touriga Nacional red varieties, higher sugar content was determined in grapes from clay-calcareous soil. Furthermore, parcels with clayey and clay-calcareous soils were related with grapes with higher volatile and phenolic contents, as well as higher antiradical activity. Besides soil type, for Touriga Nacional red variety, also altitude seems to modulate its volatile composition: higher altitude (TN-SM2, ca. 70 m, clay-calcareous soil) seems to be related with higher volatile content.

Harvest climatic conditions influenced all the 7 grape varieties oenological potential. For all varieties under study, it was found that 2010 harvest was related with higher phenolic and volatile contents, as well as higher antiradical activity, while the opposite was observed in 2011. Besides, it was found that Bical variety was very sensitive to climatic conditions since its physicochemical characteristics, as sugar content and titratable acidity, were very variable from one harvest to another. Besides the fact that Arinto variety resist to different harvest climatic conditions, the excessive dry and hot conditions of 2011 harvest allowed sesquiterpenic content to slightly decrease until technologic maturity, which may influence its aroma potential. Also, the sugar contents of Baga and Sousão were very variable from one harvest to another, which may influence the alcohol content in the resulting wines.

The knowledge of the particularities of each variety related with these factors (parcel characteristics and harvest climatic conditions) may represent a support for grape and wine producer’s decisions.

Chapter IV - Comprehensive study of variety oenological potential by using statistic tools

Comprehensive study of variety oenological potential by using statistic tools

Overview

A comprehensive study was performed by using data obtained at technologic maturity, in order to evaluate the impact of vineyard parcel characteristics and harvest climatic conditions on each variety composition and thus, in its oenological potential. Special attention was devoted to grapes collected at technologic maturity since they were the raw material for wines production that had developed all essential characteristics responsible for wine character and quality. Beyond berry weight, pH, titratable acidity, sugar content, total phenolic content, antiradical activity, and free volatile fraction, also glycosidically-linked fraction was determined for mature grapes. The knowledge of these two fractions offers a mean of evaluating the aroma potential of each variety. A comprehensive approach combining the different domains was made by using analysis of variance-simultaneous component analysis (ASCA).

III. 1. Framework

Grapes constitute the raw material for producing wines. Its technologic maturity level is one of the most deciding factors in determining wine character and quality. It is the result of the entire complex physiological and biochemical phenomena whose development is intricately related to environmental conditions (vineyard, varieties, soils, and climate). During maturation, grapes accumulate sugar while acidity falls, and accumulate secondary products of major oenological importance, such as phenolic and volatile compounds (Ribéreau-Gayon *et al.*, 2000). Mature grapes particularly contain levels of numerous free and glycosidically-linked forms of volatile compounds, which are accumulated in the berries during maturation (Sánchez-Palomo *et al.*, 2007). Free forms are volatile compounds directly involved in aroma, playing a key role in the quality and the peculiar aroma of wines. Glycosidically-linked forms, which are non-volatile and odourless (Günata *et al.*, 1985; Sánchez-Palomo *et al.*, 2007), can be released during winemaking process by enzymatic activity through the action of endogenous or exogenous β-glucosidases (López-Tamames *et al.*, 1997), giving rise to odorant compounds that play a role in the aroma characteristic of wines. Located predominantly on the grape skin (Günata *et al.*, 1985), the glycosidic aroma compounds are constituted by different aglycones, including monoterpenic compounds, C_{13} norisoprenoids, phenol derivatives, and aliphatic alcohols, associated to a sugar part represented by glucose or disaccharides (glucose-rhamnose, -arabinose and -apiose) (Bayonove, 2003). Several studies carried out on free and glycosidically-linked volatiles of different grapes recognized a relationship between the wine varietal character and grape compounds such as terpenic compounds and C_{13} norisoprenoids (Schreier *et al.*, 1976; Versini *et al.*, 1994). Thus, all the knowledge about the mature grapes volatile composition, including both free and glycosidically-linked fractions, offers a means of evaluating their aroma potential.

The grapes characteristics and composition, and wine quality depend on a complex network of variables known to influence viticulture, including environmental conditions. A comprehensive approach by using statistic tools seems to be a useful tool to evaluate each variety oenological potential. Thus, the present Chapter aims to perform a comprehensive study combining different grape parameters (berry weight, pH, acidity, volatile and phenolic composition) by using statistic tools (ASCA) applied to each variety.

IV. 2. Material and Methods

The procedures performed for grapes physicochemical parameters determination, at technologic maturity, including berry weight, pH, titratable acidity, sugar and phenolic content, and antiradical activity, and also grapes free volatile composition determination by HS-SPME/GC×GC-ToFMS, have already been described in Chapter III (Material and Methods sections).

IV. 2.1. Samples

Six *V. vinifera* grape varieties, each one from 3 parcels, collected at technologic maturity, were considered.

IV. 2.2. Determination of grapes glycosidically linked profiles by SPE/GC×GC-ToFMS

SPE procedure

The glycosidically-linked experimental procedure was adopted from a methodology previously developed in our laboratory (Rocha *et al.*, 2000). Prior to glycosidically-linked components extraction, 350 g of grape berries (sub-sample from the ca. 1000 g) from each variety and each parcel and harvest under study, at technologic maturity, were manually crushed and centrifuged at 3000 rpm, during 25 min, at 4 ºC in order to separate the solid fraction from the liquid one. The supernatant represented the liquid pulp (grape juice) fraction. Then, each supernatant was submitted to a process of solid-phase extraction (SPE), using Amberlite XAD-2 resin (20-60 mesh), obtained from Supelco, Inc. (Bellefont, PA). This resin was submitted to a pre-treatment before use: a) it was sequentially washed with methanol and ethyl acetate by Soxhlet (each one for 8h), and then dried and stored in methanol. Before the extraction procedure the resin, suspended in methanol, was poured into a glass column (50 x 1 cm i.d.). The packed column contained about 12 cm of resin. Methanol (25 mL) and ethyl acetate (25 mL) were passed through it and finally water (50 mL). The column was then ready for use. All the solvents used for the extraction of volatile compounds were analytical grade with high purity (≥ 99 %). Each supernatant (75 mL) was passed through the column resin XAD-2 and the free volatile compounds were eluted with ethyl acetate (50 mL). Subsequently, the column was rinsed with 15 mL of ultra-pure water to remove interfering substances such as sugars, acids and other water-soluble compounds. Thereafter, the retained glycosidically-linked fraction was eluted with 75 mL of methanol. This methanolic extract was evaporated under vacuum until dryness. The residue was then dissolved in 10 mL of 0.1 M phosphate-citrate buffer (100 mL of $C_6H_8O_7.H_2O$ (0.1 M) (Riedal-De-Haën) and 100 mL Na_2HPO_4 (0.05 M) (Merk) in 250 mL of ultra-pure water, pH 5.0). To release the aglycones from the glycosidically-linked compounds, a commercial enzyme (ProZym® Aroma M, obtained from Proenol,) at 100 mg L^{-1} was used and allowed to act for 42h at 35 ºC. This enzyme preparation, reported by the producer to have activity of β-glucosidase, pectinase, and poligalacturonase, was chosen for hydrolysis of the aglycones. The generated free volatile compounds from the glycosidically-linked fractions were then extracted with ethyl acetate (75 mL) and 3-octanol (8.72 μg L^{-1}) was used as internal standard. The extracts were cooled to -20 °C and dried over anhydrous sodium sulphate to separate the water from the organic phase. The excess of low-boiling solvent was removed by distillation at low pressure using a trap with liquid nitrogen. The obtained concentrate (about 1 mL) was stored in a glass screw-top vial at -20 °C. Three replicates were done for each sample under study.

GC×GC-ToFMS analysis

The analysis of the glycosidically-linked fraction of 6 *V. vinifera* varieties was carried out based on the previously described procedure on Chapter III 2 (Material and Methods section), with few modifications: 0.5 μL of the obtained extract from the glycosidically-linked fraction was injected into GC×GC–ToFMS injection port and the detector was off during 150 s. The GC×GC-ToFMS injection port was 250 °C, and the same kit of columns were used: an Equity-5 column (30 m x 0.32 mm I.D., 0.25 µm film thickness, Supelco, Inc., Bellefonte, PA, USA) was used as first-dimension (^{1}D) column and a DB-FFAP (0.79 m x 0.25 mm I.D., 0.25 µm film thickness, J&W Scientific Inc., Folsom, CA, USA) was used as a second-dimension (^{2}D) column. The ovens temperature programs were the same as previously described. Total ion chromatograms (TIC) were processed using the automated data processing software ChromaTOF (LECO) at signal-to-noise threshold of 100.

Contour plots were used to evaluate the separation general quality and for manual peak identification. For identification purposes, the mass spectrum and retention times (^{1}D and ^{2}D) of the analytes were compared with standards, when available. Also, the mass spectrum of each compound detected was compared to those in mass spectral libraries, which included an in-house library of standards and two commercial databases (Wiley 275 and US National Institute of Science and Technology (NIST) V. 2.0 – Mainlib and Replib). The identification was also supported by the experimentally determined retention index (RI) values that were compared, when available, with the values reported in the bibliography for chromatographic columns similar to the one used in the present PhD thesis for one dimensional GC with 5%-phenyl-methylpolysiloxane GC column or equivalent (Adams, 1995, 2000; Adams *et al.*, 2005; Alissandrakis *et al.*, 2007; Araujo *et al.*, 2003; Baccouri *et al.*, 2007; Beaulieu and Grimm, 2001; Buchin, 2002; Carrapiso *et al.*, 2002; Cho *et al.*, 2007; Couladis *et al.*, 2001; D'Arcy *et al.*, 1997; Dickschat *et al.*, 2005; El-Sayed *et al.*, 2005; Engel *et al.*, 2002; Engel and Ratel, 2007; Ferhat *et al.*, 2007; Flach *et al.*, 2004; Flamini *et al.*, 2002; Hazzit *et al.*, 2006; Hognadottir and Rouseff, 2003; Isidorov *et al.*, 2003; Jalali-Heravi *et al.*, 2006; Kim *et al.*, 2006; Kobaisy *et al.*, 2002; Lalel *et al.*, 2003a; Marongiu *et al.*, 2006; Mevy *et al.*, 2006; Mondello and Costa, 2006; Pino *et al.*, 2003; Pino *et al.*, 2005; Radulović *et al.*, 2012; Robinson, 2006; Sarikurkcu *et al.*, 2008; Saroglou *et al.*, 2006; Setzer *et al.*, 2006; Vasta *et al.*, 2007; Vujisic *et al.*, 2006; Zhao *et al.*, 2006; Zhao *et al.*, 2008) and for comprehensive GC×GC system with Rxi-5SilMS and VF-5MS for the ^{1}D column (Robinson *et al.*, 2011; Weldegergis *et al.*, 2011) or with the same column set used in this PhD thesis - Equity-5 ^{1}D column and a DB-FFAP for ^{2}D column (Jalali *et al.*, 2012; Jalali *et al.*, 2013; Petronilho *et al.*, 2011; Petronilho *et al.*, 2013; Rocha *et al.*, 2012; Rocha *et al.*, 2007a; Rocha *et al.*, 2013; Silva *et al.*, 2010). RI values were determined using a C_8-C_{20} *n*-alkanes series (the solvent *n*-hexane was used as C_6 standard) and calculated according to the van den Dool and Kratz equation (van den Dool and Kratz, 1963). The majority (> 85%) of the identified compounds presented similarity matches $\geq$ 850 (850/1000). The data was expressed as μg L^{-1} of 3-octanol equivalents. Three independent replicates were analysed for each variety at the different conditions under study. Reproducibility was expressed as relative standard deviation (RSD). Detail data was given in supplementary information in the CD-R.

IV. 2.3. Data processing

In order to evaluate influence of parcel characteristics and harvest conditions on the composition of mature grapes, ASCA was applied to the data set, at technologic maturity (a total of 6 varieties, each variety from 3 parcels, 3 harvests (2010-2012), a total of 137 parameters (6 general physicochemical parameters and 95 and 36 grapes volatile components determined in free and glycosidically-linked fractions, respectively), and 3 independent replicates were done), combining all studied parameters, i.e. berry weight, pH, titratable acidity, sugar content, phenolic content, antiradical activity and volatile composition, including free and glycosidically-linked fractions. All variables were mean centered and autoscaled prior to all calculations. Partitioning of original data matrix was done in accordance with the design factors, parcel and harvest:

$X = X_a + X_b + E$ (Jansen *et al.*, 2005),

where “X_a” is a matrix containing variation related to the effect of parcel, “X_b” is a matrix containing variation related to the harvest. After running PCA on each of those matrices the following ASCA model is obtained:

$X = T_a P_a' + T_b P_b' + E$ (Smilde *et al.*, 2005),

where “T” and “P” are PCA scores and loadings, respectively, for each of the sub-models, and “E” is a residual error. Number of significant PCs for each sub-model was selected using cross-validation.

Significance of each factor was assessed using a permutation test (Meyners, 2001; Vis *et al.*, 2007; Westerhuis *et al.*, 2008). Permutation consists in changing randomly the order of the rows in the data set. As a result in the permuted data set treatment levels are assigned randomly to the measurements. Permutation test consists in testing the null hypothesis that a given effect is not significant and the respective ASCA sub-model describes noise using quality-of-fit criteria. Data were permuted 2000 times and the percentage of the variance explained by each sub-model in the total model was used as quality-of-fit criterion. Variance explained by each sub-model in the total model was calculated for each sub-model using the formula:

$$E_{a,tot}(\%) = \left(1 - \frac{\sum_m \sum_n (X - T_a P'_a)^2}{\sum_m \sum_n X^2}\right) \times 100\%$$

(Smilde *et al.*, 2005),

where “$E_{a,tot}$” is the percentage of explained variance of the sub-model “a” in the total model, “T_a” and “P_a” are score and loadings for the sub-model “a”, “X” is an original data matrix, and “m” and “n” are number of variables and samples in the data set, respectively. ASCA and permutation test were implemented in MATLAB R2011a using the algorithms described in (Jansen *et al.*, 2005) and (Vis *et al.*, 2007).

IV. 3. Results and Discussion

IV. 3.1. Grapes composition (general physicochemical parameters and free volatile fraction)

The composition obtained for each variety, at maturity, was compiled in Table 5, which includes berry weight, pH, titratable acidity, sugar content and phenolic content, antiradical activity, and also free volatile composition (detail data was given in Supplementary Table S1 to S6 and S8 to S13). Results obtained from these data were already discussed in Chapter III, for each variety obtained from the different conditions under study (parcels characteristics and harvest conditions). Sousão was not considered in this chapter as this variety was only collected in one parcel, thus it is not possible to evaluate parcels characteristics effect. Table 5 summarizes the results obtained, at technologic maturity, for each variety considering the different parcels characteristics and harvest conditions under study, which combined with the results of the glycosidically-linked fraction (IV. 3.2.), were used to perform the comprehensive approach by using statistic tools.

Table 5. Synoptic table of the general physicochemical composition and free volatile fraction, obtained at technologic maturity, for *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, and Touriga Nacional grapes, each one from a set of 3 parcels and from 3 consecutive harvests.

		Parcels								
Variety	**Physicochemical parameters**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**
Arinto		**AR-VA1**			**AR-VA2**			**AR-SM1**		
	Berry weight (g)	1.3 (5)*	1.5 (4)	1.5 (3)	1.1 (5)	1.4 (3)	1.5 (3)	1.0 (7)	1.5 (3)	1.4 (3)
	pH	2.7 (2)	3.2 (1)	3.2 (0)	2.9 (1)	3.2 (0)	3.1 (1)	2.7 (1)	2.9 (1)	3.1 (0)
	Acidity (g tartaric acid L^{-1})	7.6 (1)	6.5 (2)	6.6 (3)	7.8 (1)	5.7 (2)	6.5 (3)	7.4 (2)	7.9 (2)	6.3 (4)
	Sugar content (g L^{-1})	214.8 (1)	219.3 (1)	197.8 (3)	221.0 (1)	226.1 (2)	206.3 (3)	210.0 (1)	172.3 (1)	187.0 (4)
	Phenolic content (mg GAE L^{-1})	319.1 (7)	230.5 (7)	251.5 (4)	366.1 (2)	321.0 (4)	332.8 (1)	374.4 (1)	276.7 (9)	291.6 (2)
	Antiradical activity (% DPPH rem)	85.2 (1)	84.8 (2)	88.2 (1)	68.6 (1)	70.1 (2)	80.0 (1)	70.0 (3)	73.8 (2)	82.8 (3)
	Total chromatographic area [a]	22527.9 (9)	21002.5 (9)	20962.1 (16)	27780.7 (9)	21573.9 (9)	20304.7 (14)	27871.1 (9)	23270.4 (11)	20019.0 (14)
	Total varietal chromatographic area [a]	7861.3 (8)	4435.3 (10)	5302.3 (15)	9589.9 (9)	5610.9 (9)	7353.9 (14)	10343.4 (9)	5961.6 (10)	6723.5 (13)
Bical		**BI-VA1**			**BI-VA2**			**BI-SM1**		
	Berry weight (g)	1.4 (1.0)	1.4 (1)	1.6 (1)	1.2 (2)	1.3 (4)	1.6 (3)	1.4 (3)	1.7 (3)	1.8 (3)
	pH	2.9 (2)	3.2 (1)	3.2 (1)	2.9 (0)	3.1 (0)	3.2 (1)	3.2 (0)	3.0 (1)	3.1 (1)
	Acidity (g tartaric acid L^{-1})	5.7 (1)	7.1 (1)	5.8 (2)	6.1 (1)	5.9 (1)	5.5 (3)	4.2 (1)	6.8 (2)	5.6 (1)
	Sugar content (g L^{-1})	173.4 (1)	215.3 (1)	208.5 (1)	170.0 (1)	214.8 (1)	209.7 (1)	187.0 (1)	207.4 (1)	193.2 (1)
	Phenolic content (mg GAE L^{-1})	294.1 (3)	197.5 (2)	214.1 (3)	304.4 (3)	283.5 (4)	262.0 (2)	333.9 (2)	248.7 (2)	268.8 (5)
	Antiradical activity (% DPPH rem)	81.5 (3)	89.9 (3)	89.7 (2)	84.4 (2)	81.1 (3)	86.1 (2)	71.7 (1)	81.6 (3)	83.3 (1)
	Total chromatographic area	26376.6 (9)	22642.9 (9)	13200.3 (14)	29345.9 (7)	19790.4 (9)	12977.4 (13)	27176.3 (7)	17867.6 (10)	13844.2 (12)
	Total varietal chromatographic area	4136.2 (9)	1958.6 (8)	2909.3 (13)	6232.2 (7)	2590.5 (8)	4279.3 (13)	5907.5 (7)	3127.2 (9)	4429.0 (12)
Sauvignon Blanc		**SB-SM1**			**SB-SM2**			**SB-SM3**		
	Berry weight (g)	1.3 (6)	1.4 (7)	1.6 (3)	1.6 (2)	1.8 (3)	1.9 (2)	1.5 (2)	1.5 (3)	1.7 (3)
	pH	3.2 (0)	3.1 (1)	3.0 (0)	3.2 (0)	3.0 (1)	3.0 (1)	3.1 (0)	3.0 (1)	3.0 (1)
	Acidity (g tartaric acid L^{-1})	6.6 (1)	7.6 (2)	7.1 (2)	5.2 (1)	7.1 (4)	6.9 (1)	6.9 (2)	7.6 (2)	7.2 (3)
	Sugar content (g L^{-1})	243.1 (1)	247.6 (1)	208.5 (3)	204.6 (0)	241.4 (1)	201.7 (2)	226.7 (1)	247.6 (1)	213.6 (2)
	Phenolic content (mg GAE L^{-1})	255.9 (4)	236.9 (9)	276.0 (7)	403.6 (6)	283.5 (6)	315.1 (6)	467.6 (3)	357.4 (6)	365.1 (8)
	Antiradical activity (% DPPH rem)	72.6 (2)	79.9 (2)	83.1 (2)	68.9 (1)	73.5 (3)	77.6 (1)	62.7 (2)	66.4 (2)	74.2 (2)
	Total chromatographic area	32810.8 (10)	19216.0 (8)	24669.3 (9)	32906.2 (9)	18451.7 (9)	23009.9 (10)	33529.7 (10)	19233.8 (9)	26291.0 (9)
	Total varietal chromatographic area	11026.3 (10)	3196.8 (8)	9260.5 (9)	12030.1 (8)	4068.2 (9)	11173.6 (9)	11024.3 (10)	4297.6 (9)	12815.1 (8)

		BA-VA1			BA-VA2			BA-SM1		
Baga	Berry weight (g)	1.3 (4)	1.9 (1)	1.7 (4)	1.7 (3)	1.9 (4)	2.0 (3)	2.1 (4)	1.9 (4)	1.8 (4)
	pH	3.1 (0)	3.2 (0)	3.1 (0)	3.1 (1)	3.1 (2)	2.9 (1)	3.2 (2)	3.2 (1)	3.1 (1)
	Acidity (g tartaric acid L^{-1})	5.2 (2)	6.3 (2)	5.8 (3)	4.1 (3)	6.4 (2)	5.3 (6)	5.3 (1)	7.0 (3)	5.6 (5)
	Sugar content (g L^{-1})	189.8 (2)	196.6 (1)	176.8 (3)	214.8 (2)	202.3 (1)	163.8 (2)	176.8 (1)	190.4 (2)	175.7 (3)
	Phenolic content (mg GAE L^{-1})	811.0 (3)	631.4 (17)	594.8 (15)	1270.1 (15)	869.3 (11)	726.1 (8)	686.0 (12)	517.0 (20)	494.1 (11)
	Antiradical activity (% DPPH rem)	80.9 (2)	85.6 (2)	82.0 (4)	53.5 (2)	70.3 (3)	73.8 (1)	81.0 (3)	88.6 (2)	89.6 (2)
	Total chromatographic area	18930.2 (9)	15621.7 (9)	14619.2 (12)	22188.4 (9)	17530.3 (10)	12656.8 (11)	18300.6 (10)	16296.9 (9)	26169.6 (15)
	Total varietal chromatographic area	5250.9 (9)	3409.9 (8)	4515.7 (11)	5369.9 (8)	3616.2 (9)	5855.5 (11)	4529.9 (9)	2779.9 (9)	3721.1 (14)
Castelão		**CA-SM1**			**CA-SM2**			**CA-SM3**		
	Berry weight (g)	1.8 (3)	2.0 (1)	2.0 (2)	1.9 (4)	1.8 (6)	1.8 (4)	1.9 (3)	1.9 (4)	1.9 (3)
	pH	3.3 (1)	3.2 (1)	3.1 (1)	3.2 (0)	3.1 (1)	3.1 (0)	3.3 (1)	3.2 (0)	3.2 (2)
	Acidity (g tartaric acid L^{-1})	6.0 (2)	7.2 (2)	7.5 (3)	5.2 (2)	5.8 (2)	6.3 (4)	5.5 (1)	5.7 (3)	7.1 (1)
	Sugar content (g L^{-1})	221.0 (2)	196.1 (1)	181.9 (4)	241.4 (1)	218.7 (1)	186.4 (4)	218.2 (2)	193.2 (1)	170.0 (4)
	Phenolic content (mg GAE L^{-1})	1053.4 (4)	704.9 (13)	694.1 (14)	864.0 (4)	621.6 (20)	627.5 (16)	826.1 (5)	635.2 (16)	522.0 (12)
	Antiradical activity (% DPPH rem)	64.3 (3)	69.9 (1)	70.2 (2)	68.7 (2)	72.0 (1)	75.9 (3)	77.4 (2)	81.9 (3)	89.4 (4)
	Total chromatographic area	23893.3 (10)	19971.5 (6)	22383.9 (8)	24968.2 (8)	21025.1 (6)	23389.9 (9)	22249.2 (9)	20966.8 (6)	20824.6 (12)
	Total varietal chromatographic area	8580.0 (9)	3794.6 (6)	6231.5 (7)	7489.0 (9)	3200.0 (6)	4683.7 (8)	6330.2 (9)	2547.3 (6)	3177.9 (11)
Touriga Nacional		**TN-SM1**			**TN-SM2**			**TN-SM3**		
	Berry weight (g)	1.9 (2)	1.7 (3)	2.0 (3)	1.6 (6)	1.6 (3)	1.7 (5)	1.9 (1)	**	2.0 (5)
	pH	3.2 (0)	3.2 (0)	3.2 (1)	3.3 (2)	3.3 (1)	3.3 (1)	3.3 (1)	**	3.3 (0)
	Acidity (g tartaric acid L^{-1})	4.4 (3)	5.7 (2)	5.8 (2)	4.2 (3)	5.5 (3)	5.3 (6)	4.3 (2)	**	6.1 (7)
	Sugar content (g L^{-1})	200.1 (2)	193.8 (1)	187.6 (3)	254.2 (6)	205.1 (2)	201.2 (4)	199.4 (3)	**	184.7 (2)
	Phenolic content (mg GAE L^{-1})	747.3 (2)	948.9 (13)	646.5 (13)	1341.3 (2)	1339.8 (9)	1121.4 (9)	852.0 (11)	**	501.6 (14)
	Antiradical activity (% DPPH rem)	78.3 (3)	85.0 (1)	81.2 (1)	51.9 (1)	58.4 (2)	66.9 (1)	70.0 (3)	**	86.3 (1)
	Total chromatographic area	21020.8 (9)	21401.8 (9)	18299.1 (12)	22524.6 (9)	25627.9 (11)	19017.1 (12)	20195.1 (12)	**	22080.1 (14)
	Total varietal chromatographic area	5810.1 (8)	4470.7 (9)	5517.6 (11)	7038.4 (8)	5905.2 (10)	6973.6 (12)	4073.2 (11)	**	3494.3 (12)

[a] GC×GC peak area x 10^4 given in arbitrary units (a.u.)

* Relative standard deviation (RSD, % in parentheses). Detailed information is given in Supplementary Tables S1 to S6 and S8 to S13.

** Grapes not available (grapes in an advanced rotting stage was observed).

IV. 3.2. Glycosidically-linked fraction

In the analysis of the glycosidically-linked fraction of *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão and Touriga Nacional varieties (Table 6), mono- and sesquiterpenic compounds, and C_{13} norisoprenoids were the selected chemical families. Particular attention was devoted to these compounds because of the considerable significance of these compounds to flavour and varietal character of *V. vinifera* varieties (González-Barreiro *et al.*, 2015). In this fraction a total of 32 terpenic compounds (25 mono- and 7 sesquiterpenic ones) and 4 C_{13} norisoprenoids were determined (Table 6). The number of total volatile compounds determined in the glycosidically-linked fraction was higher in Bical (28), Sauvignon Blanc (22), and Arinto (12) white varieties than in red varieties under study: 7 in Touriga Nacional, 8 in Castelão and 9 in Baga. It was found that the major class of glycosidically-linked compounds in white varieties was monoterpenic compounds; on the other hand in red varieties the number of sesquiterpenic and monoterpenic compounds was similar. Detailed information regarding each determined compound on the glycosidically-linked fraction of the 6 varieties under study was shown in Supplementary Tables S15 to S20.

Table 6. Volatile components determined for the selected chemical families, at technologic maturity, in glycosidically-linked fractions of grapes from *Vitis vinifera* L. cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, and Touriga Nacional varieties.

					$RI_{lit.}$[c]		**Ref. $RI_{lit.}$[d]**		**Varieties**					
^{1}Dtr(s),^{2}Dtr(s)[a]	**Compound**	**CAS number**	**Formula**	**$RI_{calc.}$[b]**	**1D-GC**	**GC×GC**	**1D-GC**	**GC×GC**	**Arinto**	**Bical**	**Sauvignon Blanc**	**Baga**	**Castelão**	**Touriga Nacional**
Monoterpenic compounds														
314, 0.440	α-Pinene	80-56-8	$C_{10}H_{16}$	938	939	941	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x	x	x	
344, 0.457	β-Pinene *	18172-67-3	$C_{10}H_{16}$	988	989	987	(Buchin, 2002)	(Jalali et al., 2012)	x	x		x	x	
356, 0.570	β-Myrcene	123-35-3	$C_{10}H_{16}$	1001	991	1008	(Adams, 1995)	(Jalali *et al.*, 2012)		x		x	x	
362, 0.520	3-Carene	13466-78-9	$C_{10}H_{16}$	1007	1004	1020	(Engel *et al.*, 2002)	(Jalali *et al.*, 2012)	x	x	x			
392, 0.405	*m*-Cymene	535-77-3	$C_{10}H_{14}$	1025	1020	1027	(Araujo *et al.*, 2003)	(Jalali *et al.*, 2012)		x				
398, 0.476	Limonene *	138-86-3	$C_{10}H_{16}$	1028	1029	1035	(Adams *et al.*, 2005)	(Jalali *et al.*, 2012)	x	x	x			x
404, 0.476	1,8-Cineole	470-82-6	$C_{10}H_{18}O$	1034	1032	1039	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x			
428, 0.678	Linalool oxide (isomer)		$C_{10}H_{18}O_2$	1071	1071	1078	(Saroglou *et al.*, 2006)	(Jalali *et al.*, 2012)	x	x	x			
440, 0.560	α-Terpinolene	586-62-9	$C_{10}H_{16}$	1076	1088	1097	(Adams, 2000)	(Jalali *et al.*, 2012)		x				
440, 0.790	Linalool oxide (isomer)		$C_{10}H_{18}O_2$	1076	1087	1097	(Saroglou *et al.*, 2006)	(Jalali *et al.*, 2012)		x				
452, 0.746	Linalool *	78-70-6	$C_{10}H_{18}O$	1096	1098	1108	(Adams, 2000)	(Jalali *et al.*, 2012)	x	x	x			x
470, 0.646	Hotrienol	53834-70-1	$C_{10}H_{16}O$	1113	1114	1122	(D'Arcy *et al.*, 1997)	(Jalali *et al.*, 2012)		x				
524, 0.860	Borneol	507-70-0	$C_{10}H_{18}O$	1169	1165	1172	(Adams, 1995)	(Jalali *et al.*, 2012)			x			
536, 0.715	Terpinen-4-ol	562-74-3	$C_{10}H_{18}O$	1183	1177	1181	(Adams, 2000)	(Jalali *et al.*, 2012)		x	x			
542, 0.835	α-Terpineol *	98-55-5	$C_{10}H_{18}O$	1195	1189	1206	(Adams, 2000)	(Jalali *et al.*, 2012)		x	x			
548, 0.850	Dihydrocarvone	7764-50-3	$C_{10}H_{16}O$	1197	1193	1211	(Adams, 1995)	(Jalali *et al.*, 2012)		x	x			
560, 0.850	Verbenone	80-57-9	$C_{10}H_{14}O$	1214	1204	1214	(Adams, 2000)	(Jalali *et al.*, 2012)		x	x			
566, 0.703	p-Menth-1-en-9-al	29548-14-9	C10H16O	1217	1217	1219	(Pino *et al.*, 2003)	(Jalali *et al.*, 2012)		x				
578, 0.700	*m/z* 93, 121, 119, 136 (alcohol)		$C_{10}H_{16}O$	1224	_	_	_	_		x				
584, 0.873	Geraniol (isomer) *		$C_{10}H_{18}O$	1235	1224	1235	(Vujisic *et al.*, 2006)	(Jalali *et al.*, 2012)			x			
584, 0.943	β-Citronellol *	106-22-9	$C_{10}H_{20}O$	1237	1233	1237	(Adams, 2000)	(Jalali *et al.*, 2012)			x			
590, 0.737	Geraniol (isomer) *		$C_{10}H_{18}O$	1244	1237	1242	(Ferhat *et al.*, 2007)	(Jalali *et al.*, 2012)		x	x			
596, 0.976	Citral (isomer)		$C_{10}H_{16}O$	1247	1240	1245	(Beaulieu and Grimm, 2001)	(Jalali *et al.*, 2012)		x	x			
602, 0.815	Carvone *	99-49-0	$C_{10}H_{14}O$	1251	1253	1245	(Adams, 2000)	(Jalali *et al.*, 2012)			x			
626, 0.775	Citral (isomer)		$C_{10}H_{16}O$	1274	1270	1287	(Beaulieu and Grimm, 2001)	(Rocha *et al.*, 2007a)	x	x	x			

C_{13} Norisoprenoids															
566, 0.532	*m/z* 159, 91, 131 (hydrocarbon)		$C_{13}H_{18}$	1216	_	_	_	_		x				
736, 0.648	Geranylacetone *	3796-70-1	$C_{13}H_{22}O$	1455	1449	1454	(Adams *et al.*, 2005)	(Rocha et al., 2013)	x	x		x	x	x
784, 0.717	β-Ionone *	79-77-6	$C_{13}H_{20}O$	1488	1485	1494	(Adams, 1995)	(Silva *et al.*, 2010)		x				
900, 0.894	Methyl dihydrojasmonate	24851-98-7	$C_{13}H_{22}O_3$	1661	1650	_	(Mondello and Costa, 2006)	_	x	x	x	x	x	x
Sesquiterpenic compounds														
790, 0.660	α-Farnesene	502-61-4	$C_{15}H_{24}$	1501	1508	1505	(Adams, 2000)	(Petronilho *et al.*, 2011)	x	x			x	x
796, 0.525	γ-Cadinene	39029-41-9	$C_{15}H_{24}$	1504	1513	1511	(Adams, 1995)	(Jalali *et al.*, 2013)				x		
826, 0.629	α-Calacorene	21391-99-1	$C_{15}H_{20}$	1555	1542	1554	(Adams, 2000)	(Jalali *et al.*, 2013)				x		
850, 0.751	Globulol	489-41-8	$C_{15}H_{26}O$	1594	1598	1592	(Robinson, 2006)	(Petronilho *et al.*, 2013b)			x			
912, 0.654	*m/z* 119, 91, 191, 109 (alcohol)		$C_{15}H_{26}O$	1675	_	_	_	_		x	x	x	x	x
942, 0.820	Farnesal	502-67-0	$C_{15}H_{24}O$	1731	1730	1724	(Adams, *et al.*, 2005)	(Jalali *et al.*, 2013)		x	x		x	
1036, 0.671	Ledene oxide		$C_{15}H_{24}O$	1873	1890	1867	(Lalel *et al.*, 2003a)	(Jalali *et al.*, 2013)	x	x	x	x		x

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] RI_{calc}: retention index obtained through the modulated chromatogram.

[c] RI_{lit}: retention index reported in the literature for 5% phenyl polysilphenylene-siloxane GC column or equivalents, reported for 1D-GC and GC×GC.

[d] Ref. RI_{lit}: references found in the literature for 5% phenyl polysilphenylene-siloxane GC column or equivalents, reported for 1D-GC and GC×GC.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

Notation **x** indicates that the compound was determined in the corresponding variety. Detailed data related to the glycosidically-linked fraction of the volatile compounds determined for each variety under study, at technologic maturity, each one from 3 parcels and 3 consecutive harvests was given in Supplementary Tables S15 to S20.

For rapid interpretation of the glycosidically-linked fractions determined for each variety (each one from 3 parcels and 3 consecutive harvests), a graphical representation of the total concentration of volatile compounds determined in these fractions was performed (Figure 42 to Figure 47), organized by chemical families (mono- and sesquiterpenic compounds - C_{10} and C_{15} respectively, and C_{13} norisoprenoids - C_{13}). The total concentration was obtained as the sum of the individual concentrations of all compounds detected. This allowed obtaining a rapid visual access of each variety glycosidically-linked fraction, allowing the comparison of the similarities and differences between the different parcels and harvests under study.

Arinto

Figure 42 shows the total concentration of volatile compounds determined in the glycosidically-linked fractions of Arinto variety obtained from AR-VA1, AR-VA2 and AR-SM1 parcels, from 2010, 2011 and 2012 harvests. As observed for the volatile components in free fraction (Figure 35), the glycosidically-linked fractions exhibited differences among the parcels studied and also between the 3 harvests. It was observed that grapes from AR-VA2 parcel followed by grapes from AR-SM1 have higher concentrations of total volatile compounds determined in the glycosidically-linked fraction than AR-VA1 grapes. The higher chemical group determined was monoterpenic compounds, representing ca. 59-80% of the total content determined: grapes from AR-VA2 accounted for 33.3-97.7 $\mu g\ L^{-1}$, values higher than those obtained for AR-SM1 (30.7-77.1 $\mu g\ L^{-1}$) and AR-VA1 (25.8-62.8 $\mu g\ L^{-1}$) grapes (Table S15). Considering all the parcels, limonene and linalool were the major monoterpenic compounds presented in Arinto grapes, being in lower amounts in AR-VA1 grapes. The potential contribution of each component to the aroma properties correspond to the amount in free form plus the amount in glycosidically-linked form. In 2010 linalool (36 $\mu g\ L^{-1}$) determined in grapes from AR-VA2 was above their sensory perception limit (25 $\mu g\ L^{-1}$) (Ferreira *et al.*, 2000), this may suggest its contribution with citrus, sweet and flowery odours (Marais, 1983). Besides, for all parcels, the amount of volatile compounds obtained from the glycosidically-linked fractions was higher in 2010 harvest (78.1-138.0 $\mu g\ L^{-1}$) than in 2011 (35.6-56.7 $\mu g\ L^{-1}$) and 2012 (41.9-63.7 $\mu g\ L^{-1}$). Besides, the major monoterpenic compound determined also exhibited different amounts between the 3 harvests: limonene was ca. 2.5 and 4.5 times higher in

2010 than in 2012 and 2011, respectively, while linalool was ca. 1.8 and 2.3 times higher (Table S15). These results suggest that the climatic conditions of 2010 harvest seem to favour the formation of Arinto secondary plant metabolites, such as monoterpenic compounds.

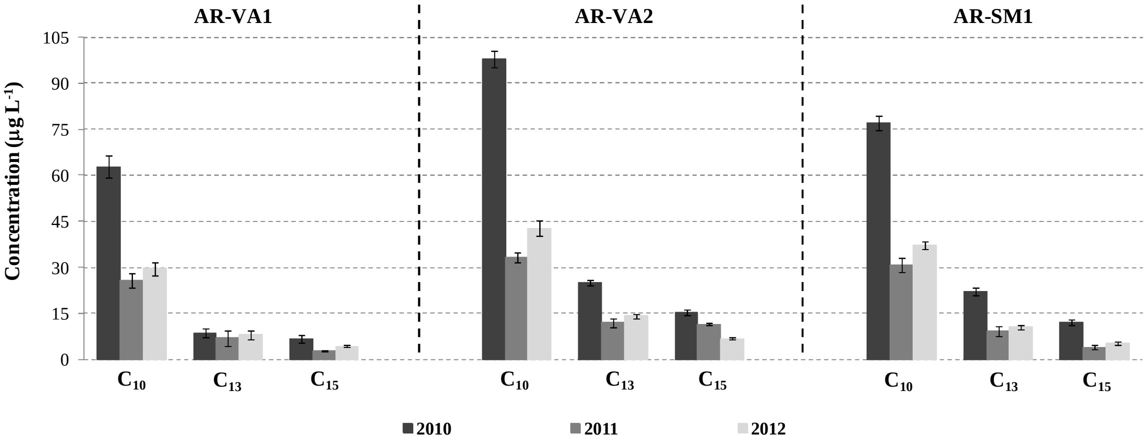


Figure 42. Terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Arinto grapes obtained from AR-VA1, AR-VA2, and AR-SM1 parcels, for 3 harvests (2010-2012). The concentrations are expressed as µg L^{-1} of 3-octanol equivalents. Detail data was given in Supplementary Table S15 in a CD-R.

Bical

The total concentrations of volatile compounds determined in the glycosidically-linked fractions of Bical variety obtained from BI-VA1, BI-VA2 and BI-SM1 parcels, from 2010-2012 harvests, organized by chemical families, were shown in Figure 43. Considering all parcels, Bical grapes exhibited monoterpenic compounds followed by C_{13} norisoprenoids as the higher chemical families determined, accounting for ca. 54-72% and 17-34%, respectively, while sesquiterpenic compounds only accounted for 9-13% (Table S16). However, the total contents determined in Bical glycosidically-linked fractions were different for grapes from the 3 parcels under study: BI-SM1 and BI-VA2 grapes accounted for 108.6-185.5 µg L^{-1} and 97.6-158.3 µg L^{-1} respectively, values that are higher than those determined for BI-VA1 (69.3-118.3 µg L^{-1}) grapes (Table S16). This trend was in accordance with the results observed for the free volatile fractions (Figure 36). Besides, for all parcels under study, the amount of total volatile compounds obtained from the glycosidically-linked fractions was higher in 2010 harvest (118.3-185.5 µg L^{-1}), than in 2011 (69.3-108.6 µg L^{-1}) and 2012 (73.1-114.1 µg L^{-1}) (Table S16). According to these results, both parcel and harvest conditions may influence grapes aroma potential, by influencing their volatile composition.

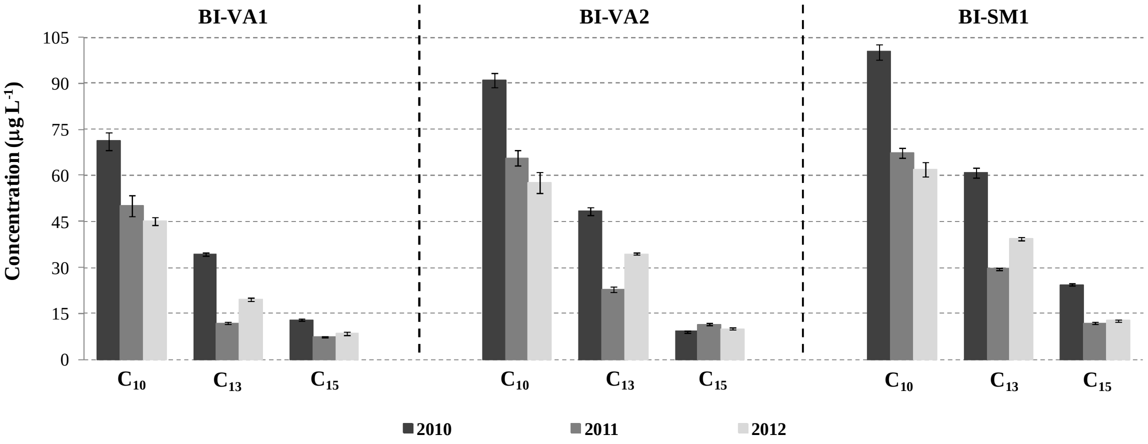


Figure 43. Terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Bical grapes obtained from BI-VA1, BI-VA2, and BI-SM1 parcels, for 3 harvests (2010-2012). The concentrations are expressed as µg L^{-1} of 3-octanol equivalents. Detail data was given in Supplementary Table S16 in a CD-R.

Sauvignon Blanc

Figure 44 shows the contents of mono- and sesquiterpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Sauvignon Blanc grapes obtained from SB-SM1, SB-SM2, and SB-SM3 parcels, for 2010, 2011, and 2012 harvests. Similar composition of Sauvignon Blanc grapes was observed between the parcels under study. Only in 2010 some differences in monoterpenic content were observed: grapes from SB-SM3 (228.5 µg L^{-1}) followed by SB-SM2 (181.9 µg L^{-1}) grapes exhibited higher monoterpenic content when compared to SB-SM1 (119.6 µg L^{-1}) (Figure 44, Table S17). These observations corroborate the results previously discussed for the free volatile fraction (Figure 37), showing that the conditions of Sauvignon Blanc parcels seem to have low influence on the volatile composition of Sauvignon Blanc grapes. Furthermore, the monoterpenic content accounted for ca. 70-82% of the total glycosidically-linked volatile content. These compounds are very important for the varietal aroma of the wines, being related essentially with citric, fruity, floral, and sweet notes (Marais, 1983). Quantitatively, considering the 3 parcels and the 3 harvests, monoterpene alcohols were the predominant chemical group determined, being two geraniol isomers the main monoterpenic compounds determined in Sauvignon Blanc grapes. Together, these compounds accounted for ca. 41 to 48% of the total monoterpenic content. These have a low sensory perception limit being important to the general enhancement of the floral and fruity notes of Sauvignon Blanc grape aromas (Marais, 1983). Besides, the harvest conditions seem to modulate Sauvignon Blanc glycosidically-linked composition since its content varied from one harvest to another, decreasing from 2010, 2012, to 2011.

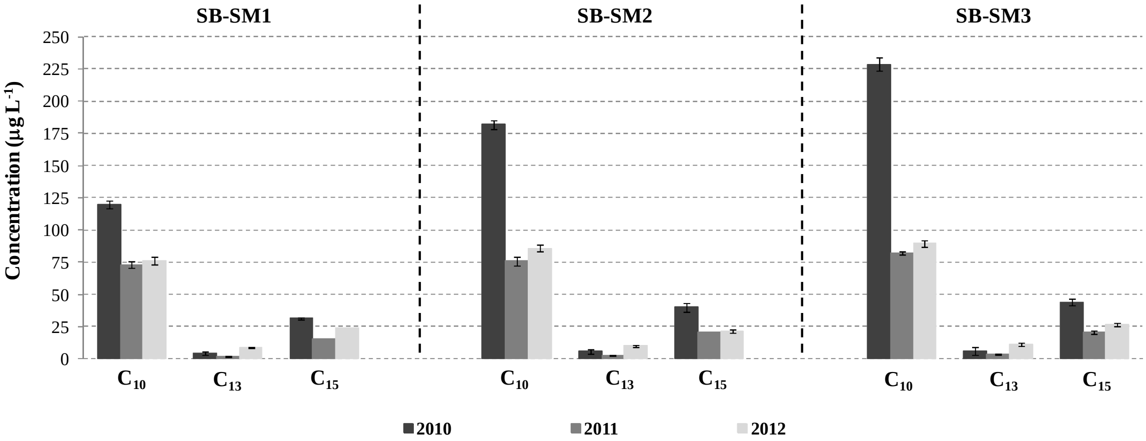


Figure 44. Terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Sauvignon Blanc grapes obtained from SB-SM1, SB-SM2, and SB-SM3 parcels, for 3 harvests (2010-2012). The concentrations are expressed as µg L^{-1} of 3-octanol equivalents. Detail data was given in Supplementary Table S17 in a CD-R.

Baga

The content of terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Baga grapes obtained from BA-VA1, BA-VA2, and BA-SM1 parcels, for 3 harvests (2010-2012) was shown in Figure 45. As this figure shows, grapes from the 3 parcels under study exhibited different volatile composition determined in the glycosidically-linked fractions: grapes from BA-SM1 accounted for 22.3-25.0 µg L^{-1}, values that are lower than those determined for BA-VA1 (33.6-51.1 µg L^{-1}) and BA-VA2 (40.5-61.4 µg L^{-1}) grapes (Table S18). Similar trend was previously described for grapes from the free volatile fractions (Figure 38). The main chemical group presented in the glycosidically-linked fraction of Baga grapes was sesquiterpenic compounds (accounting for ca. 45-63%). These decrease in the following order: grapes from BA-VA2 (20.0 to 31.7 µg L^{-1}), followed by BA-VA1 (15.7 to 23.8 µg L^{-1}), and BA-SM1 (12.3 to 14.9 µg L^{-1}). Sesquiterpenic compounds represent an important chemical group of *V. vinifera* due to their aroma properties and also bioactive effect as anti-bacterial, anti-inflammatory, antitumor activities, among others (Petronilho *et al.*, 2012; Tamemoto *et al.*, 2001). Furthermore, the amount of total volatile compounds obtained from the glycosidically-linked fractions was higher in 2010 harvest, than in 2011 and 2012. These results suggest that both parcel and harvest conditions modulate Baga grapes composition.

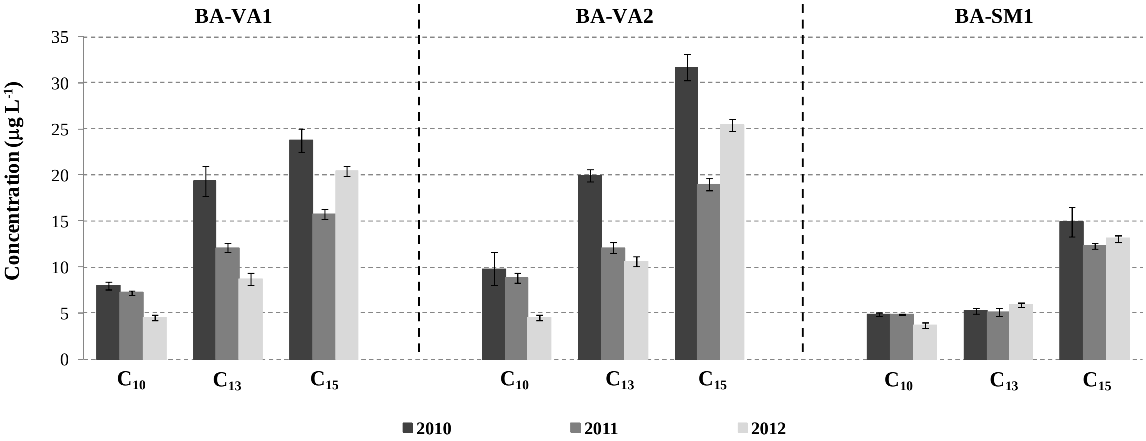


Figure 45. Terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Baga grapes obtained from BA-VA1, BA-VA2, and BA-SM1 parcels, for 3 harvests (2010-2012). The concentrations are expressed as µg L^{-1} of 3-octanol equivalents. Detail data was given in Supplementary Table S18 in a CD-R.

Castelão

Figure 46 shows the glycosidically-linked varietal volatile compounds of Castelão grapes obtained from CA-SM1, CA-SM2, and CA-SM3 parcels considering the 3 consecutive harvests. For this variety the glycosidically-linked content was higher in grapes from CA-SM1 (37.6-56.3 µg L^{-1}), followed by CA-SM2 (31.7-48.8 µg L^{-1}) and CA-SM3 (24.2-40.9 µg L^{-1}). These results are in accordance with the free volatile composition previously discussed (Figure 39). Similar with Baga, the main chemical family determined in the glycosidically-linked fraction of Castelão grapes was also the sesquiterpenic compounds, which decreased in the same order as observed for the total content: from CA-SM1 (19.9 to 26.5 µg L^{-1}) followed by CA-SM2 (17.4 to 23.3 µg L^{-1}), and CA-SM3 (15.3 to 22.0 µg L^{-1}). Sesquiterpenic compounds are described as contributors for aroma character of different matrices, where their aroma perception has been usually expressed as spicy, sweet, floral and woody aromas (Lalel *et al.*, 2003b; Minh *et al.*, 2003), thus they can contribute favourably to Castelão wine aroma characteristics. Considering the set of 3 parcels and 3 harvests, this group of compounds represents ca. 46 to 68% of the total varietal volatile content, while monoterpenic compounds represent only ca. 13-25%. Considering the 3 harvests under study different volatile compositions were determined for Castelão grapes (Figure 46): higher content was observed for grapes from 2010 harvest, principally for sesquiterpenic compounds, when compared to 2011 and 2012 harvests.

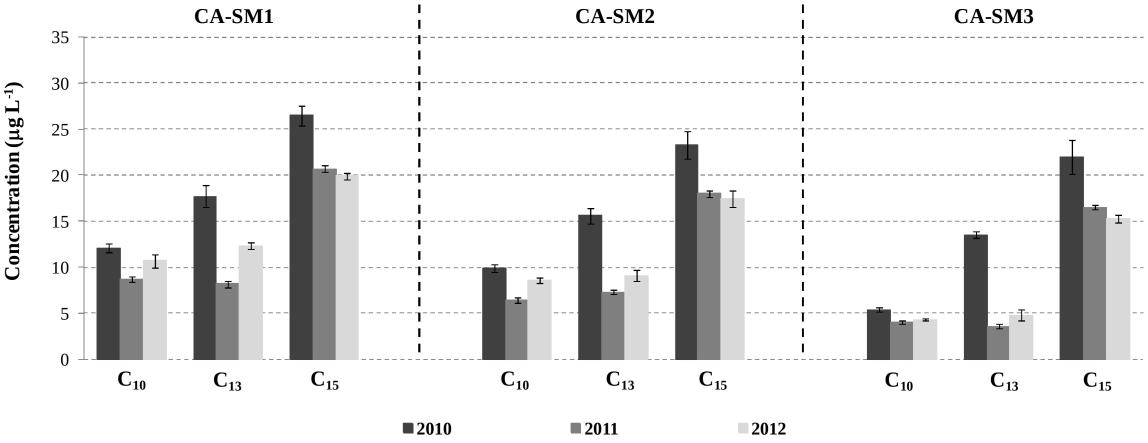


Figure 46. Terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Castelão grapes obtained from CA-SM1, CA-SM2, and CA-SM3 parcels, for 3 harvests (2010-2012). The concentrations are expressed as μg L^{-1} of 3-octanol equivalents. Detail data was given in Supplementary Table S19 in a CD-R.

Touriga Nacional

Figure 47 shows the varietal compounds determined, at technologic maturity, in glycosidically-linked fractions of Touriga Nacional grapes obtained from TN-SM1, TN-SM2, and TN-SM3 parcels, for 3 harvests (2010-2012). Different volatile contents were determined on the different glycosidically-linked fractions of grapes from the 3 parcels: TN-SM2 grapes exhibited higher contents (43.3-67.8 μg L^{-1}) while the lower ones were determined for TN-SM3 grapes (29.1-34.6 μg L^{-1}) (Table S20). Similar trend was previously observed for the free volatile fractions of Touriga Nacional (Figure 41). Besides the observed differences between the 3 parcels, the grapes exhibited sesquiterpenic compounds as the higher chemical family determined, followed by C_{13} norisoprenoids and monoterpenic compounds. These accounted for ca. 53-61%, 22-32%, and 13-18%, respectively (Table S20). These compounds were related with very pleasant aromas, thus they may have an important contribution on the varietal aroma of Touriga Nacional wines. According to

Figure 47, considering all parcels under study, the amount of total volatile compounds obtained from the glycosidically-linked fractions was higher in 2010 harvest (34.6-67.8 μg L^{-1}), than in 2011 (38.2-43.3 μg L^{-1}) and 2012 (29.-46.6 μg L^{-1}) (Table S20). In 2011, no glycosidically-linked data was available for grapes from TN-SM3 parcel, as overripe grapes were observed. According to these results, both parcel and harvest conditions influence Touriga Nacional grapes aroma potential, by influencing its grapes volatile composition.

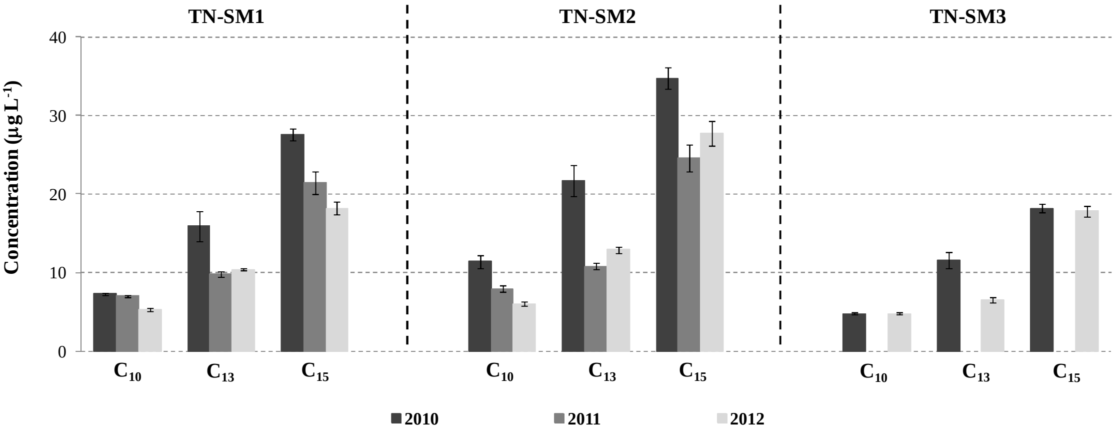


Figure 47. Terpenic compounds (C_{10} and C_{15}) and C_{13} norisoprenoids determined, at technologic maturity, in glycosidically-linked fractions of Touriga Nacional grapes obtained from TN-SM1, TN-SM2, and TN-SM3 parcels, for 3 harvests (2010-2012). The concentrations are expressed as μg L^{-1} of 3-octanol equivalents. Detail data was given in Supplementary Table S20 in a CD-R.* In 2011, at technologic maturity, no grapes were available for TN-SM3 parcel (overripe grapes).

IV. 3.3. Statistic tools to evaluate each variety oenological potential

In order to evaluate the oenological potential of *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, and Touriga Nacional, to different vineyard parcel characteristics and harvests (2010-2012), ASCA was applied for each variety, to the set of parameters measured at technologic maturity: data set combined berry weight, pH, titratable acidity, sugar content, phenolic content, antiradical activity, and volatile composition including free and glycosidically-linked factions. The significance of these factors (harvest and parcel) was assessed using a permutation test (2000 permutations). Resulting *p*-values were shown in Table 7. According to this table, the harvest effect was significant for all varieties studied (*p*-value < 0.0005) explaining ca. 54-68% of the total data set variance. On the other hand, with the exception of Sauvignon Blanc white variety, parcel characteristic effect was also significant for the varieties studied (*p*-values < 0.05) and this factor explained ca. 15-19% of the total data set variance. Harvest climatic conditions exhibited higher influence in all varieties composition than vineyard parcels, representing the largest source of the data variability (Table 7). This was already expected since parcel characteristics were highly related with harvest climatic conditions. For instance, altitude was directly associated to the resulting temperature, precipitation, and humidity (Jackson and Lombard, 1993) of the parcels, while soil may affect the microclimate by its heat-retaining and light reflecting capacity and may affect water and nutrient availability to the plant by its retaining capacity (Jackson and Lombard, 1993; Martinez *et al.*, 2011). Besides, the formation of phenolic and volatile compounds, object of study in this PhD thesis, result from plant secondary metabolism in response to several types of stress conditions, namely in response to environmental factors such as changes in temperature, humidity, sunlight, among others (Seigler, 2002).

Table 7. Significance testing of factors harvest and parcel, for *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, and Touriga Nacional varieties, determined at technologic maturity.

	***p*-values (2000 permutations)**	**Explained Variance (%)**
Factors	**Arinto**	
Harvest year	<0.0005	62.2
Parcel characteristics	0.0390	14.9
	Bical	
Harvest year	<0.0005	53.8
Parcel characteristics	0.0135	17.1
	Sauvignon Blanc	
Harvest year	<0.0005	68.2
Parcel characteristics	> 0.05 *	11.9
	Baga	
Harvest year	<0.0005	61.4
Parcel characteristics	0.040	14.8
	Castelão	
Harvest year	<0.0005	66.7
Parcel characteristics	0.041	15.5
	Touriga Nacional	
Harvest year	<0.0005	59.3
Parcel characteristics	0.0105	18.8

* *p*-value > 0.05 (no significance)

Arinto

For Arinto grapes, the effect of harvest year accounted for 62.2% of total data set variance (Table 7). First component of the score plot separates 2010 harvest (placed in PC1 positive) from the others (placed in PC1 negative), while second component separates 2011 (PC2 negative) and 2012 (PC2 positive) harvests (Figure 48a). According to the separation along PC1, in 2010, grapes with lower berry weight and pH, and with higher phenolic content and higher number and amount of volatile compounds were determined (Figure 48b). These comprised mono- and sesquiterpenic oxygen-containing compounds, as dihydromyrcenol, dihydrolinalool, nerolidol and β-eudesmol, and also C_{13} norisoprenoids, principally TDN and β-damascenone (isomer 2). On the other hand, the separation along PC2 of grapes from 2011 and 2012 harvests were mostly due to the higher content of 6 monoterpenic compounds (limonene, 1,8-cineole, linalool oxide, fenchol, borneol, and menthol) in grapes from 2012, while α-terpinolene and rose oxide (isomer 1) were higher in 2011 grapes. Varietal compounds were regarded as key odorants in grapes contributing with floral, fruity and citrus attributes. They are secondary plant metabolites whose formation is highly related to climate conditions: temperature and precipitation amount can change the amount and type of aroma compounds present in grapes (Moreno-Arribas and Polo, 2009). These results reveal that 2010 climatic conditions seem to favour the Arinto varietal compounds formation, suggesting that grapes from 2010 harvest have higher aroma potential when compared with the other two harvests. Also, 3 C_6 aldehydes (hexanal, 2-hexenal, and 2,4-hexadienal) were determined in higher amounts in grapes from 2010, while higher content of 2 C_6 alcohols (2- and 3-hexen-1-ol) were determined in grapes from 2012. The formation of C_6 aldehydes and their reduction to the corresponding alcohols depends on the content of unsaturated lipids presents in grapes and also on the activities of lipoxygenase and alcohol dehydrogenase enzymes (Bakker and Clarke, 2011). These compounds have herbaceous odours that have been related to negative effects in wines (Baumes *et al.*, 1986), however in white wines a small herbaceous perception is appreciated by the consumers (Welke *et al.*, 2012). Although Arinto variety resist to different climatic conditions, it was found that berry weight, pH, phenolic content and mainly its varietal composition were different from one harvest to another.

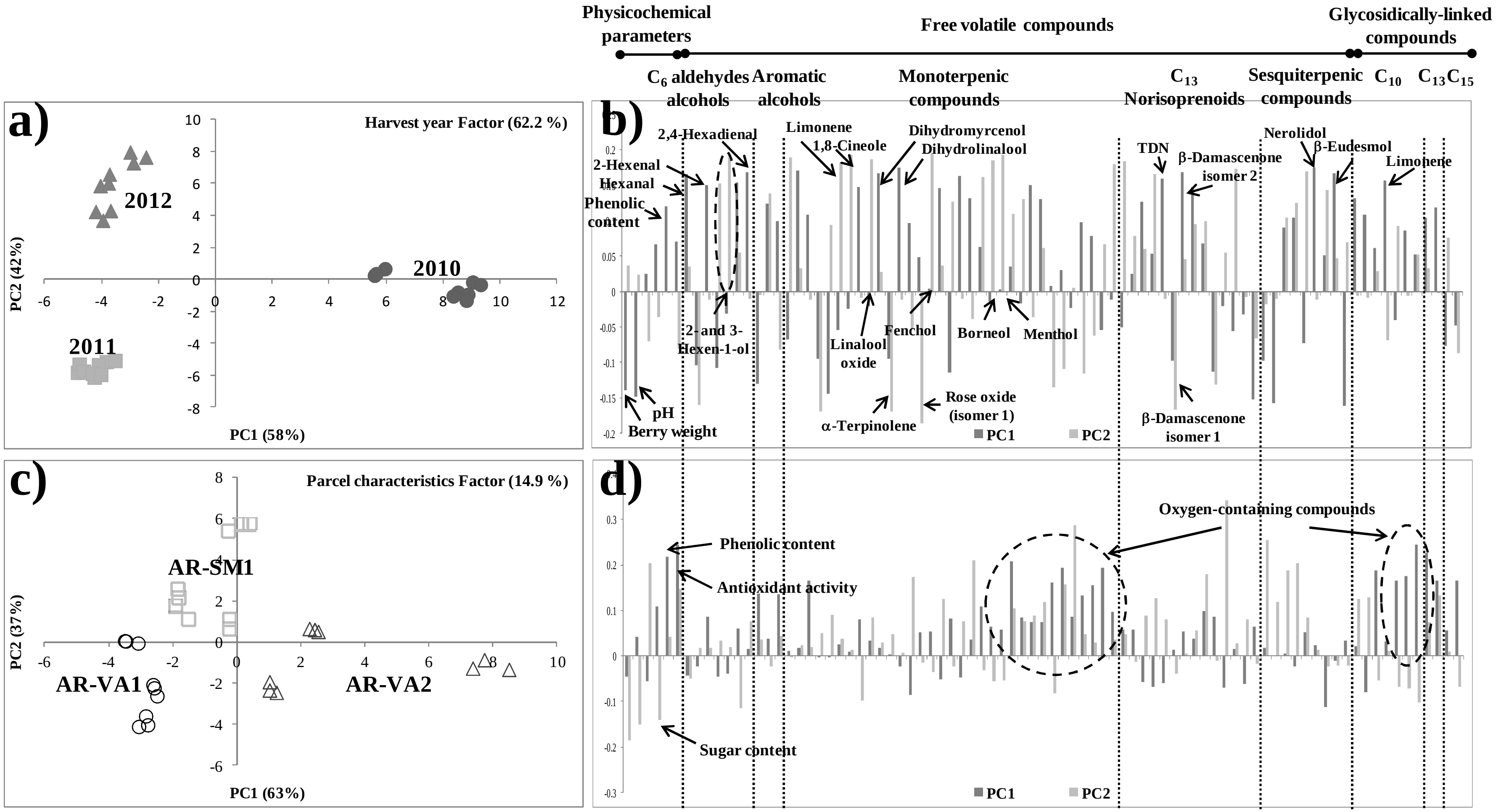


Figure 48. ASCA scores plot for (a) harvest and (c) parcel factors, and the corresponding variable loadings plot (b, and d, respectively), obtained for Arinto, at technologic maturity (significance test reported on Table 7). Each variable is normalized separately by dividing by its standard deviation value.

The effect of parcel characteristics for Arinto grapes accounted for 14.9% of the total data set variance (Table 7). Scores and loading plots were shown in Figure 48c and d, respectively. PC1 distinguishes AR-VA2, placed in the positive side of PC1, from the other 2 parcels, placed in the negative side of PC1 (AR-VA1) or near to origin (AR-SM1) (Figure 48c). The same number of total volatile compounds was found in grapes from all parcels (a total of 66 individual compounds), thus the separation observed in the loadings plot (Figure 48b) was due to the different amounts determined for most of the varietal volatile compounds, in particular, monoterpenic ones, including free and glycosidically-linked fractions, as well as total phenolic content and antiradical activity: Arinto grapes from AR-VA2 with higher contents was placed in PC1 positive and grapes from AR-VA1, with lower content, was placed in PC1 negative. Besides, sugar content also allowed to distinguish grapes from AR-SM1 from the other two parcels, having these grapes lower sugar content. These observations should be associated to the different characteristics of the 3 parcels, described in Chapter II. Soil type may influence grapes composition: soils with lower (clay-sandy soil - AR-VA2) and middle (clay-calcareous soil - AR-SM1) water-holding capacity than clayey soil seem to favour varietal volatile compounds, phenolic content, and also antiradical activity. According to these results, the oenological potential of Arinto variety is different from one parcel to another: it is expected that Arinto grapes from AR-VA2 may have higher aroma potential while AR-VA1 grapes may have the lower one. On the other hand AR-SM1 grapes with lower sugar content may produce wines with lower alcohol content.

Bical

Harvest effect on Bical grapes composition accounted for 53.8% of total data set variance (Table 7). According to the score plot all three harvests can be distinguished: 2010 harvest was placed PC1 positive and the other 2 harvests under study, 2011 and 2012, were placed in PC1 negative and near to origin, respectively (Figure 49a). According to the loading plot (Figure 49c), the separation of grapes from 2010 harvest (PC1 positive) was related principally to lower sugar content, higher total phenolic content, and higher contents of mono- and sesquiterpenic compounds determined in the free fraction and also to the monoterpenic compounds determined in the glycosidically-linked fraction. On the other hand, lower amounts of these compounds were observed for grapes from 2011 harvest, which explains its position in PC1 negative side. Besides, higher sugar content was determined in grapes from 2011 harvest and higher berry weight in grapes from 2012. Bical variety is known to be very sensible to different climatic conditions and according to these results it was found that its oenological potential varied with harvest year conditions: higher aroma potential is expected for grapes from 2010 and the opposite for grapes from 2011. Besides, higher alcohol content is expected for grapes from 2011.

The scores plot obtained for parcel factor accounting for 17.1% of variance (Table 7) was shown in Figure 49c. According to this figure, parcels were distributed along the first PC with BI-SM1 situated at the positive and BI-VA1 at the negative side of PC1 and BI-VA2 was near to origin. Analysis of loadings (Figure 49d) showed that this distribution along PC1 was related to the content of monoterpenic compounds determined in free and glycosidically-linked fractions and aromatic alcohols, and also phenolic compounds and antiradical activity: higher amounts were determined for grapes from BI-SM1, while the opposite was observed for grapes from BI-VA1. Grapes from BI-SM1 not only exhibited higher content of free volatile monoterpenic compounds, which are very important for grape aroma and quality, but also higher amounts of these compounds in the glycosidically-linked fraction. This suggests higher aroma potential for grapes from this parcel. According to this, BI-SM1 parcel characteristics seem to favour Bical grapes composition. The distribution observed along PC2 allowed to distinguish BI-VA2 (PC2 positive) from BI-VA1 (PC2 negative) (Figure 49c). According to the loadings plot (Figure 49d) this distinction was mainly due to the lower berry weight, and higher amounts of 2 monoterpenols (hotrienol and terpinen-4-ol) and 2 sesquiterpenes (aromadendrene and α-farnesene) in BI-VA2 grapes, while BI-VA1 exhibited higher amounts of ocimenol and geraniol (isomer 1). The observed differences may be related with soil type: soils with lower (clay-sandy soil - BI-SM1) and middle (clay-calcareous soil - BI-VA2) water-holding capacity than clayey soil seem to favour varietal volatile compounds, phenolic content, and also antiradical activity.

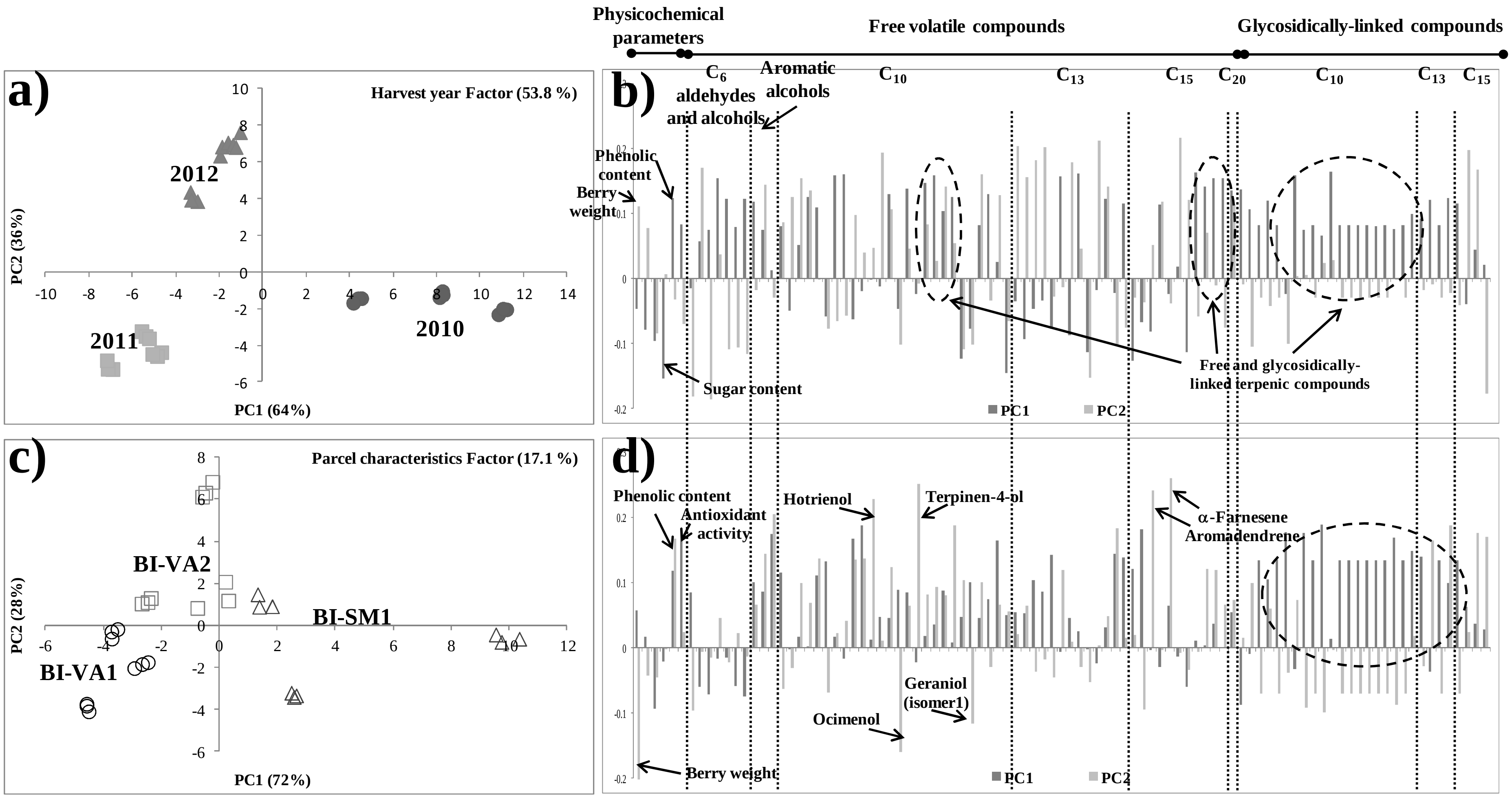


Figure 49. ASCA scores plot for (a) harvest and (c) parcel factors, and the corresponding variable loadings plot (b, and d, respectively), obtained for Bical, at technologic maturity (significance test reported on Table 7). Each variable is normalized separately by dividing by its standard deviation value.

Sauvignon Blanc variety

The comprehensive approach performed for Sauvignon Blanc grapes revealed that harvest and parcel factors explained ca. 68% and 12% of the total data set variance. However, only effect of harvest was found to be significant (*p*-value lower than 0.0005), while parcel factor had no statistical significance (*p*-value higher than 0.05) (Table 7). During maturation (Chapter III), it was determined that grapes from SB-SM2 (ca. 50 m, clay-calcareous soil) exhibited higher berry weight and lesser sugar content than grapes from SB-SM1 and SB-SM3 parcels (both at 70 m, with clayey and clay-sandy soils, respectively) (Figure 22, Table 5). Also, higher phenolic content and antiradical activity were determined for grapes from SB-SM3 followed by SB-SM2 (Figure 29, Table 5). However, at technologic maturity, the comprehensive approach using statistic tools revealed that these differences between the 3 parcels were not statistic significant, suggesting that similar Sauvignon Blanc grapes compositions and thus oenological potential, can be obtained from SB-SM1, SB-SM2 and SB-SM3 parcels.

Harvest climatic conditions influence Sauvignon Blanc grapes composition (Figure 50). According to the score plot (Figure 50a), samples were distributed along PC1: 2010 harvest was placed in PC1 positive and 2011 and 2012 harvests were placed in PC1 negative and near to origin, respectively. According to loadings plot (Figure 50b), practically all parameters studied contributed to the sample separation along the first PC: grapes from 2010 harvest had the highest content on volatile compounds, including free and glycosidically-linked ones, while grapes harvested in 2011 had higher titratable acidity values and the lower volatile content, principally related with the varietal one. From the varietal compounds (free and glycosidically-linked fractions), geraniol isomers were the higher ones determined in all parcels and harvests. In the free form, geraniol isomer 1 and 2 accounted for ca. 1% and 3-9% of the total GC×GC areas, respectively, and accounted for ca. 16-20% and 13-18% of the total amount determined in the glycosidically-linked form, respectively. Although in small amounts, linalool was the third major monoterpenol and accounted for ca. 1-3% of the total GC×GC areas and 5-9% of the total amount determined in the glycosidically-linked form. These compounds may be important to the general enhancement of the fruity, floral and citric aromas (Marais, 1983).

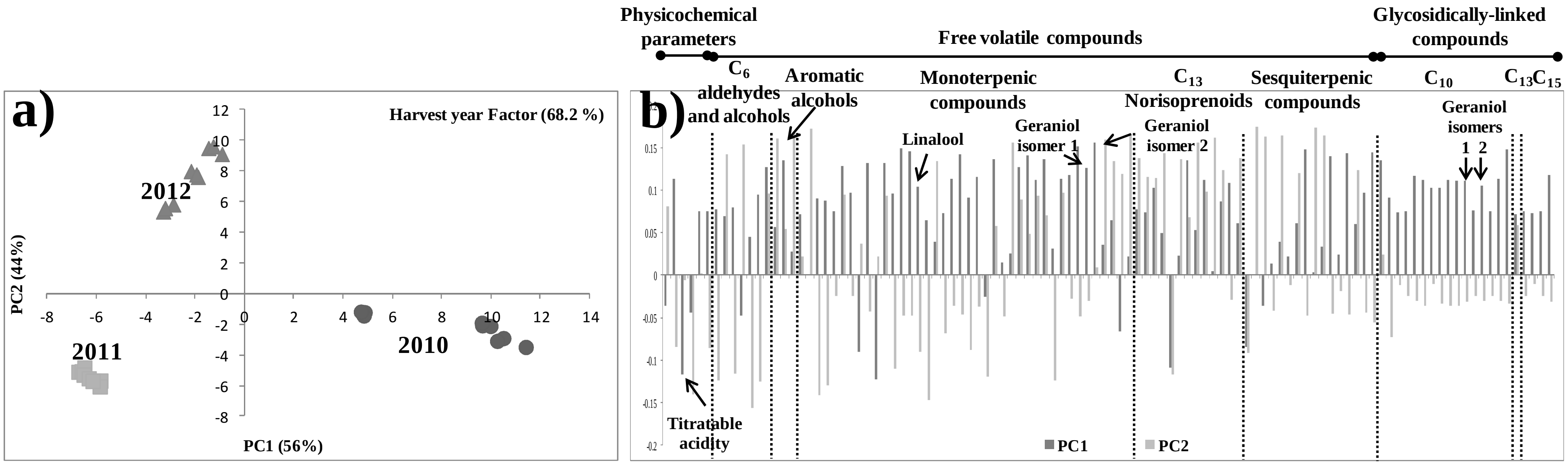


Figure 50. ASCA scores plot for harvest factor (a), and the corresponding variable loadings plot (b), obtained for Sauvignon Blanc, at technologic maturity (significance test reported on Table 7). Each variable is normalized separately by dividing by its standard deviation value.

Baga variety

The effect of harvest year accounted for 61.4% of total data set variance (Table 8). First component of scores plot separates 2010 harvest (placed in PC1 positive) from the others (placed in PC1 negative), while second component separates 2011 and 2012 harvests (Figure 51a). The loadings plot (Figure 51b) showed that the projection of 2010 harvest in PC1 positive was explained by the higher GC×GC peak area determined in grapes from this harvest, essentially of monoterpenic compounds (including hydrocarbons and oxygen-containing ones). From these, β-ocimene, α-terpinolene, rose oxide, 1-terpineol, nerol oxide, digydrocarvone, menth-1-en-9-al, and carvone were only detected in 2010 grapes. Besides, PC2 loadings showed that mainly C_{13} norisoprenoids and sesquiterpenic compounds were responsible for the separation of 2012 harvest from 2011, due to their higher GC×GC areas determined in grapes from 2012. Besides, grapes from 2011 (PC2 negative) also exhibited higher titratable acidity and sugar content when compared to 2012 and 2010 harvests.

The effect of parcels in Baga grapes accounted for 14.8% of total data set variance (Table 8). Scores and loading plots were shown in Figure 51c and d, respectively. PC1 distinguishes BA-VA1, placed in the positive side of PC1, from BA-SM1 placed in the negative side of PC1, while BA-VA2 was near to origin but in the positive side of PC1. Corresponding loadings plot (Figure 51b) showed that this separation was due to the higher sugar and phenolic contents and antiradical activity, as well as higher varietal grapes components determined on the glycosidically-linked fraction of BA-VA2 grapes, while the lower contents were determined in BA-SM1 grapes. The presence of higher amounts of varietal compounds on the glycosidically-linked fraction in BA-VA2 grapes compared with the other parcels, suggest higher aroma potential of Baga variety obtained from this parcel, due to the significant relevance of these compounds in the varietal aroma (Moreno-Arribas and Polo, 2009). Furthermore, the varietal volatile composition determined for BA-VA2 and BA-VA1 grapes was very similar, corresponding to ca. 27.5% and 26.0% of the total GC×GC areas respectively, while only 13.4% was determined for BA-SM1 grapes. Thus, their distinction based on PC1 was unclear. However, PC2 allowed distinguishing BA-VA1, placed in PC2 negative, from the other parcels (BA-SM1 placed in PC2 positive and BA-VA2 was near to origin) (Figure 51c). The loadings plot (Figure 51d) showed that the projection of BA-VA1 in PC2 negative was mainly related with the higher GC×GC peak area of some monoterpenic compounds, including α-pinene, limonene, linalool oxide, and β-citronellol. The monoterpenic compounds, if present in amounts above their sensorial perception limits, can contribute with characteristic notes: α-pinene has fresh and citrus notes, limonene has a lemon, orange and sweet notes, linalool has fruity, floral, and rosy notes, and β-citronellol exhibit rose and lemon notes (Marais, 1983). Considering the main parcels characteristics described in Chapter II, these observations should be associated to the different soils type. The soil type is highly related to the water status (Jackson and Lombard, 1993): clay-calcareous (BA-VA2) and clayey (BA-VA1) related parcels, with middle and higher water-holding capacity than clay-sandy soils seem to favour varietal volatile compounds, phenolic content, and also antiradical activity.

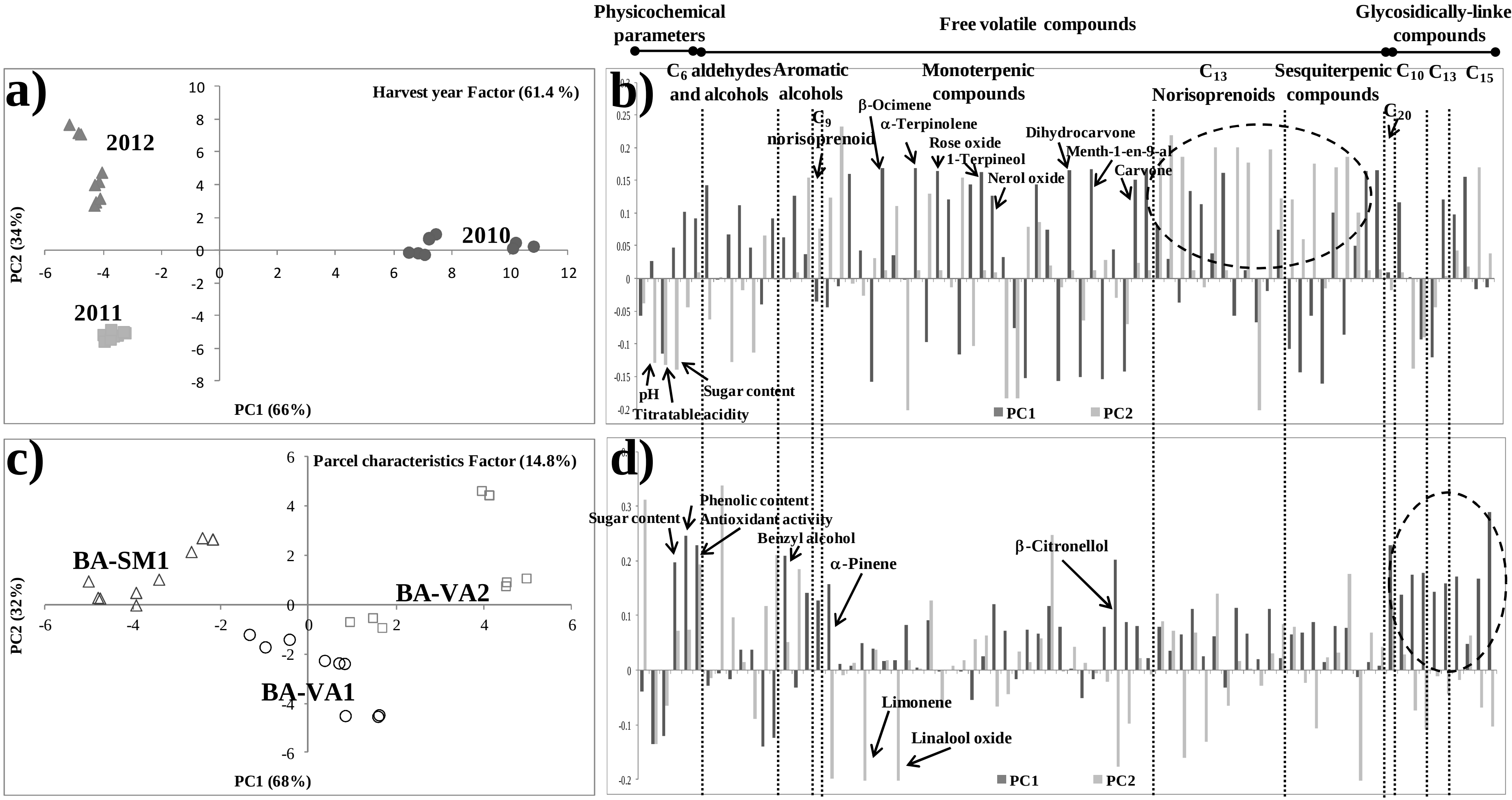


Figure 51. ASCA scores plot for (a) harvest and (c) parcel factors, and the corresponding variable loadings plot (b, and d, respectively), obtained for Baga, at technologic maturity (significance test reported on Table 7). Each variable is normalized separately by dividing by its standard deviation value.

Castelão variety

The effect of harvest on Castelão grapes composition accounted for 66.7% of the total data set variance (Table 7). According to the scores plot (Figure 52a), first PC allowed to distinguish 2010 harvest, placed in positive side of PC1, from the other 2 harvests, placed in PC1 negative side. The corresponding loadings plot (Figure 52b) showed that the separation of grapes from 2010 harvest was related with the higher pH and phenolic content of these grapes and principally with the higher volatile compounds amounts determined, principally the monoterpenic ones which represent ca. 20% of the total volatiles, while in 2011 and 2012 these chemical family represent ca. 10% and 13%, respectively. Furthermore, PC2 allowed to distinguish 2012, placed in PC2 positive, from 2011, placed in PC2 negative (Figure 52a). The loadings scatter plot (Figure 52b) showed that the projection of 2011 in PC2 negative was mainly related with the lower content of almost all parameters determined when compared to 2012, essentially the oxygen-containing monoterpenic compounds (free and glycosidically-linked fractions). Besides, 2012 grapes (PC2 positive) exhibited higher titratable acidity and lower sugar content. The results from the 3 consecutive harvests showed that, besides the fact that Castelão was well adapted to regions with maritime influence (as Bairrada Appellation), its grapes composition is modulated by the different climatic conditions of each harvest.

The scores plot for the factor parcel accounted for 15.5% of the total data set variance (Table 7, Figure 52b). The different parcels were distributed along the first PC with CA-SM1 situated at the positive side and CA-SM3 at the negative side. Analysis of loadings (Figure 52d) showed that this distribution along PC1 was related to the higher titratable acidity, antiradical activity and higher content of some oxygen-containing monoterpenic compounds (dihydromyrcenol, hotrienol, and nerol oxide) and also higher varietal content determined on the glycosidically-linked fraction of CA-SM1 grapes, when compared with CA-SM3 grapes. Also, along PC2, it was possible to distinguish CA-SM2 from the other parcels (PC2 negative), which was characterized essentially by lower berry weight and pH, and higher amounts of sugar, C_6 alcohols and aldehydes, and some varietal compounds as linalool oxide, hotrienol, α-terpineol, vitispirane, and β-damascenone. According to these results clay-calcareous (CA-SM1) and clayey (CA-SM2) soils with higher water-holding capacity than clay-sandy soil seem to be related with higher varietal volatile composition of Castelão grapes.

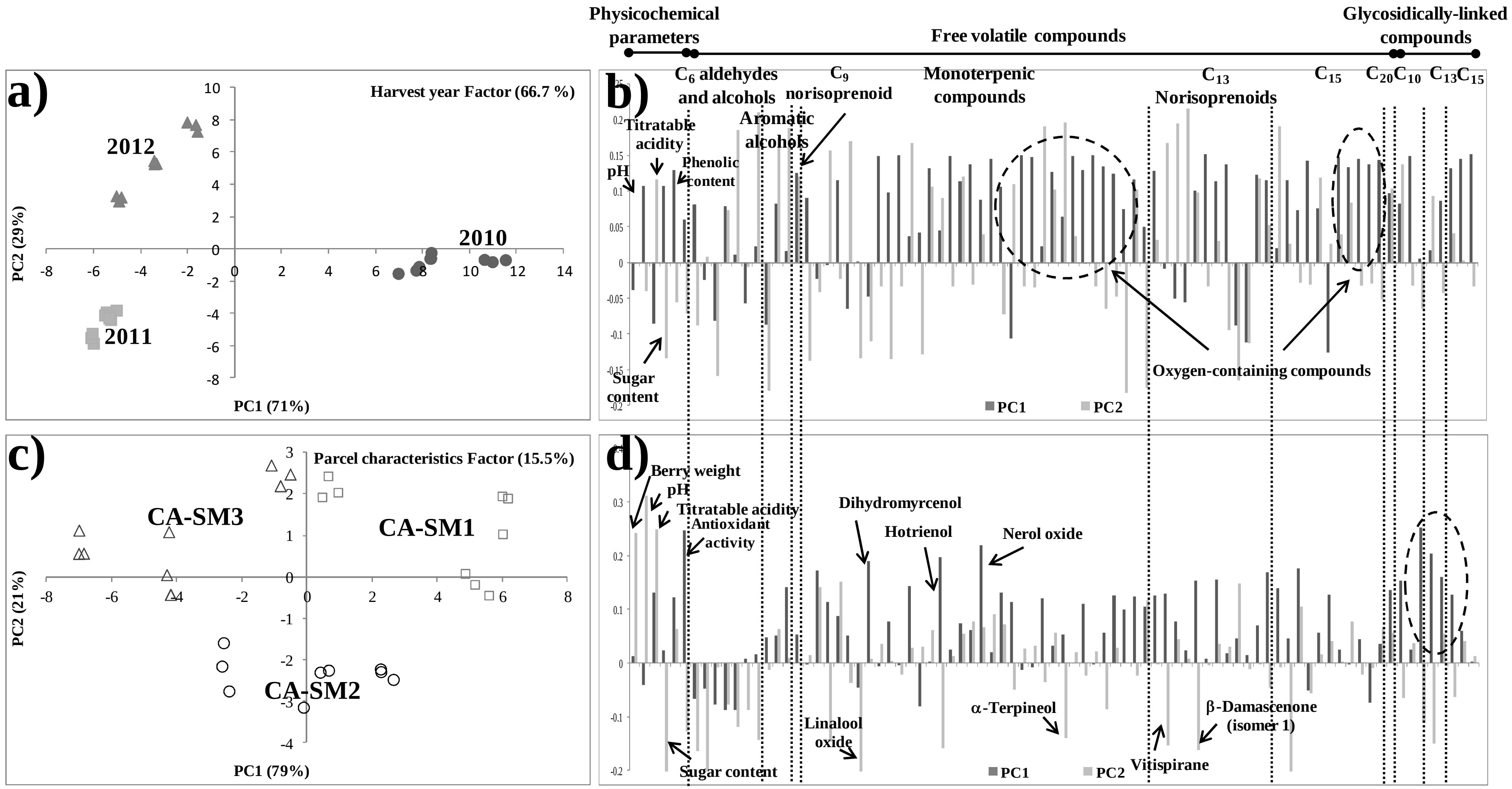


Figure 52. ASCA scores plot for (a) harvest and (c) parcel factors, and the corresponding variable loadings plot (b, and d, respectively), obtained for Castelão, at technologic maturity (significance test reported on Table 7). Each variable is normalized separately by dividing by its standard deviation value.

Touriga Nacional variety

For Touriga Nacional variety harvest accounted for 59.3% of the total data set variance (Table 7). First component of the score plot allowed to separate 2010 harvest, placed in PC1 positive side, from 2012 and 2011 harvests placed in PC1 negative side and near to origin, respectively (Figure 53a). The loadings plot (Figure 53b) showed that essentially aromatic alcohols, C_{13} norisoprenoids and monoterpenic compounds in the free and glycosidically-linked forms, contributed to discriminate harvests along PC1. In grapes from 2010 harvest, higher content of aromatic alcohols was determined, accounting for ca. 4% of the total GC×GC areas, while only ca. 2.5% was determined in 2011 and 2012. Higher amounts of 2 isomers of β-damascenone and geranylacetone were also found in grapes from 2010. Besides, two monoterpenic compounds, β-ocimene and dihydrolinalool, were only determined in this harvest. On the other hand, higher titratable acidity and lower amounts of monoterpenic compounds were determined in grapes from 2012 harvest. However, 5,6-epoxy-β-ionone was only determined in grapes from this harvest. Touriga Nacional requires good sunlight exposures for a long period in order to develop its volatile characteristics. As 2012 was fresh and rainy this may explain the lower volatile content determined in this harvest. In grapes from 2011 harvest was found two monoterpenones (verbenone and carvone) and one C_{13} norisoprenoid (vitispirane) which were not determined in 2010 and 2012 harvests. The C_6 alcohols and aldehydes were found to be higher in grapes form 2011, accounting for ca. 78% of the total GC×GC areas and ca. 73% for grapes from the other harvests. Considering all harvest under study, for Touriga Nacional the higher terpenic compound determined was linalool, accounting for 20-27% of the total monoterpenic GC×GC areas. Previous studies also identified linalool as a major monoterpenic compound determined in Touriga Nacional wines, being considered an important varietal compound in the aroma of its wines (Oliveira *et al.*, 2006; Pinho *et al.*, 2007).

The effect of parcel on Touriga Nacional grapes accounted for 18.8% of the total data set variance (Table 7). Scores and loadings plots were shown in Figure 53c and d, respectively. According to the scores plot of the two first principal components, PC1 distinguishes TN-SM2 (PC1 positive) from the other 2 parcels, TN-SM1 and TN-SM3, both placed in PC1 negative. Corresponding loadings plot (Figure 53d) showed that grapes from TN-SM2 (PC1 positive) had lower berry weigh and higher sugar and phenolic contents, antiradical activity, and content of monoterpenic compounds, mainly the oxygen-containing ones. Besides, PC2 distinguishes TN-SM1 (PC2 negative) from TN-SM3, which was characterized by higher amount of varietal compounds, including mono- and sesquiterpenic compounds, and also C_{13} norisoprenoids. Linalool, the major monoterpenic compound determined in all parcels, accounted for ca. 27% and 25% in grapes from TN-SM2 followed by TN-SM1 respectively, values higher than those found for TN-SM3 grapes (ca. 14%). According to the characteristics of these parcels described in Chapter II, higher altitude and clay-calcareous soil of TN-SM2 parcel seem to be related with higher varietal aroma potential of Touriga Nacional red variety, and also higher grapes phenolic content and antiradical activity.

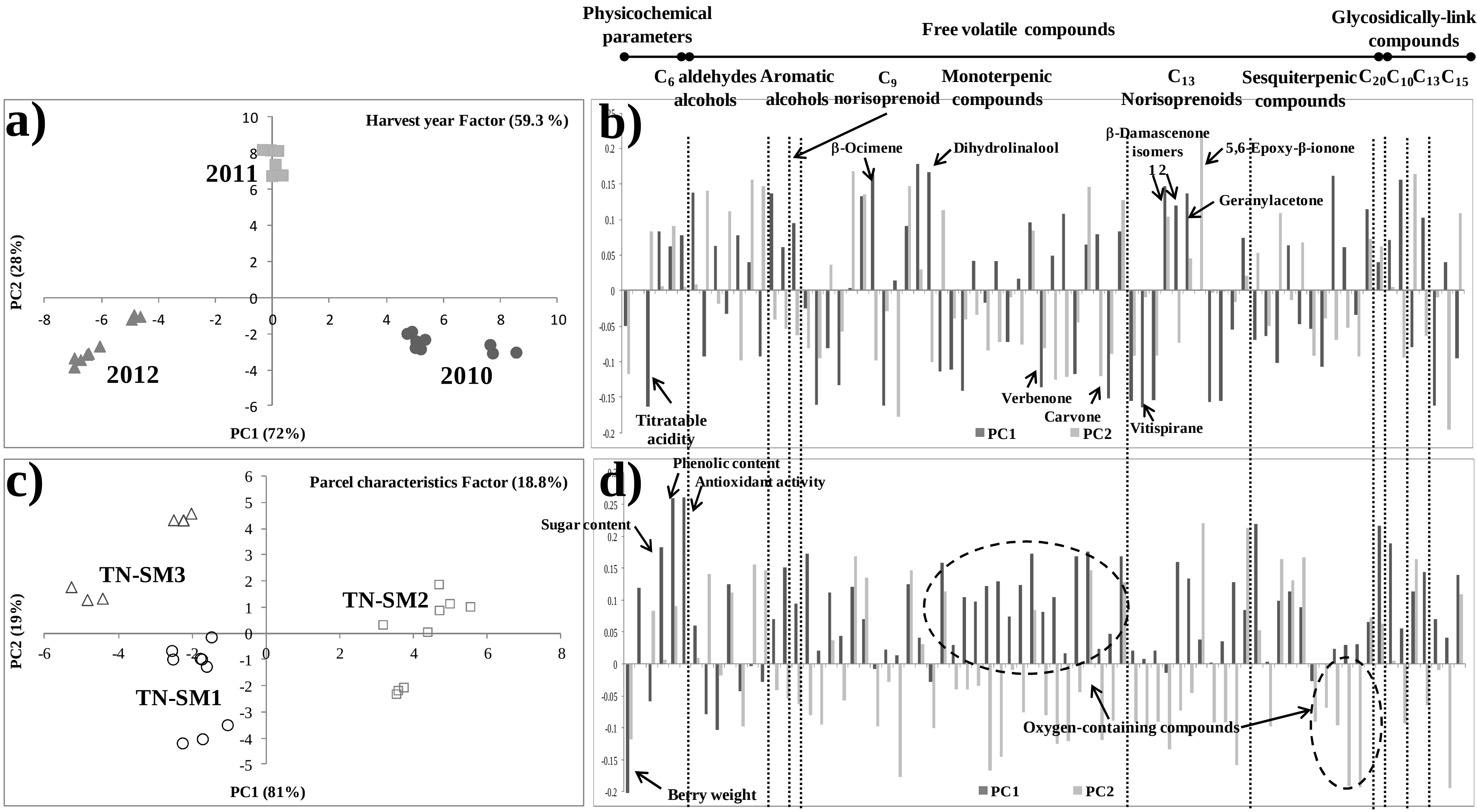


Figure 53. ASCA scores plot for (a) harvest and (c) parcel factors, and the corresponding variable loadings plot (b, and d, respectively), obtained for Touriga Nacional, at technologic maturity (significance test reported on Table 7). Each variable is normalized separately by dividing by its standard deviation value.

IV. 4. Concluding remarks

The comprehensive approach allowed identifying the effect of harvest and parcelling characteristics on grapes composition allowing the ascription of the oenological potential of each variety, on the different conditions under study, as initially proposed. Each variety showed different adaptation behaviour to the edaphoclimatic conditions under study, revealing the unique character of each variety.

Harvest was the main factor that influences grapes composition (53% to 68% of the total data set variance). Considering all varieties, the moderate climatic conditions (2010) promoted higher phenolic and volatile contents, while the opposite was observed for 2011 harvest. However, fresh and rainy climatic conditions (2012) promoted lower monoterpenic content in Touriga Nacional. This is a variety that requires good sunlight exposures for a long period in order to develop its volatile characteristics.

Parcel characteristics also influence grapes compositions explaining ca. 15-19% of the total data set variance. According to the obtained results, the volatile and phenolic contents and antiradicalar activity were higher in clay-sandy followed by clay-calcareous soils for Arinto and Bical varieties. For Sauvignon Blanc the parcel characteristics had no significant effect. For Baga, Castelão, and Touriga Nacional red varieties, the phenolic and varietal volatile contents and antiradicalar activity were higher in clay-calcareous followed by clayey soils. Furthermore, sugar content was higher in Baga and Touriga Nacional and titratable acidity was higher in Castelão. Besides, also altitude seems to modulate Touriga Nacional grapes composition.

The results obtained allowed to identify a specific behaviour for each variety regarding harvest year climatic conditions and also parcels characteristics. Thus, in order to better visualise the uniqueness of each variety, and to better compare their compositions, a synoptic figure was constructed, joining the entire generated grape data at technologic maturity, considering the variability associated to the 3 harvest years and 3 parcels characteristics (Figure 54). According to this figure, Sauvignon Blanc was the variety with higher sugar content and higher varietal volatile compounds followed by Arinto, while the opposite was observed for Bical grapes. Varietal compounds are related with fruity, sweety and floral aromas, thus their higher content on Sauvignon Blanc may explain the aroma sensory attributes of this variety (Chapter II). For the red varieties, Baga and Castelão had similar titratable acidity, sugar, phenolic and glycosidically-linked volatile compounds.

From the red varieties under study, Sousão grapes exhibited higher titratable acidity and phenolic content, and lower varietal volatile content, which may suggest higher colour and lower aroma potential for this variety. This may explain why this variety is used as "teinturier" to blend with other varieties poor in colour but rich in aromas. Besides, Touriga Nacional grapes had lower titratable acidity and its total phenolic content was near to Sousão variety.

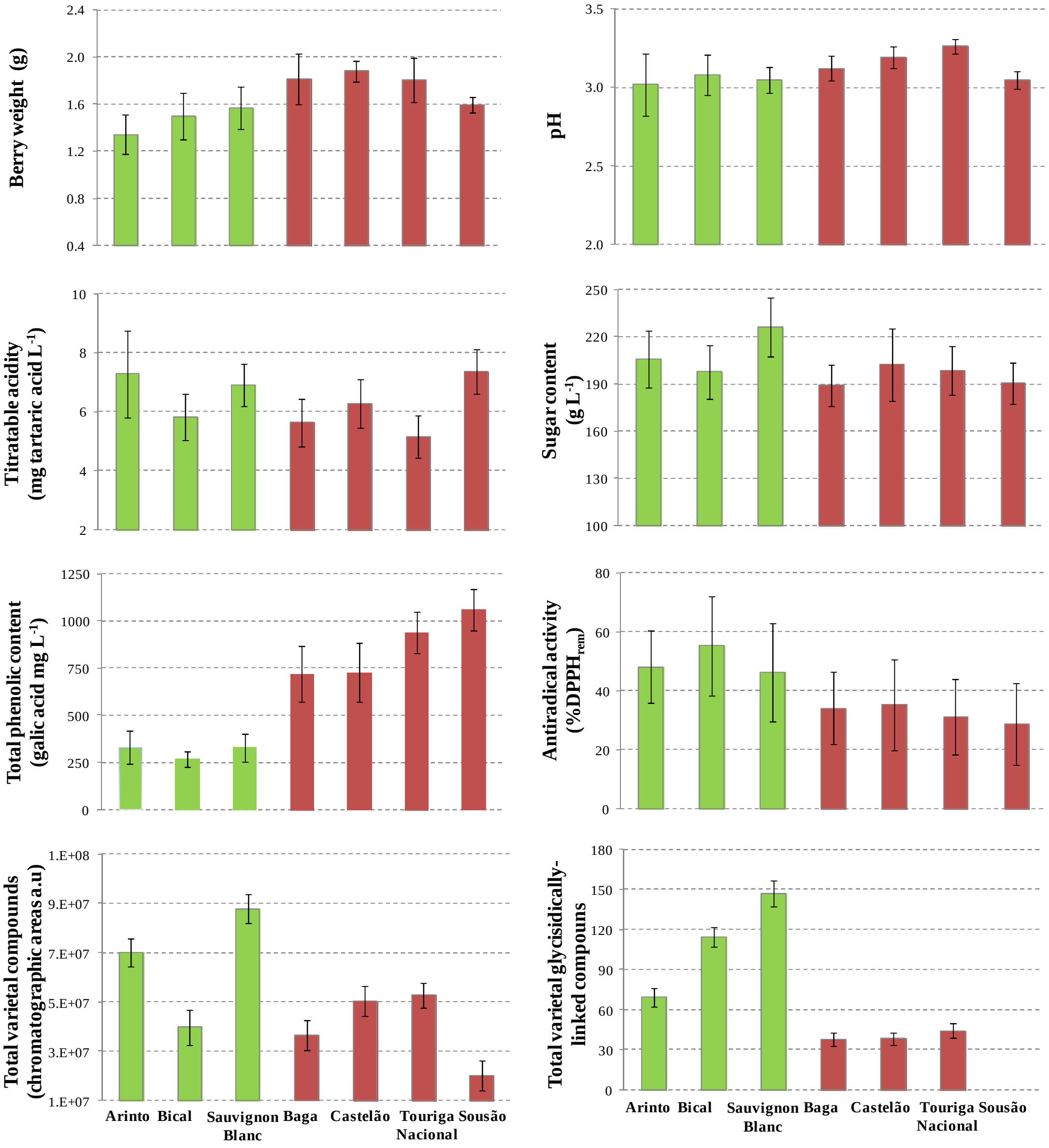


Figure 54. Synotic figure of all the parameters obtained at technologic maturity for grapes from *V. vinifera* cv. Arinto, Bical, Sauvignon Blanc, Baga, Castelão, Touriga Nacional and Sousão.
* Glycosidically-linked fraction was not determined for Sousão variety.

Chapter V - Combining sensory and molecular data provided by instrumental analysis to explain wine aroma properties

Combining sensory and molecular data provided by instrumental analysis to explain wine aroma properties

Overview

Aroma plays an important role in wine quality and is related to volatile compounds and aroma sensory perceptions. This can yield new insights in the aroma fingerprint to be used for the distinction of wines based on their aroma properties. The aim of the present research study was to establish the aroma properties of 6 monovarietal wines. A novel approach, named aroma network, that links volatile compounds (instrumental analysis) and aroma data to key odour active molecules was used. For this, several chemical families that are known to contribute for wine aroma perceptions, namely esters, carbonyl compounds, alcohols, acids, terpenic compounds, lactones, C_{13} norisoprenoids, volatile phenols, and thiols, were considered. Wines from Bairrada Appellation were selected as a case study. The economic agents of this Appellation aim to greater enhance the appreciation and the consumption of Bairrada wines in markets worldwide. For the present study, 3 white (Arinto, Bical, and Sauvignon Blanc) and 3 red monovarietal commercial wines (Baga, Castelão and Touriga Nacional varieties) were selected.

This network approach is a tool that can be used as an aroma fingerprint to explain the aroma properties of wines worldwide. The comprehensive approach can yield new insights in wine related science.

V. 1. Framework

Wine quality concept is very complex, being related with several intrinsic (defined by the drinking experience, namely pleasure, aroma, flavour, and mouthfeel) and extrinsic factors (winemaking, price, origin, bottle form, and colour), that influence consumers choice (Hopfer and Heymann, 2014; Sáenz-Navajas *et al.*, 2013). From these, aroma is considered one of the most significant factors to establish wine quality and character and therefore, for determining consumer's acceptance. A relationship between the wine character and its volatile composition has already been recognized by several researchers worldwide, and hundreds of volatile compounds belonging to different chemical classes, namely alcohols, esters, acids, aldehydes, ketones, lactones, terpenoids, and volatile phenols, have already been identified in different wine varieties (Rocha *et al.*, 2000; Rocha *et al.*, 2007b; Vázquez *et al.*, 2002; Vilanova *et al.*, 2010). As these compounds produce an effect on consumer's sensory perceptions, both volatile composition and sensory properties are essential to determine wine aroma characteristics.

Gas chromatography (GC) based techniques provide an effective tool for the wine volatile compounds determination. However, without aroma sensory analysis by a trained panel, the mere knowledge about the volatile composition of a wine is insufficient to explain the whole wine aroma properties (Álvarez *et al.*, 2011). Thus, due to the high economic value of the wine-product worldwide, the exploitation of innovative approaches allowing to combine sensory and instrumental data (volatile composition), and explaining the wine aroma properties are extremely important. One of these approaches is the network-based approach. This approach was introduced as a network that captures the flavour compounds shared by culinary ingredients, identifying a series of statistically significant patterns that characterize the way humans choose the ingredients they combine in their food (Ahn *et al.*, 2011). Firstly, a bipartite network-based approach consisting of two different nodes need to be built, being one node the aroma notes, and the other the volatile compounds. Each volatile compound can present one or different aroma notes, contributing to the complexity of the aroma. Then, a projection of the bipartite network-based approach is performed, known as aroma network. For the determination of the aroma networks, the amount of each compound determined for each specific note, is considered. In this aroma network two nodes are linked if they share at least one aroma note, and the thickness of the line used between aroma notes is proportional to the number of shared compounds (Ahn *et al.*, 2011).

V. 2. Material and Methods

V. 2.1. Wines under study

Six young monovarietal wines (all of them with less than 1 year) from 2010 harvest were studied: Vitis vinifera L. cv. Arinto (12.0 alcohol % v/v), Bical (13.0 alcohol % v/v), Sauvignon Blanc (12.5 alcohol % v/v), Baga (12.5 alcohol % v/v), Castelão (14.0 alcohol % v/v), and Touriga Nacional (15.0 alcohol % v/v) varieties. The wines were produced in Manuel dos Santos Campolargo, Herdeiros company, from Bairrada Appellation (Portugal). These varieties are recommended for QWPSR (quality wine produced in specified region) of Bairrada Appellation. Also, Baga is the most cultivated variety in this Appellation and represents 90% of the total red Bairrada vineyard. Also, vineyards of Arinto and Bical varieties represent 20% (10% for each one) of the total white vineyard. Briefly, the white grape varieties were pressed and decanted. Then, must fermentation occurred with batonnage in barrels, without temperature control for Arinto and Bical. In the case of Sauvignon Blanc musts, fermentation occurred in still vats with controlled temperature (12 ºC). The red grape varieties were crushed and de-stemmed. Fermentation occurred in small vats using mechanical pressing and, at the end of fermentation, they were passed to barrels. All samples were bottled (3 bottles of 0.75 L for each wine variety), sulfited and stored at 4 ºC in the dark, until analysis.

V. 2.2. Reagents and Standards

Dichloromethane, HPLC quality, was from Fisher Scientific (Loughborough, U.K.), methanol of LiChrosolv quality was from Merck (Darmstadt, Germany), absolute ethanol (ACS quality) was purchased from Panreac (Barcelona, Spain), and pure water was obtained from a Milli-Q purification system (Millipore, Billerica, MA). LiChrolut EN resins and polypropylene cartridges were obtained from Merck (Darmstadt, Germany). The aroma chemical standards were supplied by Aldrich (Gillingham, U.K.), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO), Lancaster (Strasbourg, France), PolyScience (Niles, IL), ChemService (West Chester, PA), Interchim (Monlucüon, France), International

Express Service (Allauch, France), and Firmenich (Geneva, Switzerland). α,α,α-Tris-(hydroxymethyl)-methylamine (Tris) 99.9% was obtained from Aldrich-España (Madrid, Spain), and cysteine 99% and phydroxymercuribenzoic acid were from Sigma (St. Louis, MO). The chemical standards used were 2-butanol, 4-methyl-2-pentanol, 2-octanol, and 4-hydroxy-4-methyl-2-pentanone, supplied by Merck (Darmstadt, Germany), PolyScience (Miles, USA), and Aldrich (Gillingham, UK), respectively. *n*-Hexane for organic trace analysis (UniSolv) was from Merck (Darmstadt, Germany). Diethyl ether for instrumental analysis and mercaptoglycerol were from Fluka (Buchs, Switzerland). Anhydrous sodium sulfate was for analysis ACS-ISO quality from Panreac (Barcelona, Spain). Ethylenediaminetetraacetic acid disodium salt 2-hydrate (EDTA), L-cystein hydrochloride hydrate 99%, 1,4-dithioerythritol, octafluoronaphthalene 96% (OFN) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were from Aldrich (Steinheim, Germany). *O*-Methylhydroxylamine hydrochloride purum >98% and 2,3,4,5,6-Pentafluorobenzyl bromide (PFBBr) were from Fluka (Buchs, Switzerland). 4-Mercapto-4-methyl-2-pentanone and 3-mercaptohexylacetate were from Oxford Chemicals (Hartlepool, UK). 2-Furfurylthiol and 3-mercaptohexanol were from Lancaster (Strasbourg, France). 2-Methyl-3-furanthiol and 2-methyl-3-tetrahydrofuranthiol were from Aldrich (Steinheim, Germany). Benzylmercaptan, 2-phenylethanethiol and 4-methoxy-α-toluenethiol were from Fluka (Buchs, Switzerland). Bond Elut-ENV resins, prepacked in a 50 mg cartridge (1 mL total volume) and semi-automated SPE Vac Elut 20 station were from Varian (Walnut Creek, CA, USA).

V. 2.3. Determination of wine volatile components based on gas chromatographic techniques

Wines are composed by several hundreds of volatile compounds that belong to different chemical classes (namely alcohols, esters, acids, lactones, terpenoids, volatile phenols), present at different concentration ranges, which increase the complexity of their determination in one single analysis. Figure 55 represents a workflow of the experimental procedures used for the extraction techniques and gas chromatographic analysis for a comprehensive determination of the wines volatile composition.

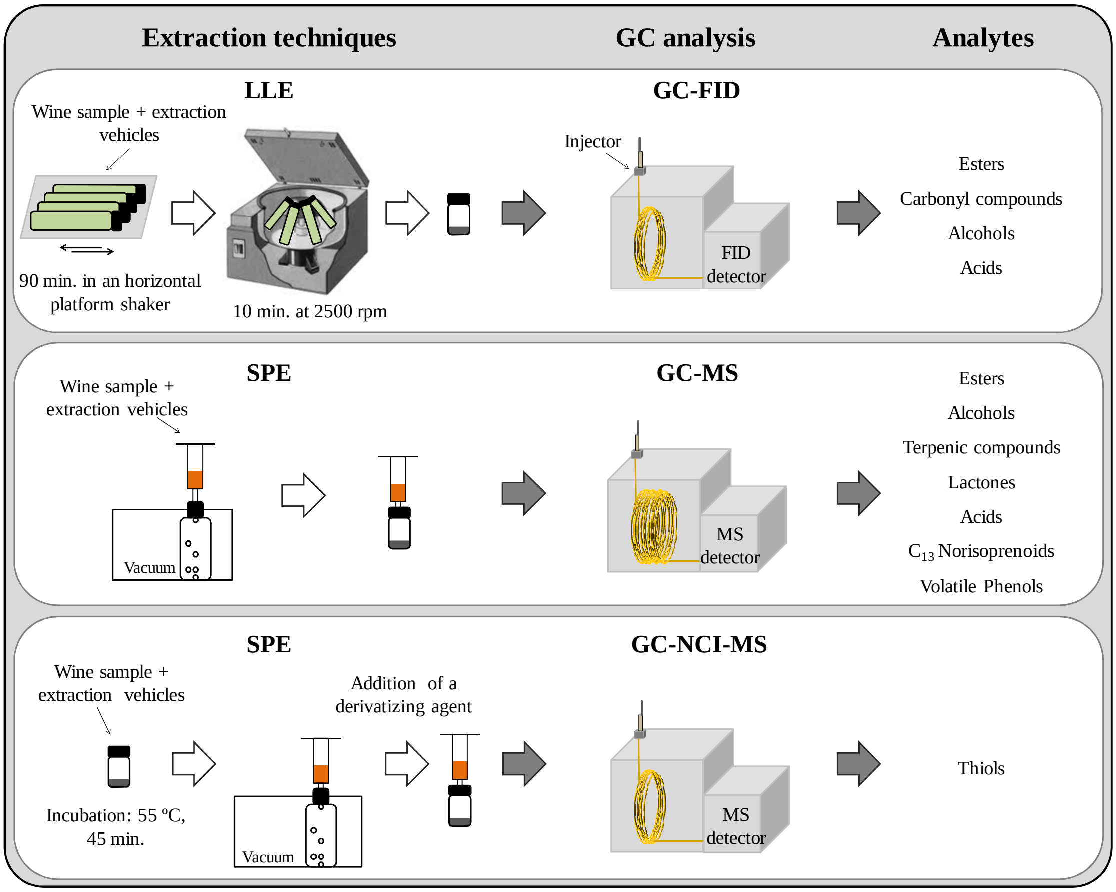


Figure 55. Workflow of the experimental procedures used for the extraction and gas chromatographic analysis for a comprehensive determination of the wines volatile composition.

a) Determination of esters, carbonyl compounds, alcohols and acids by liquid-liquid extraction (LLE)/GC-FID - According to the LLE/GC–FID methodology already proposed (Ortega *et al.*, 2001), to 10 mL screw-capped centrifuge tubes were added 4.1 g of ammonium sulphate ($(NH_4)_2SO_4$), 2.7 mL of wine, 6.3 mL of water, 20 µL of internal standard solution (2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, ethyl heptanoate, heptanoic acid and 2-octanol at 200 µg/mL in ethanol) and 0.25 mL of dichloromethane. The tubes were shaken in a horizontal platform shaker (Heidolph Promax 1020), during 90 min, and then centrifuged at 2500 rpm for 10 min. Once the phases had been separated, the dichloromethane phase was recovered (*ca.* 150 µL) with a 500 µL syringe and transferred to a 300 µL vial. This extract was then analyzed by GC with FID detection. A Varian CP-3800 gas chromatograph was used. The column (30 m × 0.32 mm I.D., 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA) was a Carbowax 20M, preceded by a 3 m x 0.33 mm uncoated pre-column. The temperature program was as follows: 40 ºC for 5 min, then raised at 4 ºC/min up to 102 ºC, then raised at 2 ºC/min up to 112 ºC, then raised 3 ºC/min up to 125 ºC for 5 min, then raised at 3 ºC/min up to 160 ºC and finally raised 6 ºC/min up to 200 ºC for 30 min. Injector and detector were both kept at 250 ºC. Carrier gas was H_2 at 2.2 mL/min, the split flow was 1.20 mL/min, and the injection (2 μL) was performed in split mode. Identification of each volatile compound was confirmed by the coincidence of the retention times of each compound by the corresponding chemical standard. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs built by the analysis of synthetic wines containing known amounts of the analytes. Each sample was extracted in duplicate.

b) Determination of terpenic compounds, lactones, C_{13} norisoprenoids, volatile phenols, and also some esters, alcohols, and acids by solid-phase extraction (SPE)/GC–ion trap–MS - According to the previously developed SPE/GC–ion trap–MS methodology (López *et al.*, 2002), 50 mL of wine sample containing 26 μL of surrogated standards solution (surrogates were isopropyl propanoate, 3-octanone, heptanoic acid and β-damascone), was passed through a 200 mg LiChrolut EN cartridge at about 2 mL/min. The SPE cartridge had been previously conditioned with 4 mL of dichloromethane, 4 mL of methanol and, finally, with 4 mL of a water–ethanol mixture (12%, v/v). The sorbent was dried by letting air pass through (0.6 bar, 10 min). Analytes were recovered by elution with 1.6 mL of dichloromethane-1% methanol. An internal standard solution (2-octanol, 4-methyl-2-pentanol, and 4-hydroxy-4-methyl-2-pentanone in dichloromethane) was added to the eluted sample. The extract was then analyzed by GC with ion trap MS. The GC was a Star 3400CX fitted to a Saturn 4 electronic impact ion trap mass spectrometer from Varian. The column used was a DB-WAXetr (60 m × 0.25 mm I.D., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA), and was preceded by a 3 m x 0.25 mm uncoated (deactivated, intermediate polarity) pre-column. The carrier gas was He at 1.5 mL/min. The temperature program was as follows: 40 ºC for 5 min, raised to 220 ºC at 2 ºC/min. A 1079 septum-equipped programmable injector (SPI) from Varian was used. The initial temperature of this injector was 40 ºC for 0.3 min and was then raised to 250 ºC at 200 ºC/min. Three microlitres of sample were injected in splitless mode. A 35–220 *m/z* mass range was recorded in full scan acquisition mode. Identification of each volatile compound was confirmed by the coincidence of the retention times and mass spectra of each compound with the corresponding chemical standard. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs obtained from the GC–MS analysis of dichloromethane solutions containing known amounts of the analytes and of the internal standards. Each wine sample was extracted in duplicate.

c) Determination of thiols using SPE/GC–negative chemical ionization (NCI)–MS - Based on the developed SPE/GC–NCI–MS methodology for the determination of thiols (Mateo-Vivaracho *et al.*, 2008). In a 40 mL screw capped vial, spiked 25 mL of wine with 0.2 g of EDTA (5 g/L) and 0.6 g of L-cystein clorhydrate (0.1 M Cys) and shake for 2 min. After this, 10 mL of the wine was transferred to a 20 mL volumetric flask, spiked with 15 μL of an ethanolic solution containing 1400 μg/L of 2-phenylethanethiol as internal standard, and was shaken to ensure a complete dissolution, and make up to volume with the wine. This volume was transferred to a 24 mL screw-capped vial to which 0.2 g of *o*-methylhydroxylamine was added, the mixture stirred for 15 s, and the vial purged gently with pure nitrogen, sealed, and incubated in a water bath at 55 ºC for 45 min. Six milliliters of this incubated sample were then loaded onto a 50 mg Bond Elut-ENV SPE cartridge (previously conditioned with 1 mL of dichloromethane, 1 mL of methanol and 1mL of water). Some wine volatile components (namely esters, alcohols, acids) were removed by rinsing with 4 mL of a 40 % methanol–water solution 0.2 M in phosphate buffer at pH 7.7 and after this, with 1 mL of water. A second internal standard was added to the cartridge (20 μL of an ethanolic solution containing 150 μg/L of 4-methoxy-α-toluenethiol and 200 μL of water) and loaded onto the cartridge. Thiols retained in the cartridge were directly derivatized by passing 1 mL of an aqueous solution of DBU (6.7%) and 50 μL of a 2000 mg/L solution of PFBBr in hexane, and letting the cartridge imbibed with the reagent for 20 min at room temperature (*ca*. 25 ºC). Excess of reagent is removed by adding 100 μL of a 2000 mg/L solution of mercaptoglycerol in 6.7% DBU aqueous solution, and letting the cartridge react again for more 20 min at room temperature. The cartridge was then rinsed with 4 mL of a 40 % methanol/water solution 0.2 M in H_3PO_4 and with 1 mL of water. Derivatized analytes were finally eluted with 600 μL of a solvent mixture (hexane 25% in diethylether) containing 22.5 μg/L of the internal standard (OFN). The eluate was finally washed with five 1 mL volumes of brine (200 g/L NaCl water solution), transferred to a standard 2 mL autosampler vial and spiked with a small amount of anhydrous sodium sulfate. Four microliters of this sample was directly injected into the GC-negative chemical ionization (NCI) MS system. A Shimadzu QP-2010 Plus gas chromatograph with a quadrupole mass spectrometric detection system was used, with an Optic 3 injector from ATAS-GL (Veldhoven, The Netherlands). The initial temperature of the injector was 65 ºC and after 40 s it was heated at 5 ºC/s to 260 ºC, remaining at this temperature until the end of the analysis. The carrier gas was He, flowing through the column initially at 0.82 mL/min. Seventeen seconds after the injection the flow was increased to 1 mL/min for 4 min. After this period it was fixed at 3mL/min. The split valve was opened at the first 17 s of analysis (split flow 100 mL/min), closed at the following 3 min, and opened again for the rest of the analysis (split flow 50 mL/min). The column was a Factor Four capillary column DB-5MS (20 m × 0.18 mm I.D., 0.18 μm film thickness, Varian, Walnut Creek, CA, USA). The column initial temperature was 40 ºC for 4.35 min, heated to 140 ºC at 25 ºC/min, then to 180 ºC at 15 ºC/min, then to 210 ºC at 30 ºC/min and finally to 300 ºC at 250 ºC/min; remaining at that temperature for 10 min. The ion source was operated in NCI mode using methane at 2 bars as reagent gas. The temperature of the ion source was 220 ºC and the interface was kept at 280 ºC. The analytes and internal standards ions were acquired in single ion monitoring (SIM) mode from minute 5.5 to minute 18 at 0.18 s/point: OFN was quantified with *m/z* 272; 2-methyl-3-furanthiol and 2-furfurylthiol were quantified with *m/z* 274; 4-mercapto-4-methyl-2-pentanone was quantified with *m/z* 160; 3-mercapto-1-hexanol was quantified with *m/z* 133; 3-mercaptohexyl acetate was quantified with *m/z* 175; and benzylmercaptan was quantified with *m/z* 284. Finally, the quantification of the internal standards was carried out with *m/z* 135 and 314 for 2-phenylethanethiol and 4-methoxy-α-toluenethiol, respectively. To obtain the concentration data, the corresponding analyte peak relative areas were simply divided by the slopes calculated for solutions containing known amounts of the analytes.

V. 2.4. Wine aroma sensory analysis

The sensory panel was composed of eight females and five males, between the ages of 23 and 68, all of them belonging to the laboratory for Flavour Analysis and Analytical Chemistry of Zaragoza (Spain), with long experience in sensory analysis. Five specific 1 hour training sessions were carried out. In the first one, judges generated descriptive terms for Bairrada wines. In sessions 2 and 3, different aroma standards were presented and discussed by the panel, where a total of 15 aroma terms were selected for the following descriptive analysis: 11 for white wines (fermentation, tree and tropical fruits, citric, herbaceous, fusel, toasted, oxidized, flowery, sweet, and woody) and 10 for the red ones (fermentation, sweet fruits (which include tree, tropical, and berry fruits), herbaceous, fusel, toasted, oxidized, flowery, spicy, lactic, and reduction). In sessions 4 and 5, panelists scored the intensity of each attribute using a 7 point scale: 0 = no odor, 1 = weak and low intense odor, 2 = clear perceptive and intense odor, 3 = extremely intense odor; half values were allowed. After the training period, each panelist participated individually in one session per day, to evaluate the wine samples. In all cases, wines (20 mL, ca 20 °C, 2 glasses) were presented in coded, tulip-shaped glasses covered by glass dishes and presented in a random order. The data processed was a mixture of intensity and frequency of detection ("modified frequency" - MF), which was calculated with the following formula:

$MF(\%) = \sqrt{F(\%)I(\%)}$ (Dravnieks, 1985); where F(%) is the detection frequency of an aroma attribute expressed as percentage of total number of judges (n = 13) and I (%) is the average intensity expressed as percentage of the maximum intensity.

V. 2.5. Data processing

A heatmap representation of the full wine volatile data set (6 wine varieties, total of volatile components, 2 independent replicates), normalized by maximum, was performed by using the Unscrambler® X (30-day trial version - CAMO Software AS, Oslo, Norway).

Based on the wine volatile composition a bipartite network was built, consisting of two different nodes: one node corresponded to the volatile components determined in wines and the other represented the corresponding aroma descriptors (all aroma descriptors are indicated for each volatile compound). A total of 19 aroma notes were found (citric, sweet, woody, flowery, honey, coconut, tropical fruit, tree fruit, berry fruit, fermentation, toasted, spicy, fusel/alcohol, vanilla, herbaceous, lactic (cheese, butter), oxidized, reduction (animal, leather), and tobacco) for the wine components determined. Then, a projection of this bipartite network was performed, known as aroma network. The aroma network of each one of the 6 monovarietal wines was constructed based on the determined bipartite network and on the determined odor activity values – OAVs, described as the ratio between the concentration of each volatile component in a wine sample (Callejon *et al.*, 2010) and its odor threshold value (the lowest concentration of a compound in vapour phase which can be detected by smell). The percentage of the OAV of each wine component that contributed for each individual aroma note was determined, and represented in pie charts. Only for these representations, the mean between the 2 replicates were considered. In these pie charts, each colour represents an aroma note, and the arc length/central angle of each sector (aroma note), is proportional to the quantity (OAV) it represents. The thickness of the line corresponds to the number of shared compounds found in the studied wines.

Aroma sensory data was analyzed by two-way analysis of variance (ANOVA), in which wine varieties and judges were considered as the factors (significance level was determined according to the p-value obtained). ANOVA was applied to the sub-set of white wines (Arinto, Bical, and Sauvignon Blanc) and to the sub-set of red ones (Baga, Castelão, and Touriga Nacional), and notation * indicate significance at $p < 0.05$ and ** $p < 0.01$. The SPSS software for Windows, version 5.0, from SPSS Inc. (Chicago, IL, USA) was used.

V. 3. Results and Discussion

V. 3.1. Wine volatile components determination

A total of 71 volatile compounds, distributed over 9 chemical families, including esters, alcohols, acids, carbonyl compounds, terpenic compounds, C_{13} norisoprenoids, lactones, phenols and thiols, were determined in the wines under study (Table 8 and Table 9 for white and red wines, respectively). Besides their quantification, the already known odour threshold values (Campo *et al.*, 2006; Gómez-Míguez *et al.*, 2007) and the OAVs for each determined compound were also considered (details in Tables 8 and 9).

Table 8. Quantitative wine volatile components determination for the 3 Bairrada white wines studied based on the different GC techniques used, organized by chemical families, odor threshold, content, and odor active values (OAV).

		Arinto				**Bical**				**Sauvignon Blanc**			
Compound	**Odor threshold (µg/L) [a]**	**Content* (µg/L) n=2**		**OAV [b]**		**Content (µg/L) n=2**		**OAV**		**Content (µg/L) n=2**		**OAV**	
Esters													
Ethyl acetate (a)	12264	13422.5	13483.6	1.09	1.10	12401.2	12458.4	1.01	1.02	18700.6	19540.8	1.52	1.59
Ethyl propanoate (a)	10	94.9	92.3	9.49	9.23	103.8	107.7	10.38	10.77	94.1	97	9.41	9.70
Ethyl butyrate (a)	20	91.7	96.1	4.59	4.81	68.1	70.1	3.41	3.51	99.5	100.1	4.98	5.01
Isoamyl acetate (a)	30	226.2	231.1	7.54	7.70	18.4	18	0.61	0.60	289.8	301.1	9.66	10.04
Ethyl hexanoate (b)	14	307.6	296.2	21.97	21.16	174	180.9	12.43	12.92	293.7	279.9	20.98	19.99
Hexyl acetate (b)	1500	17.8	17.1	0.01	0.01	n.d.	n.d.	_	_	58.6	53.8	0.04	0.04
Ethyl lactate (c)	154636	8561.4	8428.3	0.06	0.05	7288.2	6926.6	0.05	0.04	10892.2	11225.6	0.07	0.07
Ethyl octanoate (d)	5	193.6	184.2	38.72	36.84	100.1	104	20.02	20.80	198.1	201.6	39.62	40.32
Ethyl decanoate (d)	200	60.3	68.1	0.30	0.34	60.5	69	0.30	0.35	58.9	62.3	0.29	0.31
Diethyl succinate (e)	200000	301.3	330.7	0.00	0.00	245	211.2	0.00	0.00	359.8	363.6	0.002	0.002
Phenylethyl acetate (d)	250	187.4	185.1	0.75	0.74	n.d.	n.d.	_	_	244.9	230.7	0.98	0.92
Ethyl isobutyrate (e)	15	22.5	23.3	1.50	1.55	32	34	2.13	2.27	17.6	16.9	1.17	1.13
Isobutyl acetate (e)	1600	26.3	25	0.02	0.02	46.8	45	0.03	0.03	71.5	74.5	0.04	0.05
Butyl acetate (e)	1880	5.2	5	0.003	0.003	1.9	1.9	0.00	0.00	0.4	0.4	0.000	0.000
Ethyl 2-methylbutyrate (e)	18	3	3.2	0.17	0.18	4.1	4	0.23	0.22	4.2	4.1	0.23	0.23
Ethyl isovalerate (e)	3	6	6.2	2.00	2.07	4.2	4.1	1.40	1.37	5.1	5.3	1.70	1.77
Ethyl furoate (e)	16000	10	9.9	0.001	0.001	4.1	4	0.00	0.00	12.5	13	0.001	0.001
Ethyl dihydrocinnamate (e)	1.6	n.d.	n.d.	_	_	0.2	0.2	0.13	0.13	0.1	0.1	0.06	0.06
Methyl vanillate (e)	3000	5.1	4.8	0.002	0.002	6	6.3	0.00	0.00	4.7	4.8	0.002	0.002
Ethyl vanillate (e)	990	10.9	10.7	0.011	0.011	8.8	9.2	0.01	0.01	9.8	10.2	0.01	0.01

Carbonyl compounds													
Acetaldehyde (f)	500	824.3	830.1	1.65	1.66	640.3	638.5	1.28	1.28	714.9	712.9	1.43	1.43
Diacetyl (f)	100	733.2	729.7	7.33	7.30	957.7	952.7	9.58	9.53	724.8	711.2	7.25	7.11
Acetoin (f)	150000	2441.6	2773.9	0.02	0.02	2238.5	2357.3	0.01	0.02	2200.7	2059.4	0.01	0.01
Phenylacetaldehyde (e)	1	33.5	32.7	33.50	32.70	25.1	24.0	25.10	24.00	12.6	12.1	12.60	12.10
Alcohols													
Isobutanol (f)	40000	10123.9	11013.7	0.25	0.28	10292.4	10396.8	0.26	0.26	11651.2	11986.8	0.29	0.30
1-Butanol (f)	150000	115.3	121.2	0.00	0.00	144.5	146.4	0.00	0.00	117.1	119.2	0.00	0.00
Isoamyl alcohol (f)	30000	10966.9	10329.1	0.37	0.34	10338.4	10345.6	0.34	0.34	16990.6	17318.0	0.57	0.58
1-Hexanol (a)	8000	422.9	444.4	0.05	0.06	704.0	744.2	0.09	0.09	643.6	678.1	0.08	0.08
(Z)-3-Hexenol (a)	400	13.5	14.4	0.03	0.04	27.7	25.4	0.07	0.06	14.1	16.4	0.04	0.04
Benzyl alcohol (e)	200000	28.1	26.5	0.00	0.00	9.2	10.0	0.00	0.00	107.6	101.6	0.00	0.00
Phenylethanol (e)	14000	20485.0	21527.3	1.46	1.54	15685.2	15369.3	1.12	1.10	17561.5	17748.8	1.25	1.27
Terpenic compounds													
Linalool (e)	25	15.6	15.2	0.624	0.608	10.3	10.0	0.41	0.40	11.6	11.4	0.46	0.46
Linalool acetate (e)	unknown	0.1	0.1	_	_	0.2	0.2	_	_	0.2	0.2	_	_
α-Terpineol (e)	250	8.4	8.1	0.034	0.032	3.6	3.6	0.01	0.01	5.0	4.9	0.02	0.02
β-Citronelol (e)	100	3.2	3.3	0.032	0.033	2.6	2.5	0.03	0.03	2.7	2.9	0.03	0.03
Geraniol (e)	20	6.1	6.3	0.305	0.315	4.4	4.8	0.22	0.24	5.1	5.2	0.26	0.26
Lactones													
γ-Butyrolactone (c)	35000	2373.7	2617.2	0.07	0.08	2051.7	1996.7	0.06	0.06	2548.4	2606.0	0.07	0.07
(E)-Whiskylactone (e)	790	0.7	0.7	0.00	0.00	1.1	1.1	0.00	0.00	1.5	1.4	0.00	0.00
δ-Octalactone (e)	400	17.8	18.0	0.05	0.05	n.d.	n.d.	_	_	n.d.	n.d.	_	_

γ-Nonalactone (e)	30	2.2	2.2	0.07	0.07	3.4	3.2	0.11	0.11	3.3	3.4	0.11	0.11
γ-Decalactone (e)	88	304.2	312.3	3.46	3.55	308.1	316.2	3.50	3.59	301.7	309.4	3.43	3.52
δ-Decalactone (e)	386	47.5	46.6	0.12	0.12	41.9	40.2	0.11	0.10	22.8	21.7	0.06	0.06
Acids													
Acetic acid (c)	200000	100069.0	100445.0	0.50	0.50	137267.3	145240.8	0.69	0.73	127332.8	127888.5	0.64	0.64
Isobutyric acid (c)	230	235.1	239.1	1.02	1.04	347.1	339.3	1.51	1.48	170.0	158.8	0.74	0.69
Butyric acid (c)	173	679.3	725.1	3.93	4.19	643.6	704.4	3.72	4.07	656.1	672.2	3.79	3.89
Isovaleric acid (d)	33.4	278.2	262.9	8.33	7.87	285.0	299.9	8.53	8.98	230.0	257.4	6.89	7.71
Hexanoic acid (d)	420	2406.7	2577.5	5.73	6.14	2595.7	2789.0	6.18	6.64	3440.2	3458.3	8.19	8.23
Octanoic acid (d)	500	2084.9	2072.8	4.17	4.15	2384.3	2340.9	4.77	4.68	2103.7	2133.1	4.21	4.27
Decanoic acid (d)	1000	390.2	340.0	0.39	0.34	291.0	288.1	0.29	0.29	374.0	380.9	0.37	0.38
Norisoprenoids													
β-Damascenone (e)	0.05	11.6	12.0	232.00	240.00	3.3	3.4	66.00	68.00	9.3	9.1	186.00	182.00
β-Ionone (e)	0.09	0.3	0.4	3.33	4.44	0.5	0.5	5.56	5.56	0.3	0.3	3.33	3.33
Volatile Phenols													
Guaiacol (e)	9.5	0.4	0.4	0.04	0.04	0.9	0.9	0.09	0.09	0.1	0.1	0.01	0.01
Eugenol (e)	6	4.0	3.9	0.67	0.65	5.2	4.9	0.87	0.82	0.4	0.4	0.07	0.07
o-Cresol (e)	3	0.7	0.7	0.02	0.02	1.1	1.1	0.04	0.04	0.6	0.6	0.02	0.02
m-Cresol (e)	68	0.4	0.4	0.01	0.01	0.6	0.6	0.01	0.01	0.1	0.1	0.00	0.00
4-Ethylguaiacol (e)	33	201.7	198.5	6.11	6.02	123.5	128.9	3.74	3.91	154.8	149.9	4.69	4.54
4-Propylguaiacol (e)	unknown	0.7	0.8	_	_	0.2	0.2	_	_	0.03	0.02	_	_
4-Ethylphenol (e)	440	616.9	604.1	1.40	1.37	233.8	243.2	0.53	0.55	118.9	117.7	0.27	0.27
4-Vinylguaiacol (e)	1100	3.2	3.2	0.00	0.00	8.1	8.5	0.01	0.01	13.3	14.2	0.01	0.01

4-Vinylphenol (e)	180	135.1	132.0	0.75	0.73	129.1	134.8	0.72	0.75	221.5	213.9	1.23	1.19
4-Allyl-2,6-dimethoxiphenol (e)	120	31.6	32.2	0.03	0.03	34.5	32.0	0.03	0.03	0.4	0.4	0.00	0.00
Acetovanillone (e)	1000	56.2	53.1	0.06	0.05	19.1	18.3	0.02	0.02	27.7	28.8	0.03	0.03
Thiols													
Methionol (e)	1000	1213.5	1250.3	1.21	1.25	1447.8	1479.1	1.45	1.48	1703.1	1713.7	1.70	1.71
2-Methyl-3-furanthiol (g)	0.0050	0.239	0.198	47.80	39.60	0.637	0.687	127.40	137.40	0.703	0.813	140.60	162.60
2-Furfurylthiol (g)	0.0004	n.d	n.d.	_	_	0.003	0.003	6.50	7.75	n.d.	n.d.	_	_
4-Mercapto-4-methyl-2-pentanone (h)	0.0008	0.019	0.014	23.75	17.50	0.017	0.020	21.25	25.00	0.012	0.019	15.00	23.75
3-Mercaptohexyl acetate (h)	0.0042	n.d.	n.d.	0.00	0.00	0.002	0.000	0.38	0.07	0.001	0.001	0.33	0.26
3-Mercapto-1-hexanol (g)	0.0600	0.031	0.030	0.52	0.50	0.090	0.093	1.50	1.55	0.047	0.052	0.78	0.87
Benzylmercaptan (g)	0.0003	0.009	0.010	30.00	33.33	0.066	0.065	220.00	216.67	0.002	0.002	8.00	6.33

[a] Odor threshold values previously reported in the literature (for mixtures of ethanol/water): Campo et al., 2006 and Gómez-Míguez et al., 2007.

[b] OAV: Odor activity value was calculated by dividing the determined concentration of each wine component by its odor threshold value.

*concentration of wines volatile components was obtained by dividing the chromatographic area of each volatile component by the area of the corresponding internal standard: (a) 4-methyl-2-pentanol, (b) ethyl heptanoate; (c) 4-hydroxy-4-methyl-2-pentanone; (d). heptanoic acid; (e) 2-octanol; (f) 2-butanol; (g) 4-methoxi-α-toluenothiol; (h) 1,4-dithioerythritol octafluoronaphthalene (OFN). Then, the corresponding analyte relative area was divided by the slope determined in the calibration graphs for each volatile compound (data not shown).

n.d. - not detected.

Table 9. Quantitative wine volatile components determination for the 3 Bairrada red wines studied based on the different GC techniques used, organized by chemical families, odor threshold, content, and odor active values (OAV).

		Baga				**Castelão**				**Touriga Nacional**			
Compound	**Odor threshold (µg/L) [a]**	**Content* (µg/L) n=2**		**OAV [b]**		**Content (µg/L) n=2**		**OAV [b]**		**Content (µg/L) n=2**		**OAV [b]**	
Esters													
Ethyl acetate (a)	12264	49262.7	48035.2	4.02	3.92	56271.9	51631.6	4.59	4.21	64360.2	61364.1	5.25	5.00
Ethyl propanoate (a)	10	202.9	215.2	20.29	21.52	270.1	300.3	27.01	30.03	242.3	238.9	24.23	23.89
Ethyl butyrate (a)	20	168.4	157.2	8.42	7.86	196.7	180.4	9.84	9.02	270.5	295.2	13.52	14.76
Isoamyl acetate (a)	30	234.3	214.4	7.81	7.15	514.1	511.7	17.14	17.06	384.6	361.9	12.82	12.06
Ethyl hexanoate (b)	14	596.6	546.9	42.62	39.06	591.1	551.3	42.22	39.38	683.9	622.4	48.85	44.46
Ethyl lactate (c)	154636	109213.7	110090.0	0.71	0.71	85691.8	83621.7	0.55	0.54	77143.7	72395.1	0.50	0.47
Ethyl octanoate (d)	5	267.0	268.8	53.40	53.76	238.3	225.5	47.66	45.11	288.8	293.4	57.76	58.68
Ethyl decanoate (d)	200	71.7	74.5	0.36	0.37	70.2	68.0	0.35	0.34	55.1	53.3	0.28	0.27
Diethyl succinate (e)	200000	5700.9	5476.4	0.03	0.03	6268.4	6225.0	0.03	0.03	3042.0	3181.5	0.02	0.02
Phenylethyl acetate (d)	250	124.6	128.1	0.50	0.51	213.0	199.3	0.85	0.80	147.7	149.4	0.59	0.60
Ethyl isobutyrate (e)	15	35.6	35.4	2.37	2.36	44.3	45.4	2.95	3.03	42.0	39.9	2.80	2.66
Isobutyl acetate (e)	1600	70.6	70.1	0.04	0.04	88.5	84.0	0.06	0.05	125.7	119.7	0.08	0.07
Butyl acetate (e)	1880	7.6	7.2	0.00	0.00	3.5	3.6	0.00	0.00	7.3	7.6	0.00	0.00
Ethyl 2-methylbutyrate (e)	18	5.5	5.7	0.31	0.31	6.9	7.0	0.39	0.39	5.1	5.2	0.28	0.29
Ethyl isovalerate (e)	3	7.2	7.3	2.42	2.45	9.7	10.2	3.22	3.40	5.0	5.3	1.66	1.78
Ethyl furoate (e)	16000	2.1	2.1	0.00	0.00	2.9	2.8	0.00	0.00	1.3	1.3	0.00	0.00
Ethyl dihydrocinnamate (e)	1.6	0.4	0.4	0.23	0.24	0.5	0.5	0.32	0.30	0.7	0.7	0.41	0.41
Ethyl cinnamate (e)	1.1	0.8	0.8	0.72	0.76	1.3	1.3	1.14	1.21	1.4	1.4	1.27	1.30
Methyl vanillate (e)	3000	16.8	17.1	0.01	0.01	32.5	34.8	0.01	0.01	57.8	55.3	0.02	0.02
Ethyl vanillate (e)	990	659.8	650.9	0.67	0.66	743.9	763.4	0.75	0.77	1241.1	1174.7	1.25	1.19

Carbonyl compounds													
Acetaldehyde (f)	500	1000.6	1068.9	2.00	2.14	1015.1	1070.9	2.03	2.14	556.7	542.0	1.11	1.08
Diacetyl (f)	100	846.0	860.6	8.46	8.61	518.2	567.0	5.18	5.67	324.9	297.6	3.25	2.98
Acetoin (f)	150000	163.6	173.5	0.00	0.00	97.8	95.6	0.00	0.00	199.3	193.3	0.00	0.00
Phenylacetaldehyde (e)	1	14.6	14.0	14.59	13.98	14.3	15.1	14.30	15.11	14.0	15.3	14.02	15.25
Alcohols													
Isobutanol (f)	40000	79016.2	79109.2	1.98	1.98	73644.9	69250.0	1.84	1.73	63013.2	66004.1	1.58	1.65
1-Butanol (f)	150000	1530.4	1536.6	0.01	0.01	1187.7	1161.1	0.01	0.01	1517.1	1384.8	0.01	0.01
Isoamyl alcohol (f)	30000	353652.7	353078.0	11.79	11.77	312121.0	292658.1	10.40	9.76	266522.1	255224.0	8.88	8.51
1-Hexanol (a)	8000	1520.5	1483.8	0.19	0.19	1289.7	1234.8	0.16	0.15	1432.7	1356.7	0.18	0.17
(Z)-3-Hexenol (a)	400	27.1	29.0	0.07	0.07	45.8	49.5	0.11	0.12	34.6	34.8	0.09	0.09
Benzyl alcohol (e)	200000	23.1	21.5	0.00	0.00	73.8	77.6	0.00	0.00	57.5	56.9	0.00	0.00
Phenylethanol (e)	14000	81537.0	86042.4	5.82	6.15	71136.2	76894.0	5.08	5.49	53554.3	56785.9	3.83	4.06
Terpenic compounds													
Linalool (e)	25	5.4	5.6	0.22	0.22	9.2	9.1	0.37	0.36	30.7	29.2	1.23	1.17
Linalool acetate (e)	unknown	0.4	0.5	_	_	0.5	0.5	_	_	0.4	0.4	_	_
α-Terpineol (e)	250	2.0	1.9	0.01	0.01	4.8	4.6	0.02	0.02	14.8	15.3	0.06	0.06
β-Citronelol (e)	100	8.6	7.9	0.09	0.08	12.0	11.5	0.12	0.12	13.5	12.5	0.13	0.12
Geraniol (e)	20	8.3	8.1	0.41	0.41	16.0	16.3	0.80	0.82	41.5	39.4	2.08	1.97
Lactones													
γ-Butyrolactone (c)	35000	15236.1	16609.5	0.44	0.47	14132.7	13245.8	0.40	0.38	8537.7	8203.2	0.24	0.23
(E)-Whiskylactone (e)	790	0.7	0.7	0.00	0.00	1.0	1.0	0.00	0.00	1.5	1.4	0.00	0.00
γ-Nonalactone (e)	30	34.0	35.8	1.13	1.19	39.0	38.1	1.30	1.27	25.1	27.2	0.84	0.91

γ-Decalactone [(e)]	88	662.4	678.0	7.53	7.70	628.0	609.3	7.14	6.92	704.7	701.7	8.01	7.97
δ-Decalactone [(e)]	386	52.6	56.8	0.14	0.15	45.8	41.6	0.12	0.11	58.8	55.8	0.15	0.14
Acids													
Acetic acid [(c)]	200000	559820.7	591825.6	2.80	2.96	675261.9	609621.8	3.38	3.05	798360.9	734655.1	3.99	3.67
Isobutyric acid [(c)]	230	2260.8	2099.5	9.83	9.13	2118.0	2015.0	9.21	8.76	2012.8	1975.4	8.75	8.59
Butyric acid [(c)]	173	1088.9	1049.5	6.29	6.07	1290.1	1198.0	7.46	6.92	1464.1	1386.2	8.46	8.01
Isovaleric acid [(d)]	33.4	1369.7	1459.9	41.01	43.71	1589.7	1447.6	47.60	43.34	1150.4	1198.5	34.44	35.88
Hexanoic acid [(d)]	420	2569.8	2548.7	6.12	6.07	2408.3	2350.8	5.73	5.60	2678.7	2594.9	6.38	6.18
Octanoic acid [(d)]	500	2312.8	2373.9	4.63	4.75	1990.7	1893.3	3.98	3.79	1975.3	1865.1	3.95	3.73
Decanoic acid [(d)]	1000	681.7	670.2	0.68	0.67	520.3	505.3	0.52	0.51	408.8	424.9	0.41	0.42
Norisoprenoids													
β-Damascenone [(e)]	0.05	2.7	2.6	54.02	52.60	2.2	2.1	44.52	42.81	3.0	2.9	59.27	57.23
β-Ionone [(e)]	0.09	0.6	0.6	6.95	6.70	0.6	0.6	6.24	6.18	0.5	0.5	5.39	5.83
Volatile Phenols													
Guaiacol [(e)]	9.5	10.5	11.1	1.11	1.17	3.7	4.0	0.39	0.42	6.1	5.8	0.64	0.61
Eugenol [(e)]	6	21.9	22.1	3.64	3.69	18.1	17.3	3.02	2.89	3.4	3.5	0.57	0.59
o-Cresol [(e)]	31	4.8	4.5	0.16	0.14	4.64	4.46	0.15	0.14	3.8	3.7	0.12	0.12
m-Cresol [(e)]	68	1.6	1.7	0.02	0.03	1.4	1.3	0.02	0.02	1.8	1.7	0.03	0.03
4-Ethylguaiacol [(e)]	33	5.1	5.4	0.15	0.16	1.7	1.6	0.05	0.05	7.2	6.9	0.22	0.21
4-Propylguaiacol [(e)]	unknown	0.1	0.1	_	_	0.1	0.1	_	_	n.d.	n.d.	_	_
4-Ethylphenol [(e)]	440	6.0	5.9	0.01	0.01	5.6	5.3	0.01	0.01	3.7	4.3	0.01	0.01
4-Vinylguaiacol [(e)]	1100	21.1	20.0	0.02	0.02	8.4	7.9	0.01	0.01	12.7	11.5	0.01	0.01
2,6-Dimethoxyphenol [(e)]	120	17.3	16.6	0.03	0.03	22.63	21.82	0.04	0.04	20.0	19.7	0.04	0.03

4-Vinylphenol (e)	180	2.4	2.3	0.01	0.01	3.16	3.41	0.02	0.02	1.5	1.2	0.01	0.01
4-Allyl-2,6-dimethoxyphenol (e)	120	13.6	14.1	0.11	0.12	15.10	14.88	0.13	0.12	10.1	10.6	0.08	0.09
Acetovanillone (e)	1000	334.4	328.9	0.33	0.33	211.3	215.6	0.21	0.22	278.0	266.8	0.28	0.27
Thiols													
Methionol (e)	1000	4123.5	4389.2	4.12	4.39	3336.8	3324.5	3.34	3.32	2749.1	2629.5	2.75	2.63
2-Methyl-3-furanthiol (g)	0.0050	0.188	0.196	37.60	39.20	0.388	0.431	77.60	86.20	0.347	0.408	69.40	81.60
2-Furfurylthiol (g)	0.0004	0.002	0.002	4.00	5.75	0.002	0.002	4.50	5.50	0.002	0.002	5.75	5.00
4-Mercapto-4-methyl-2-pentanone (h)	0.0008	0.022	0.024	27.50	30.00	0.022	0.019	27.50	23.75	0.010	0.012	12.50	15.00
3-Mercaptohexyl acetate (h)	0.0042	0.001	0.002	0.31	0.45	0.002	0.002	0.52	0.52	0.001	0.002	0.21	0.38
3-Mercapto-1-hexanol (g)	0.0600	0.103	0.106	1.72	1.77	0.102	0.104	1.70	1.73	0.047	0.022	0.78	0.37
Benzylmercaptan (g)	0.0003	0.004	0.004	14.33	13.33	0.008	0.010	27.33	31.67	0.003	0.004	11.33	13.33

[a] Odor threshold values previously reported in the literature (for mixtures of ethanol/water): Campo et al., 2006 and Gómez-Míguez et al., 2007.

[b] OAV: Odor activity value was calculated by dividing the determined concentration of each wine component by its odor threshold value.

*concentration of wines volatile components was obtained by dividing the chromatographic area of each volatile component by the area of the corresponding internal standard: (a) 4-methyl-2-pentanol, (b) ethyl heptanoate; (c) 4-hydroxy-4-methyl-2-pentanone; (d). heptanoic acid; (e) 2-octanol; (f) 2-butanol; (g) 4-methoxi-α-toluenothiol; (h) 1,4-dithioerythritol octafluoronaphthalene (OFN). Then, the corresponding analyte relative area was divided by the slope determined in the calibration graphs for each volatile compound (data not shown).

n.d. - not detected.

In order to make easier the analysis of the data set concerning the volatile pattern of the 6 monovarietal Bairrada wines, a heatmap representation was performed (Figure 56), corresponding to the graphical representation of the data from Tables 8 and 9. Two independent replicates for each wine were presented. Different intensities correspond to differences in the normalized (by maximum) concentrations of each wine component. The heatmap allows a rapid visual access of each wine volatile profile and the relative comparison of the 6 monovarietal wines.

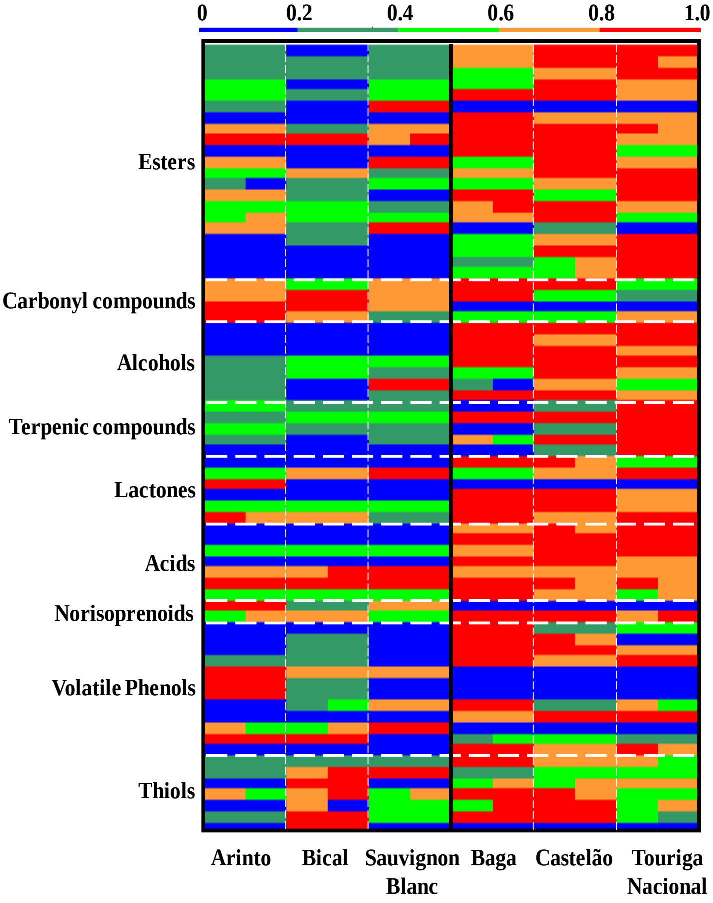


Figure 56. Heatmap representation corresponding to the volatile composition of the 6 *Vitis vinifera* L. monovarietal wines from Bairrada Appellation: 3 white varieties (Arinto, Bical, and Sauvignon Blanc) and 3 red ones (Baga, Castelão, and Touriga Nacional). Two independent replicates for each wine were presented. Different intensities correspond to the normalized (by maximum) concentrations of each wine component (detail data was reported on Supplementary Tables 8 and 9 for white and red wine varieties, respectively).

The heatmap shows that, although the identified chemical families are the same for all Bairrada wines under study, the volatile composition determined for white and red wines is different, having the red wines greater amounts of almost all of the determined volatile compounds (Figure 56). For example, the most abundant red wine components are esters, alcohols, and acids (mainly C_4–C_{10} fatty acids). These compounds produced during alcoholic fermentation, play an important role in the aroma of wines (Lambrechts and Pretorius, 2000). Covering all the identified chemical families, esters represent the largest one (a total of 21 compounds were determined), and these compounds have already been described as important compounds in young wine aroma, associated to their fruity and sweet notes (Escudero *et al.*, 2004; Falqué and Fernández, 1999; Gómez-Míguez *et al.*, 2007). Considering all the wines studied, Bical presents the lowest amount of esters, while Touriga Nacional presents the higher amount (Figure 56). On the other hand, alcohols and acids are quantitatively the largest groups of volatile compounds determined, although their composition was different between the wine varieties: higher amount was determined for red varieties, principally on Baga red wine, while the lower amount was found for Bical white wine. Among these, higher amounts of isoamyl alcohol, phenylethanol, isobutanol, and acetic acid were determined. Quantitatively, isoamyl alcohol accounts for more than 50% of all alcohols determined in all the red wines under study, being higher in Baga and lower in Touriga Nacional wines. Isoamyl alcohol is considered the main aliphatic alcohol synthesized by yeast during fermentation, comprising 40-70% of the total alcohol fraction (Lambrechts and Pretorius, 2000). Furthermore, the alcohols with six carbons such as 1-hexanol and (*Z*)-3-hexen-1-ol were determined at concentrations under their odor threshold values in all the analysed Bairrada wines (Tables 8 and 9), which are recognized as having a negative effect on wine aroma quality, when their concentrations are above their odour threshold values (Ferreira *et al.*, 1995). Furthermore, the presence of aromatic alcohols, as benzyl alcohol and phenylethanol, are associated with positive notes, and these were determined in all varieties studied, suggesting their contribution to the aroma characteristics of these wines. For instance, in all varieties, phenylethanol was over its odor threshold level (14 mg/L). This compound has already been quantified in Bical (Rocha *et al.*, 2005) and Sauvignon Blanc wines (Benkwitz *et al.*, 2012), and similar amounts were determined (ca. 16 and ca. 18 mg/L for Bical and Sauvignon Blanc, respectively) when compared with those previously reported (ca. 20 mg/L and 17-43 mg/L, respectively). A9 total of six lactones were also identified and quantified on the studied monovarietal wines, being the most abundant γ-butyrolactone, principally in the red wines, ranging from 8.2 mg/L in Touriga Nacional wine to 16.6 mg/L in Baga wine (Table 9). Furthermore, the heatmap also allows to infer that carbonyl compounds prevail in white wines, principally in Arinto and Bical. These are related to young white wine oxidation (Escudero *et al.*, 2002).

Other important compounds, such as volatile phenols, have been determined. 4-Ethylphenol, 4-ethylguaiacol, and 4-vinylphenol were the most abundant volatile phenols determined on the white wines; principally in Arinto wine, while for the red ones was acetovanillone. Although volatile phenols can contribute positively to the aroma of wines, they are better known for their contribution to off-flavours such as animal or leather notes, resulting essentially from high concentrations of ethylphenols. At concentrations above 1.74 mg/L, 4-ethylphenol is regarded as negative quality factor (Suárez *et al.*, 2007). However, as the maximum concentration of this compound in these set of wines was ca. 0.6 mg/L in Arinto wine, this off-flavour is not expected.

Varietal compounds, including C_{13} norisoprenoids and terpenic compounds, were also determined. Two C_{13} norisoprenoids were quantified: α-damascenone and β-ionone. α-Damascenone was found at the higher amount, mainly in Arinto and Sauvignon Blanc white wines, and Touriga Nacional red wine. This compound has already been reported as having a determinant role in the Arinto wine aroma profile (Rocha *et al.*, 2006b). Among the quantified monoterpenic compounds, linalool, α-terpineol and also geraniol were the most abundant ones. This level of monoterpenols was higher in Touriga Nacional wine than in the other red wines studied (Figure 56). In fact, wines from Touriga Nacional have already been considered richer in terpenol compounds (Pinho *et al.*, 2007). From the white wines under study, the lowest amount in terpenic compounds was found in Bical wine (Figure 56, Table 8). The terpenic profile of Bical wine has already been quantitatively determined and this wine was found to be pouring in terpenic compounds (Rocha *et al.*, 2005). Furthermore, the major monoterpenols determined in Arinto wine were linalool and α-terpineol, which are also in accordance with a previous study (Rocha *et al.*, 2006b).

Regarding the volatile thiols, methionol, 2-methyl-3-furanthiol and also 3-mercapto-1-hexanol were the most abundant thiols found in the Bairrada wines studied, in agreement with the data reported for Zalema wines from Spain (Gómez-Míguez *et al.*, 2007). For white wines, Bical followed by Sauvignon Blanc, exhibited higher amounts of these compounds, and for the red wines, the lower amount was determined in Touriga Nacional. These compounds have already been considered important aroma compounds in Sauvignon Blanc wines (Benkwitz *et al.*, 2012).

According to these results, the volatile profiles determined for the wines under study (Figure 56), can contribute to the distinction of these varieties based on their different chemical compounds amount and composition. The detail knowledge of each wine volatile composition is essential to explain differences between their aroma properties.

V. 3.2. Wine aroma sensory analysis

The aroma sensory evaluation of the 6 monovarietal Bairrada wines, described by the trained panel, is expressed in Figure 57.

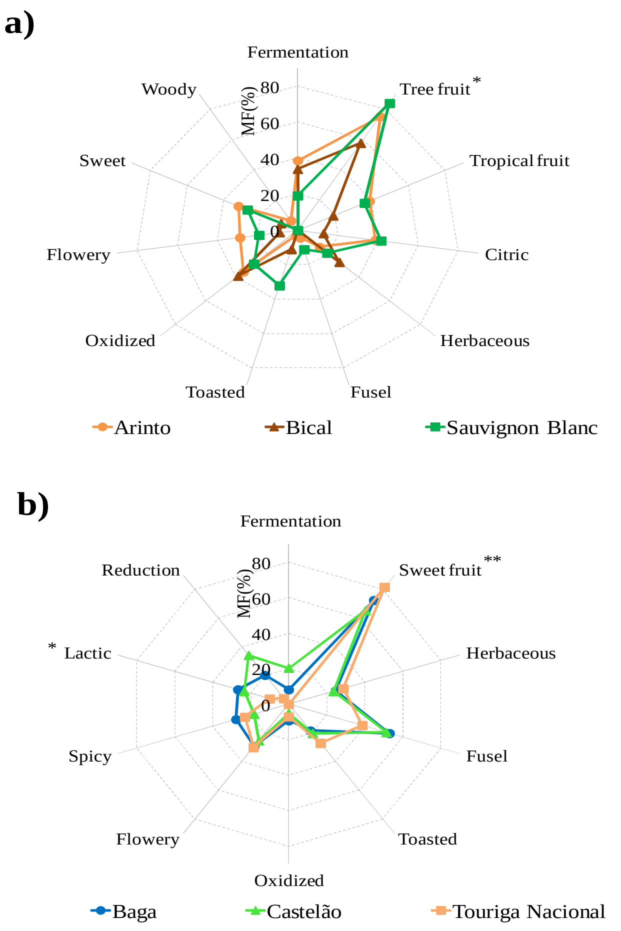


Figure 57. Aroma sensory data expressed as modified frequency - MF (%) - of the 6 monovarietal wines from Bairrada Appellation: a) 3 white (Arinto, Bical, and Sauvignon Blanc) and b) 3 red (Baga, Castelão, and Touriga Nacional) ones, based on the 11 and 10 sensory terms, for white and red wines, respectively, selected by the trained panel (13 judges). In order to determine discriminant sensory terms ANOVA was applied to the sub-set of white wines and to the sub-set of red ones. * $p < 0.05$ and ** $p < 0.01$.

The aroma of the white wines (Figure 57a) are described as fruity (tree and tropical fruits), citric, herbaceous, toasted, oxidized, fermented, flowery, and sweety. Particularly, Arinto and Sauvignon Blanc wines are characterized by tree (apple, pear) and tropical (banana, pineapple) fruits and also citric notes, although Arinto wine also exhibited flowery and sweety notes. Sauvignon Blanc wine also exhibited the highest MF (%) values related to toasted notes and the lowest ones for fermented notes, while for Arinto wine the opposite was observed. The sensory aroma of Bical wine is characterized essentially by tree fruit notes, and also herbaceous and oxidized notes (Figure 57a). On the other hand, the aroma of red varieties (Figure 57b) is characterized as sweet fruits, which included tree (apple, pear), tropical (banana, pineapple), and berry fruits (strawberry, raspberry, blackberry), herbaceous, fusel, toasted, flowery, spicy, lactic, reduction, and fermented. Aroma sensory analysis revealed that Touriga Nacional wine exhibited the higher MF (%) values related to sweet fruits (including, tree, tropical, and berry fruits) and toasted and flowery notes. The lower MF (%) values were related to lactic, fusel, and reduction notes, while Castelão and Baga wines had similar MF (%) of these attributes. Furthermore, Baga wine exhibited spicy and lactic notes, while Castelão wine is characterized by fermented and reduction notes. According to ANOVA analysis performed for the sub-set of white and to the sub-set of red wines, the most discriminative terms are tree fruit notes ($p < 0.05$) for white wines (Figure 57a), and sweet fruits ($p < 0.01$) and lactic notes ($p < 0.05$) for the red wines (Figure 57b).

V. 3.3. Aroma network construction for the 6 wines from Bairrada Appellation

A bipartite network-based approach consisting of two different nodes was built (Ahn *et al.*, 2011) (Figure 58), in order to explain the wine aroma properties: one node represents the 71 volatile compounds that are quantified in the studied wines and the other represents the 19 aroma notes determined for these wine components (citric, sweet, woody, flowery, honey, coconut, tropical, tree and berry fruits, fermentation, toasted, spicy, fusel/alcohol, vanilla, herbaceous, lactic (cheese, butter), oxidized, reduction (animal, leather), and tobacco). Figure 58 reveals that the aroma of the wines under study was very complex, with several volatile compounds sharing at least two or more aroma notes (represented in bold), which would contribute to explain the different wines aroma properties.

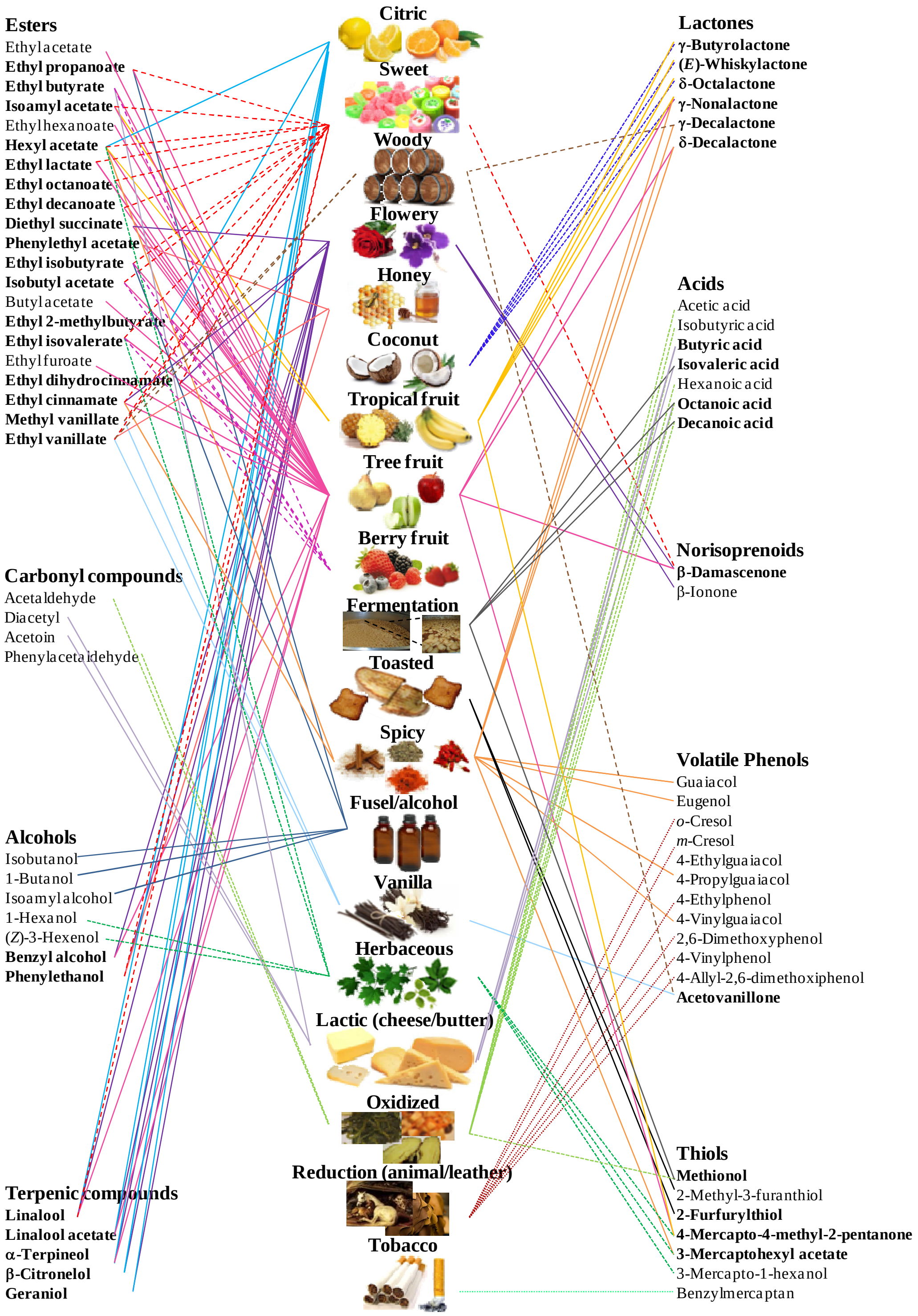


Figure 58. Bipartite network-based approach that explains wine aromas, constructed using the volatile composition of the 6 white and red wines from Bairrada Appellation: central column represents the wine aroma notes, and lateral ones correspond to the volatile compounds that exhibit that notes. Bold names indicate the volatile compounds that shared at least two or more aroma notes.

Then, a projection of the bipartite network-based approach was performed - the aroma network (Figure 59). For the determination of the aroma networks of the wines under study, the determined OAVs for each wine compound were considered. In this aroma network two nodes (Bairrada wine aroma notes) are linked if they shared at least one aroma note, and the thickness of the line is proportional to the number of shared compounds (Ahn *et al.*, 2011).

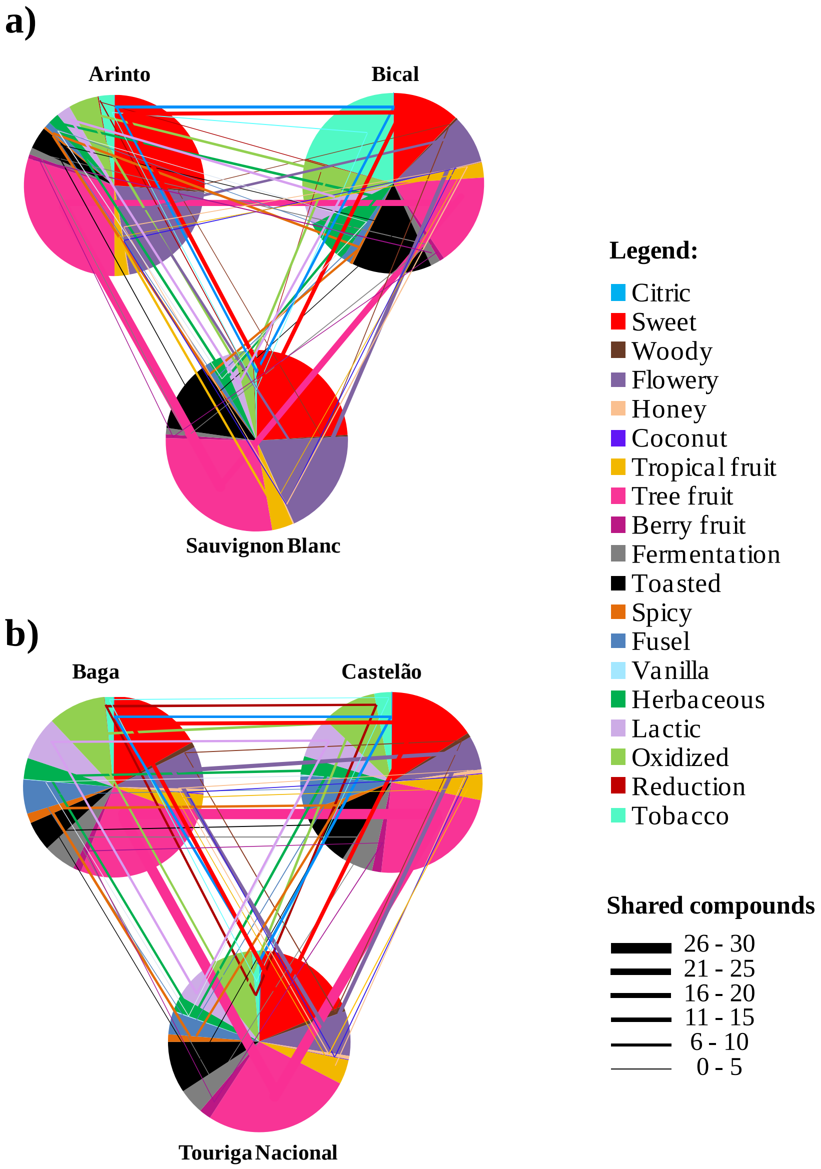


Figure 59. Aroma networks determined for the 6 wines under study, based on the bipartite network: a) 3 white wines and b) 3 red wines. Each colour represents an aroma note. The arc length/central angle of each pie sector (aroma note) is proportional to the sum of the related odor active value (OAV), and the thickness of each line corresponds to the number of compounds that explain each aroma note (shared compounds). For pie charts representation, the mean between 2 replicates were considered.

Specific aroma networks were found for the wines under study, which are in accordance with the sensory perceptions. The aroma notes were connected between them, although their intensity and the number of shared compounds were different (Figure 59).

Arinto wine

According to the aroma network determined for Arinto wine (Fig. 5a), higher fractions of tree fruit, flowery and sweet aroma notes were found. These are greatly related with esters and C_{13} norisoprenoids, principally with ethyl octanoate (OAV ca. 37 - 39), ethyl hexanoate (OAV ca. 21 - 22) and β-damascenone (OAV ca. 232 – 240). Beyond the fact that β-damascenone was determined in the other white wines, its OAV was higher in Arinto wine than in Sauvignon Blanc (OAV 182-186), and is 3.5 times higher than in Bical (OAV 66 - 68) wine. This is a compound well known for their contribution with sweet and flowery aromas to the wines. Its higher OAV in Arinto wine may explain the higher MF (%) values determined by the trained panel. In addition, in the determined aroma networks, the fractions related to fermentation aroma notes are very small when compared with the aroma sensory analysis (Fig. 3a) previously referred by the trained panel. Fermentation aroma notes are mainly related with the presence of some acids (isovaleric, octanoic, and decanoic acids) and also with methionol. The reason that may justify its lower fraction determined on the aroma networks (Fig. 5a) may be explained by the fact that these compounds exhibited lower OAVs (varying from ca. 0.3 to 8) (Table 8).

Bical wine

The aroma network of Bical wine revealed that this wine is characterized by tree fruit, sweet and floral notes, as determined for Arinto wine, or even for Sauvignon Blanc (Figure 59a), however these aromas are less intense when compared to these wines. This could be explained by the fact that the OAVs determined essentially for esters and C_{13} norisoprenoids and also for phenylethanol, were lower when compared to the other white wines. This may clarify why this wine was considered the least fruity wine, as sensory described by the panel (Figure 57a). Furthermore, Bical wine also presents higher fractions of oxidized and herbaceous aromas. This is in accordance to the referred aroma sensory analysis, obtained by the trained panel, and can be explained by the higher OAVs determined for acids (Rocha *et al.*, 2004), alcohols (Ferreira *et al.*, 1995; Rapp and Versini, 1991), and also some thiols (Gómez-Míguez *et al.*, 2007). Furthermore, higher tobacco notes related with benzylmercaptan, were also determined in the aroma network of Bical wine. The OAVs (220 - 217) determined for this compound in Bical wine is ca. 7 and 35 times higher than in Arinto (30 - 33) and Sauvignon Blanc (6 - 8) wines, respectively.

Sauvignon Blanc wine

Similarly to the aroma observed for Arinto, Sauvignon Blanc wine is also characterized by tree fruit, sweet and flowery aromas, sharing 25, 14 and 10 aroma compounds with this wine. These are essentially related to esters, which are present at concentration above their odor threshold (Table 8). Sauvignon Blanc wine exhibited ethyl dihydrocinnamate, hexyl acetate, and phenylethyl acetate in its composition, all described as contributing with tree fruit and sweet aroma notes. However, these compounds were not determined or had lower OAVs when compared with Arinto and Bical white wines, justifying their lower content in fruit and sweet aromas, when compared to Sauvignon Blanc wine (Table 8). Sauvignon Blanc wine also exhibited toasted aromas (Figure 59a) and these can be explained by the presence of several thiols, namely 2-methyl-3-furanthiol, whose OAV is higher than those of the other white wines (OAVs 140 - 163). In addition, in the determined aroma networks, the fractions related to tropical fruit and citric aroma notes are smaller when compared with the aroma sensory analysis (Figure 57a) previously referred by the trained panel. Tropical aroma notes are mainly related with the presence of some esters (isoamyl acetate, hexyl acetate), lactones (δ-octalactone, (*E*)-whiskylactone) and thiols (4-mercapto-4-methyl-2-pentanone), while citric ones are related with terpenic compounds. Great part of these aroma compounds exhibited OAVs lower than one (Table 8), which can justify their lower fractions determined on the aroma networks (Figure 59a).

Baga wine

Concerning the aroma network of Baga wine, tree fruit, and sweet aromas are the predominant ones (Figure 59b). These are related essentially with ester compounds, principally ethyl octanoate and ethyl hexanoate, both with OAVs higher than 39, and also with β-damascenone (OAV ranging from 52 to 54) (Table 9). On the other hand, also some lactic, oxidized and fusel/alcohol aromas were determined, but with lower fractions when compared with fruity and sweet ones. Lactic and oxidized aromas are mainly explained by the higher OAVs determined for some acids, principally isovaleric, butyric, isobutyric, and octanoic acids, while fusel aromas were mainly explained by the higher OAVs determined for isoamyl alcohol and isobutanol (Table 9). Besides, in the determined aroma networks, the fraction related to spicy aroma notes is smaller when compared with the aroma sensory results (trained panel) (Figure 57b). Spicy notes are related with the presence of some lactones (γ-nonalactone, γ and δ-decalactone), phenols (guaiacol, eugenol, 4-propylguaiacol, and 4-vinylguaiacol) and also with 3-mercaptohexyl acetate. However, from these compounds only γ-nonalactone, γ-decalactone, and eugenol present OAVs higher than one (varying from ca. 1 to 8) (Table 9), which may justify its lower aroma network fraction (Figure 59b).

Castelão wine

The aroma network determined for Castelão wine was shown on Figure 59b. According to this figure, similarly to Baga wine, Castelão presented tree fruit and sweet aromas, although present in lower amounts. Besides the fact that these wines shares 14 aroma compounds related with these aromas, the lower fruity and sweet aromas of Castelão wine are mainly explained by the lower OAVs (Table 9) determined for ester compounds. Similarly to the observed for Baga wine, Castelão also exhibited oxidized and lactic notes, which are related with acids, whose OAVs determined were very similar to those found in Baga wine. Besides, similar fusel aromas were determined for Baga and Castelão wines related with the higher OAVs determined for isoamyl alcohol and isobutanol. These wines share 4 aroma compounds. Furthermore, Castelão wine also presents toasted aromas and this is explained by the higher OAV determined for 2-methyl-3-furanthiol (OAV from ca. 77 to 86).

Touriga Nacional wine

The aroma network of Touriga Nacional revealed that this wine presents the higher fraction of tree fruit aromas (Figure 59b). This can be explained principally by the higher OAVs found for esters and also for β-damascenone, when compared with the other wines under study. Aroma sensory analysis also revealed that Touriga Nacional wine is the fruitiest from the studied red wines. In addition, the aroma sensory analysis refers that the descriptor well scored in all red wines is sweet fruit and this included tree, tropical, and berry fruits aromas. Thus, considering these 3 aromas determined in the aroma networks of the red wines, the higher fraction is found in Touriga Nacional wine (Figure 59b). This is in accordance with the aroma sensory perceptions of the trained panel (Figure 57b). Touriga Nacional also exhibited sweet and oxidized notes, although these fractions are very similar among the 3 red wines studied. Similar to Castelão wine, Touriga Nacional presents toasted aromas, mainly explained by the higher OAV determined for 2-methyl-3-furanthiol (OAV form ca. 69 to 82). Furthermore, flowery aromas, principally related with C13 norisoprenoids (β-damascenone and α-ionone) were also a characteristic of the aroma network of Touriga Nacional. In addition, for the determined wine aroma networks, the fractions related to spicy aromas are smaller when compared with the aroma sensory analysis (Figure 57b, trained panel). These aromas are mainly related with volatile phenols and lactones. This can be explained by the fact that great part of these aroma compounds exhibited OAVs lower than one (Table 9), justifying the lower fractions determined on the red wine aroma networks (Figure 59b).

V. 4. Concluding remarks

The aroma network-based approach was done linking molecular data related to volatile composition and aroma sensory data about the key odour active molecules. This allowed to identify different wine aroma properties and infer about the consumer sensory perception. It was found that aroma properties differ from one wine variety to another, which revealed their specificities: while Arinto and Sauvignon Blanc wine exhibited higher tree fruit, sweet and flowery aroma, related with higher OAVs essentially of ester compounds and C_{13} norisoprenoids, the opposite was obtained for Bical wine, corroborating the aroma sensory perceptions (trained panel). Sauvignon Blanc also exhibited higher toasted aromas (higher OAVs related with thiols, mainly with 2-methyl-3-furanthiol). Touriga Nacional exhibited higher tree, tropical, and berry fruits notes (sensory described as sweet fruits), toasted and flowery aromas, while these are similar for the other red wines. It is important to point out that besides Bairrada wines, this aroma network approach is a tool that can be used as an aroma fingerprint and may explain the aroma properties of wines worldwide. This comprehensive approach can yield new insights in wine related science.

Chapter VI - Conclusions and future perspectives

Conclusions and future perspectives

In this PhD thesis, the evaluation of the variety oenological potential regarding Bairrada Appellation conditions has been exploited. It is known that grapes characteristics and composition, and thus wine quality, depend on a complex network of variables known to influence viticulture, including different parcels of the vineyards and also harvest year conditions. In this PhD thesis a sampling plan was implemented, which included the selections of 7 *V. vinifera* varieties, 3 parcels with different characteristics for each variety, and 3 consecutive harvest years. In general the grape sampling was performed from half-*véraison* to technologic maturity. Several parameters were used to evaluate the variety oenological potential: berry weight, pH, acidity, sugar and phenolic contents, antiradical activity, and volatile composition, including free and glycosidically-linked fractions.

Each parameter was evaluated during maturation, revealing the unique character of each variety, namely the different adaptation behaviour to the edaphoclimatic conditions. A comprehensive approach using the data from technologic maturity, based on statistic tools, was performed for each variety. These revealed that harvest year was the main factor that influences grapes composition (53% to 68% of the total variance). Grapes from moderate climatic conditions (2010) were related with higher phenolic and volatile contents, while the opposite was observed for 2011 harvest. Besides, fresh and rainy climatic conditions (2012) promoted lower monoterpenic content in Touriga Nacional. Parcel characteristics also influenced grapes composition explaining ca. 15-19% of the total data set variance. Looking for white varieties, volatile and phenolic contents and antioxidant activity were higher in clay-sandy followed by clay-calcareous soils for Arinto and Bical, while for Sauvignon Blanc the parcel characteristics had no significant effect. For the red varieties, phenolic and varietal volatile contents and antiradical activity were higher in clay-calcareous followed by clayey soils, and sugar content was higher in Baga and Touriga Nacional and titratable acidity was higher in Castelão.

Besides study of grapes composition, an aroma network-approach was used for aroma properties assessment from 6 monovarietal wines from the varieties under study. This approach allowed to identify different wine aroma properties and to infer about the consumer sensory perceptions. Specific aroma networks were determined for each variety: Arinto and Sauvignon Blanc wine exhibited higher tree fruity, sweety and flowery aroma, related with higher OAVs essentially of ester compounds and C_{13} norisoprenoids, while the opposite was obtained for Bical wine, corroborating the aroma sensory perceptions (trained panel). Sauvignon Blanc also exhibited higher toasted aromas. Furthermore, Touriga Nacional exhibited higher tree, tropical, and berry fruits notes (sensory described as sweet fruits), toasted and flowery aromas, while these are similar for the other red wines under study. It is important to point out that besides Portuguese Bairrada wines, this aroma network approach is a tool that can be used as an aroma fingerprint to explain the aroma properties of wines worldwide.

In conclusion, the grape and wine data generated under the present PhD thesis, in the context of Bairrada Appellation, shows the unique character of each variety, and may be used by growers and wine producers as a support for decision-making based on objective criteria, increasing the sustainability in this sector. It is important to point out that in order to implement sustainable viticulture, the wine producers of each region need to know the potentialities of their varieties to go further in this field. In addition, the knowledge generated under this PhD thesis may be explored to implement sustainable programs regarding the valuation of non-renewable natural resources. These programs are in line with the main concerns of official organisms and are essential to save the natural resources for future generations. It is expected that wines produced from sustainably farmed grapes may be distinguished and valuated by the consumers.

The present PhD thesis opens new fields of work, namely, the:

i) application of ASCA models to other varieties obtained from Bairrada Appellation;

ii) creation of a database of these natural resources, in order to obtain fast and reliable information that helps the winemaker decision;

iii) application of sustainable viticulture concept to other Appellations, in order to know the potentialities of the varieties of other locations.

Chapter VII - References

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Table S1. Physicochemical parameters of Arinto variety, through its maturation process, from 3 parcels with different characteristics, from 2010 to 2012.

ARINTO	**AR-VA1**		**AR-VA2**		**AR-SM1**		**AR-VA1**		**AR-VA2**		**AR-SM1**		**AR-VA1**		**AR-VA2**		**AR-SM1**	
	2010																	
	18/08						25/08						01/09					
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Berry weight (g)	0.8	8	0.6	5	0.8	3	0.9	2	0.6	8	1.0	3	1.3	3	1.0	1	1.0	4
pH	2.5	1	2.4	2	2.5	0	2.5	1	2.5	1	2.7	1	2.7	1	2.6	2	2.7	1
Titratable acidity (g tartaric acid L^{-1})	30.2	1	34.2	4	27.7	2	19.8	1	18.5	2	13.4	2	8.3	3	9.6	3	10.9	2
Sugar content (g L^{-1})	44.2	4	55.0	5	57.2	5	91.2	2	119.6	1	99.7	2	190.4	2	178.5	2	162.6	1
Phenolic content (mg GAE L^{-1})	519.9	2	694.9	1	536.2	2	402.8	6	489.2	3	356.3	3	330.9	3	388.4	2	446.0	1
Antioxidant activity (% DPPHrem)	72.3	1	63.4	2	60.8	1	83.2	3	56.4	3	63.0	2	81.8	2	61.0	1	68.0	1
	08/09						15/09						22/09					
Berry weight (g)	1.3	5	1.0	3	1.0	7	1.4	4	1.1	5	1.0	3	-	-	1.1	3	-	-
pH	2.7	2	2.8	1	2.7	1	3.0	1	2.9	1	2.9	0	-	-	2.9	1	-	-
Titratable acidity (g tartaric acid L^{-1})	7.6	1	6.4	2	7.4	2	6.6	2	7.8	1	7.3	2	-	-	7.4	2	-	-
Sugar content (g L^{-1})	214.8	1	218.7	1	210.2	1	211.4	1	221.0	1	205.7	1	-	-	211.9	1	-	-
Phenolic content (mg GAE L^{-1})	319.1	7	481.6	1	374.4	1	343.0	3	366.1	2	566.5	1	-	-	538.8	3	-	-
Antioxidant activity (% DPPHrem)	85.2	1	58.9	2	70.0	3	86.6	0	68.6	1	57.7	1	-	-	63.5	1	-	-
	2011																	
	01/08						08/08						16/08					
Berry weight (g)	1.4	9	1.0	4	1.6	3	1.2	1	1.2	1	1.2	1	1.3	6	1.2	3	1.5	3
pH	2.4	1	2.3	2	2.3	2	2.6	1	2.5	0	2.5	1	2.6	1	2.7	1	2.6	1
Titratable acidity (g tartaric acid L^{-1})	25.4	1	28.9	4	31.7	5	16.0	0	17.0	1	22.7	1	14.6	1	10.2	2	15.2	1
Sugar content (g L^{-1})	104.8	3	96.9	5	71.4	8	121.8	3	132.0	2	86.1	4	119.6	1	169.4	2	131.5	2
Phenolic content (mg GAE L^{-1})	332.4	4	314.6	2	311.2	3	313.4	10	280.9	3	295.6	6	290.3	6	294.1	3	283.1	3
Antioxidant activity (% DPPHrem)	79.8	3	77.2	0	78.0	1	90.3	2	73.4	1	76.5	4	87.9	1	70.6	2	74.9	0
	22/08						29/08						05/09					
Berry weight (g)	1.4	1	1.2	2	1.4	2	1.6	4	1.4	8	1.3	3	1.5	4	1.3	2	1.5	3
pH	3.0	1	2.7	1	2.8	1	3.0	0	2.9	1	2.9	1	3.0	1	3.0	1	2.9	1
Titratable acidity (g tartaric acid L^{-1})	9.1	2	11.0	1	12.4	1	8.9	1	9.6	1	11.0	1	7.4	1	6.6	1	7.9	2
Sugar content (g L^{-1})	188.7	1	175.7	2	149.0	2	201.2	1	198.3	1	167.7	2	206.8	1	205.7	2	172.3	1
Phenolic content (mg GAE L^{-1})	287.7	3	307.0	5	294.5	5	303.6	5	304.7	5	307.8	3	261.6	10	322.5	7	276.7	9
Antioxidant activity (% DPPHrem)	86.5	1	73.9	0	75.6	1	91.3	4	72.8	3	74.7	2	88.8	0	71.9	1	73.8	2
	12/09						19/09						26/09					
Berry weight (g)	1.4	2	1.2	4	-	-	1.6	3	1.4	5	-	-	1.5	4	1.4	3	-	-
pH	3.0	0	2.9	1	-	-	3.2	1	3.2	1	-	-	3.2	1	3.2	0	-	-
Titratable acidity (g tartaric acid L^{-1})	7.2	1	7.6	2	-	-	7.3	2	6.1	3	-	-	6.5	2	5.7	2	-	-
Sugar content (g L^{-1})	198.3	1	215.3	4	-	-	213.6	1	228.4	1	-	-	219.3	1	226.1	2	-	-

Phenolic content (mg GAE L^{-1})	235.0	4	343.4	2	-	-	253.2	8	327.8	5	-	-	230.5	7	321.0	4	-	-
Antioxidant activity (% DPPHrem)	88.1	1	74.0	3	-	-	86.5	3	71.3	1	-	-	84.8	2	70.1	2	-	-
	2012																	
	27/08						03/09						11/09					
Berry weight (g)	1.1	1	1.2	3	1.2	3	1.1	1	1.2	3	1.2	7	1.1	6	1.2	3	1.6	2
pH	2.7	1	2.6	1	2.4	1	2.7	0	2.7	1	2.6	0	3.0	1	2.8	1	2.8	1
Titratable acidity (g tartaric acid L^{-1})	24.6	4	27.8	2	26.3	1	14.4	2	14.0	2	13.7	2	8.5	3	10.8	2	12.0	3
Sugar content (g L^{-1})	103.1	3	105.4	3	82.7	3	143.4	2	132.6	3	126.9	4	163.8	3	162.6	3	158.1	3
Phenolic content (mg GAE L^{-1})	318.8	3	371.9	2	340.6	3	297.1	4	350.1	3	323.9	4	280.4	4	351.5	2	302.2	3
Antioxidant activity (% DPPHrem)	92.7	2	86.4	2	86.8	2	91.2	1	85.0	2	84.6	4	90.0	2	83.3	3	83.9	1
	18/09						25/09						02/10					
Berry weight (g)	1.2	5	1.4	5	1.5	3	1.4	2	1.5	6	1.7	2	1.5	3	1.5	3	1.4	3
pH	3.1	0	3.0	0	2.8	1	3.2	0	3.1	0	3.1	1	3.2	0	3.1	1	3.1	0
Titratable acidity (g tartaric acid L^{-1})	7.6	2	8.7	2	9.4	3	7.1	5	6.8	3	6.9	2	6.6	3	6.5	3	6.3	4
Sugar content (g L^{-1})	180.2	4	184.2	3	173.4	2	184.7	3	204.0	3	176.2	3	197.8	3	206.3	3	187.0	4
Phenolic content (mg GAE L^{-1})	270.5	7	328.7	3	307.6	4	269.9	4	337.6	2	319.5	3	251.5	4	332.8	1	291.6	2
Antioxidant activity (% DPPHrem)	87.6	3	83.1	1	83.7	3	88.6	4	81.4	2	82.4	1	88.2	1	80.0	1	82.8	3

* Gray color indicate maturity

Table S2. Physicochemical parameters of Bical variety, through its maturation process, from 3 parcels with different characteristics, from 2010 to 2012.

BICAL	BI-VA1		BI-VA2		BI-SM1		BI-VA1		BI-VA2		BI-SM1		BI-VA1		BI-VA2		BI-SM1	
	2010																	
	02/08						09/08						16/08					
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Berry weight (g)	1.1	4	1.0	3	1.0	2	1.1	4	1.0	3	1.0	5	1.4	3	1.0	7	1.4	4
pH	2.6	1	2.7	0	2.9	0	2.6	1	2.7	1	3.0	0	2.8	1	2.9	0	3.2	0
Titratable acidity (g tartaric acid L^{-1})	15.9	1	15.5	0	12.8	1	15.0	1	15.1	1	11.3	1	7.7	1	8.6	1	6.8	1
Sugar content (g L^{-1})	101.4	2	115.6	1	119.6	2	108.8	2	113.9	1	124.1	1	163.2	1	156.4	1	163.2	1
Phenolic content (mg GAE L^{-1})	254.4	2	263.4	2	270.6	4	254.4	4	258.1	2	287.3	3	229.7	2	249.1	2	272.9	2
Antioxidant activity (% DPPHrem)	80.2	2	86.0	1	84.2	0	83.8	0	87.7	3	84.6	1	88.3	1	87.3	0	82.5	2
	23/08						30/08						06/09					
Berry weight (g)	1.3	3	0.8	4	0.8	5	1.7	4	1.2	2	1.3	3	1.4	1	1.2	3	1.3	3
pH	3.1	0	3.2	0	3.2	0	2.9	0	2.9	1	3.2	0	2.9	2	2.9	0	3.2	0
Titratable acidity (g tartaric acid L^{-1})	6.0	1	6.5	1	7.4	2	5.7	1	6.2	1	5.6	1	5.7	1	6.1	1	4.2	2
Sugar content (g L^{-1})	170.6	1	165.5	1	153.0	1	171.7	1	167.7	2	184.7	1	173.4	1	169.4	1	187.6	1
Phenolic content (mg GAE L^{-1})	241.1	4	262.3	1	278.6	2	235.4	3	335.4	1	335.8	2	294.1	3	304.4	3	333.9	2
Antioxidant activity (% DPPHrem)	86.3	2	85.2	2	88.2	0	86.5	1	79.9	1	73.5	3	81.5	3	84.4	2	71.7	1
	13/09																	
Berry weight (g)	1.3	3	1.2	2	1.3	6												
pH	3.0	0	3.2	0	3.2	0												
Titratable acidity (g tartaric acid L^{-1})	5.7	2	5.4	1	4.4	2												
Sugar content (g L^{-1})	169.4	2	171.1	2	183.6	1												
Phenolic content (mg GAE L^{-1})	295.6	4	299.4	2	343.0	5												
Antioxidant activity (% DPPHrem)	79.9	1	79.4	1	78.1	3												
	2011																	
	27/07						03/08						10/08					
Berry weight (g)	1.4	3	1.0	1	1.3	1	1.2	2	1.1	3	1.3	4	1.5	1	1.6	2	1.6	3
pH	2.5	1	2.6	1	2.7	1	2.7	1	2.9	1	3.0	1	2.9	1	2.9	1	3.0	1
Titratable acidity (g tartaric acid L^{-1})	13.8	1	12.9	4	11.6	2	10.5	1	7.7	1	7.2	2	7.2	2	7.1	2	6.8	2
Sugar content (g L^{-1})	137.1	3	138.8	3	140.0	2	157.5	2	159.2	2	174.0	1	177.9	1	196.6	1	207.4	1
Phenolic content (mg GAE L^{-1})	188.1	5	227.8	3	227.5	2	205.1	3	249.4	2	224.1	1	213.4	2	261.6	2	248.7	2
Antioxidant activity (% DPPHrem)	88.7	1	87.4	2	88.4	3	90.0	2	85.9	4	83.5	1	88.5	1	84.3	1	81.6	3
	17/08						24/08						31/08					
Berry weight (g)	1.4	1	1.5	1	-	-	-	-	1.3	5	-	-	-	-	1.3	4	-	-
pH	3.2	1	3.1	0	-	-	-	-	3.1	0	-	-	-	-	3.1	0	-	-
Titratable acidity (g tartaric acid L^{-1})	7.1	1	7.1	1	-	-	-	-	6.8	2	-	-	-	-	5.9	1	-	-

Sugar content (g L^{-1})	215.3	1	213.1	1	-	-	-	-	221.0	1	-	-	-	-	214.8	1	-	-
Phenolic content (mg GAE L^{-1})	197.5	2	255.9	2	-	-	-	-	305.9	4	-	-	-	-	283.5	4	-	-
Antioxidant activity (% DPPHrem)	89.9	3	84.6	2	-	-	-	-	84.7	1	-	-	-	-	81.1	3	-	-
	07/09																	
Berry weight (g)	-	-	1.3	1	-	-												
pH	-	-	3.2	0	-	-												
Titratable acidity (g tartaric acid L^{-1})	-	-	5.6	3	-	-												
Sugar content (g L^{-1})	-	-	222.1	0	-	-												
Phenolic content (mg GAE L^{-1})	-	-	302.8	4	-	-												
Antioxidant activity (% DPPHrem)	-	-	86.8	2	-	-												
	2012																	
	16/08						23/08						30/08					
Berry weight (g)	1.3	2	1.0	1	1.5	6	1.5	3	1.3	6	1.7	3	1.7	3	1.3	4	1.6	3
pH	2.7	1	2.7	1	2.8	1	2.9	1	3.0	1	3.1	1	2.9	1	2.9	1	3.0	1
Titratable acidity (g tartaric acid L^{-1})	12.8	2	12.8	2	11.8	2	8.8	1	8.0	1	8.1	2	7.7	1	7.1	2	6.9	2
Sugar content (g L^{-1})	149.0	3	141.7	2	141.1	1	170.6	2	175.7	1	157.0	2	200.0	1	191.0	1	172.3	2
Phenolic content (mg GAE L^{-1})	259.0	4	271.2	7	300.1	3	260.0	3	246.7	6	294.7	1	238.9	4	264.4	2	274.6	2
Antioxidant activity (% DPPHrem)	93.3	4	88.6	3	86.0	1	91.5	0	86.6	2	86.1	3	90.1	3	86.5	1	83.9	0
	06/09						13/09											
Berry weight (g)	1.6	1	1.6	3	1.8	3	1.5	4	1.5	3	1.7	1						
pH	3.2	1	3.2	1	3.1	1	3.2	1	3.2	1	3.1	2						
Titratable acidity (g tartaric acid L^{-1})	5.8	2	5.5	3	5.6	1	5.5	2	5.6	1	5.3	3						
Sugar content (g L^{-1})	208.5	1	209.7	1	193.2	1	213.6	1	209.7	2	202.3	1						
Phenolic content (mg GAE L^{-1})	214.1	3	262.0	2	268.8	5	204.2	2	243.0	4	262.7	3						
Antioxidant activity (% DPPHrem)	89.7	2	86.1	2	83.3	1	89.1	3	85.1	2	83.2	2						

* Gray color indicate maturity

Table S3. Physicochemical parameters of Sauvignon Blanc variety, through its maturation process, from 3 parcels with different characteristics, from 2010 to 2012.

SAUVIGNON BLANC	**SB-SM1**		**SB-SM2**		**SB-SM3**		**SB-SM1**		**SB-SM2**		**SB-SM3**		**SB-SM1**		**SB-SM2**		**SB-SM3**	
	2010																	
	28/07						04/08						11/08					
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Berry weight (g)	1.0	3	1.2	2	1.2	5	1.1	4	1.4	6	1.3	2	1.4	4	1.7	4	1.50	2
pH	2.7	1	2.6	1	2.7	1	2.7	1	2.7	0	2.7	1	3.1	0	3.0	1	3.17	0
Titratable acidity (g tartaric acid L^{-1})	17.7	1	20.0	0	14.3	2	12.5	2	14.3	1	12.5	2	6.3	1	7.8	1	6.5	1
Sugar content (g L^{-1})	133.7	2	92.9	3	145.6	1	175.1	1	130.9	1	172.8	1	223.8	0	155.3	2	210.8	2
Phenolic content (mg GAE L^{-1})	302.5	3	420.6	3	400.9	2	303.6	3	345.6	2	410.0	2	290.0	4	330.5	1	424.4	5
Antioxidant activity (% DPPHrem)	73.0	0	65.2	5	66.9	2	79.5	3	66.8	1	60.3	2	78.1	2	72.1	1	62.7	5
	18/08						25/08											
Berry weight (g)	1.3	6	1.6	2	1.5	2	1.3	3	1.7	2	1.5	3						
pH	3.2	0	3.2	0	3.1	0	3.1	0	3.1	0	3.1	1						
Titratable acidity (g tartaric acid L^{-1})	6.6	1	5.2	1	6.9	2	4.7	3	4.2	2	6.5	2						
Sugar content (g L^{-1})	243.1	1	204.6	0	226.7	1	285.6	1	242.0	1	253.3	1						
Phenolic content (mg GAE L^{-1})	255.9	4	403.6	6	467.6	3	341.9	3	389.6	5	464.2	4						
Antioxidant activity (% DPPHrem)	72.6	2	68.9	1	62.7	2	76.1	3	61.2	5	58.6	2						
	2011																	
	22/07						29/07						05/08					
Berry weight (g)	1.4	5	1.4	3	1.5	2	1.4	4	1.4	4	1.5	1	1.5	6	1.7	6	1.4	1
pH	2.6	1	2.6	2	2.6	1	2.8	1	2.7	1	2.9	0	2.9	1	2.9	1	2.9	1
Titratable acidity (g tartaric acid L^{-1})	13.4	3	13.5	1	12.9	4	9.3	1	9.3	1	8.5	2	7.6	2	7.3	1	7.4	2
Sugar content (g L^{-1})	174.0	2	172.8	2	179.6	2	215.3	1	185.3	1	229.5	2	225.5	1	207.4	2	226.7	1
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	12/08						19/08											
Berry weight (g)	1.4	7	1.8	3	1.5	3	1.5	3	1.8	5	1.5	1						
pH	3.1	1	3.0	1	3.0	1	3.0	0	3.0	1	3.0	1						
Titratable acidity (g tartaric acid L^{-1})	7.6	2	7.1	4	7.6	2	7.4	1	7.4	2	7.4	2						
Sugar content (g L^{-1})	247.6	1	241.4	1	247.6	1	243.1	1	230.6	1	242.0	1						

Phenolic content (mg GAE L^{-1})	236.9	9	283.5	6	357.4	6	-	-	-	-	-	-						
Antioxidant activity (% DPPHrem)	79.9	2	73.5	3	66.4	2	-	-	-	-	-	-						
	2012																	
	02/08						09/08						16/08					
Berry weight (g)	1.5	2	1.5	2	1.4	3	1.5	4	1.6	3	1.5	3	1.6	5	1.9	3	1.7	2
pH	2.5	1	2.6	1	2.5	1	2.8	1	2.8	1	2.8	1	2.8	1	2.9	1	2.8	1
Titratable acidity (g tartaric acid L^{-1})	15.1	4	16.5	3	14.3	3	10.1	3	9.8	2	9.6	1	9.1	1	8.7	3	8.5	3
Sugar content (g L^{-1})	131.5	3	117.3	4	123.0	4	178.5	2	168.3	1	184.2	3	191.5	1	178.5	2	204.0	2
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	23/08						30/08											
Berry weight (g)	1.7	2	2.0	2	1.7	3	1.6	3	1.9	2	1.7	3						
pH	2.9	2	2.9	1	2.9	2	3.0	0	3.0	1	3.0	1						
Titratable acidity (g tartaric acid L^{-1})	7.4	4	7.4	2	7.4	4	7.1	2	6.9	1	7.2	3						
Sugar content (g L^{-1})	201.7	2	197.8	2	208.0	1	208.5	3	201.7	2	213.6	2						
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	276.0	7	315.1	6	365.1	8						
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	83.1	2	77.6	1	74.2	2						

* Gray color indicate maturity

Table S4. Physicochemical parameters of Baga variety, through its maturation process, from 3 parcels with different characteristics, from 2010 to 2012.

BAGA	**BA-VA1**		**BA-VA2**		**BA-SM1**		**BA-VA1**		**BA-VA2**		**BA-SM1**		**BA-VA1**		**BA-VA2**		**BA-SM1**	
	2010																	
	20/08						27/08						03/09					
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Berry weight (g)	0.7	3	1.0	3	1.0	5	1.5	4	2.0	1	1.6	3	1.6	3	1.7	2	1.8	2
pH	2.5	0	2.6	1	2.4	0	3.0	0	3.0	0	2.9	1	2.9	0	2.9	0	2.8	1
Titratable acidity (g tartaric acid L^{-1})	35.2	0	29.6	0	28.1	0	7.0	1	9.1	1	8.4	1	5.4	1	7.0	3	5.1	1
Sugar content (g L^{-1})	54.4	3	62.9	3	59.5	3	143.9	2	141.1	1	162.1	1	171.7	1	153.0	1	181.9	1
Phenolic content (mg GAE L^{-1})	1504.2	1	1361.7	1	1517.8	1	437.5	4	743.6	3	401.9	6	367.8	6	632.2	4	520.1	4
Antioxidant activity (% DPPHrem)	41.6	3	52.1	2	30.9	3	93.0	1	82.8	2	90.6	1	94.2	3	81.5	1	82.2	2
	10/09						17/09						24/09					
Berry weight (g)	1.4	4	1.5	3	1.9	3	1.3	4	2.0	2	1.9	3	1.5	8	1.8	2	1.8	1
pH	2.9	0	3.0	1	2.9	0	3.1	0	3.1	0	3.0	1	3.2	0	3.1	0	3.1	0
Titratable acidity (g tartaric acid L^{-1})	5.7	2	6.3	1	5.1	1	5.2	2	4.9	2	5.9	1	5.3	1	4.6	2	5.8	1
Sugar content (g L^{-1})	162.1	1	173.4	1	191.5	1	189.8	2	224.4	2	180.8	1	185.3	1	211.9	1	191.0	1
Phenolic content (mg GAE L^{-1})	724.6	7	575.4	9	547.3	4	811.0	3	861.0	1	424.6	3	828.4	2	869.3	2	503.4	4
Antioxidant activity (% DPPHrem)	85.1	1	84.8	1	84.9	0	80.9	2	73.3	3	89.9	1	82.7	3	67.9	2	90.1	1
	01/10						08/10											
Berry weight (g)	-	-	1.7	3	2.1	4	-	-	1.8	3	1.9	2						
pH	-	-	3.1	1	3.2	2	-	-	3.2	0	3.2	0						
Titratable acidity (g tartaric acid L^{-1})	-	-	4.1	3	5.3	1	-	-	3.6	2	4.6	2						
Sugar content (g L^{-1})	-	-	214.8	2	176.8	1	-	-	196.0	1	179.6	1						
Phenolic content (mg GAE L^{-1})	-	-	1270.1	15	686.0	12	-	-	933.0	3	654.9	2						
Antioxidant activity (% DPPHrem)	-	-	53.5	2	81.0	3	-	-	64.4	2	75.3	1						
	2011																	
	08/08						16/08						22/08					
Berry weight (g)	1.4	2	1.2	4	1.2	1	1.7	2	1.3	2	1.7	1	1.6	3	1.7	3	1.7	4
pH	2.6	1	2.3	2	2.6	1	2.9	1	2.5	0	2.7	1	2.8	0	2.7	0	2.7	0
Titratable acidity (g tartaric acid L^{-1})	18.2	0	26.4	0	19.0	1	12.2	1	17.7	1	19.0	1	13.0	1	13.1	1	14.4	1
Sugar content (g L^{-1})	107.1	5	59.5	3	77.1	3	143.9	3	96.9	2	100.9	4	147.9	1	132.6	1	110.5	2
Phenolic content (g GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	29/08						05/09						12/09					
Berry weight (g)	1.7	1	1.7	5	1.8	4	1.8	4	1.9	3	1.6	11	1.8	6	2.1	9	1.8	2
pH	3.0	1	2.9	0	2.9	2	3.1	0	2.9	1	2.9	1	3.0	1	2.8	1	2.9	1
Titratable acidity (g tartaric acid L^{-1})	10.9	1	9.5	1	8.0	1	7.9	1	7.1	2	7.8	1	6.4	2	7.6	2	10.4	1
Sugar content (g L^{-1})	153.0	1	145.1	2	150.7	2	168.3	1	157.0	2	146.8	1	185.3	1	171.1	1	141.1	2
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19/09						26/09						03/10					
Berry weight (g)	1.6	2	1.9	3	1.8	5	1.6	5	2.1	2	1.8	2	1.9	1	1.9	4	1.9	4
pH	3.1	1	3.0	1	2.9	1	3.2	0	3.1	0	3.1	0	3.2	0	3.1	2	3.2	1
Titratable acidity (g tartaric acid L^{-1})	5.5	3	5.8	2	8.8	1	4.9	3	5.6	2	7.2	2	6.3	2	6.4	2	7.0	3
Sugar content (g L^{-1})	184.2	1	201.7	1	143.4	2	193.8	2	186.4	1	163.2	2	196.6	1	202.3	1	190.4	2
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	631.4	17	869.3	11	517.0	20
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	85.6	2	70.3	3	88.6	2
								2012										
	24/08						31/08						07/09					
Berry weight (g)	1.3	4	1.6	3	1.5	3	1.3	2	1.8	3	1.7	3	1.5	1	1.4	7	1.9	5
pH	2.6	1	2.6	1	3.0	1	2.6	1	2.7	1	2.9	0	2.9	1	3.1	0	3.0	1
Titratable acidity (g tartaric acid L^{-1})	20.5	1	19.8	2	17.5	1	11.6	3	8.4	5	10.0	3	7.1	5	6.5	4	5.8	2
Sugar content (g L^{-1})	97.5	6	81.0	3	98.6	8	125.8	4	128.6	2	132.0	2	140.0	2	147.9	3	159.2	4
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14/09						21/09						28/09					
Berry weight (g)	1.8	4	1.7	5	1.7	1	1.6	3	2.0	3	1.8	4	1.6	3	-	-	-	-
pH	3.1	0	2.9	0	3.0	1	2.9	1	2.9	1	3.1	1	3.1	0	-	-	-	-
Titratable acidity (g tartaric acid L^{-1})	7.4	2	6.4	5	5.7	3	6.9	3	5.3	6	5.6	5	6.0	4	-	-	-	-
Sugar content (g L^{-1})	150.7	2	145.1	2	153.0	1	140.0	2	163.8	2	175.7	3	161.5	3	-	-	-	-
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	726.1	8	494.1	11	-	-	-	-	-	-
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	73.8	1	89.6	2	-	-	-	-	-	-
	05/10																	
Berry weight (g)	1.7	4	-	-	-	-												
pH	3.1	0	-	-	-	-												
Titratable acidity (g tartaric acid L^{-1})	5.8	3	-	-	-	-												
Sugar content (g L^{-1})	176.8	3	-	-	-	-												

Phenolic content (mg GAE L^{-1})	594.8	15	-	-	-	-
Antioxidant activity (% DPPHrem)	82.0	4	-	-	-	-

* Gray color indicate maturity

Table S5. Physicochemical parameters of Castelão variety, through its maturation process, from 3 parcels with different characteristics, from 2010 to 2012.

CASTELÃO	**CA-SM1**		**CA-SM2**		**CA-SM3**		**CA-SM1**		**CA-SM2**		**CA-SM3**		**CA-SM1**		**CA-SM2**		**CA-SM3**	
	2010																	
	20/08						27/08						03/09					
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Berry weight (g)	0.9	2	0.8	5	1.2	4	1.5	3	1.2	3	1.4	3	1.6	3	1.6	2	1.5	2
pH	2.4	0	2.4	1	2.5	0	2.8	0	2.9	1	3.1	0	3.0	1	2.9	0	3.0	1
Titratable acidity (g tartaric acid L^{-1})	29.7	0	29.3	0	28.1	0	9.9	2	8.7	1	8.0	1	6.3	2	6.2	1	6.1	1
Sugar content (g L^{-1})	44.2	4	51.0	3	64.0	4	142.8	1	181.9	1	174.0	1	164.9	1	193.8	2	174.5	1
Phenolic content (mg GAE L^{-1})	1458.7	2	1516.3	2	1095.1	2	1063.3	1	829.2	2	645.8	1	1046.6	1	513.3	1	883.7	1
Antioxidant activity (% DPPHrem)	44.5	2	45.2	2	56.5	1	63.9	0	77.7	1	85.1	0	64.8	1	86.7	0	73.9	1
	10/09						17/09						24/09					
Berry weight (g)	1.9	4	1.6	7	1.6	2	1.8	3	1.9	4	1.9	3	2.1	4	1.8	1	-	-
pH	3.0	0	3.0	1	3.0	0	3.3	1	3.2	0	3.3	1	3.4	0	3.3	0	-	-
Titratable acidity (g tartaric acid L^{-1})	5.2	1	4.7	2	5.5	1	6.0	2	5.2	2	5.5	1	4.6	2	4.3	3	-	-
Sugar content (g L^{-1})	215.9	1	238.0	1	185.3	1	221.0	2	241.4	1	218.2	2	227.2	1	255.6	1	-	-
Phenolic content (mg GAE L^{-1})	1037.5	2	714.0	2	870.8	1	1053.4	4	864.0	4	826.1	5	1098.1	1	1042.8	3	-	-
Antioxidant activity (% DPPHrem)	65.6	1	76.9	0	74.5	1	64.3	3	68.7	2	77.4	2	65.9	1	65.7	1	-	-
	2011																	
	08/08						16/08						22/08					
Berry weight (g)	1.2	2	0.9	6	1.2	3	1.6	2	1.3	1	1.7	2	1.6	2	1.3	3	1.8	6
pH	2.6	1	2.4	1	2.6	1	2.8	1	2.6	1	2.7	1	3.0	0	2.8	1	2.9	1
Titratable acidity (g tartaric acid L^{-1})	20.0	1	22.7	1	18.2	1	13.6	1	13.8	1	12.2	1	12.2	1	10.0	1	9.2	2
Sugar content (g L^{-1})	85.6	3	91.2	2	111.6	3	124.7	2	143.4	1	136.6	2	145.1	2	160.9	2	154.1	2
Phenolic content (mg GAE L^{-1})	1007.2	5	840.5	16	693.6	14	741.3	9	650.4	12	405.7	29	697.3	4	706.4	19	514.0	24
Antioxidant activity (% DPPHrem)	61.2	1	76.3	1	73.4	1	74.6	0	85.3	0	92.1	2	71.8	2	86.8	0	91.1	0
	29/08						05/09						12/09					
Berry weight (g)	1.6	2	1.3	1	1.5	2	1.9	4	1.4	3	1.7	3	2.1	1	1.5	3	1.6	1
pH	3.0	1	3.0	1	3.1	0	3.1	1	3.0	1	3.3	1	2.9	1	2.9	1	3.0	0
Titratable acidity (g tartaric acid L^{-1})	11.2	1	8.3	1	8.8	1	8.3	2	7.9	3	6.3	2	7.6	2	6.9	3	5.7	2
Sugar content (g L^{-1})	149.6	1	179.1	1	153.0	1	166.6	2	188.7	2	174.0	2	168.3	1	194.4	1	207.4	2
Phenolic content (mg GAE L^{-1})	787.5	11	624.6	12	628.3	12	653.4	4	626.1	3	608.7	7	701.9	2	593.6	6	476.9	6
Antioxidant activity (% DPPHrem)	70.5	1	84.4	1	92.2	1	69.6	0	74.7	1	87.3	2	69.1	2	77.3	0	85.6	1
	19/09						26/09						03/10					

Berry weight (g)	2.0	1	1.6	3	1.6	2	-	-	1.8	6	1.9	4	-	-	-	-	1.8	3
pH	3.2	1	3.0	1	3.2	0	-	-	3.1	1	3.2	0	-	-	-	-	3.4	0
Titratable acidity (g tartaric acid L^{-1})	7.2	2	6.8	1	5.8	1	-	-	5.8	2	5.7	3	-	-	-	-	5.0	2
Sugar content (g L^{-1})	196.1	1	197.8	1	191.5	1	-	-	218.7	1	193.2	1	-	-	-	-	200.6	2
Phenolic content (mg GAE L^{-1})	704.9	13	661.0	14	626.1	3	-	-	621.6	20	635.2	16	-	-	-	-	613.3	9
Antioxidant activity (% DPPHrem)	69.9	1	73.3	1	83.3	0	-	-	72.0	1	81.9	3	-	-	-	-	83.7	1
	2012																	
	24/08						31/08						07/09					
Berry weight (g)	1.7	3	1.5	4	1.8	1	1.7	2	1.6	3	1.6	1	1.8	2	1.5	5	1.7	4
pH	2.8	1	2.6	1	2.7	1	2.9	1	2.8	0	2.8	0	3.2	0	3.0	0	2.9	0
Titratable acidity (g tartaric acid L^{-1})	21.8	1	20.1	1	20.9	1	10.7	1	8.8	4	10.4	3	8.5	5	7.2	2	8.3	3
Sugar content (g L^{-1})	111.6	6	89.5	5	113.3	4	141.1	4	182.5	3	156.4	2	171.1	3	175.7	3	159.8	3
Phenolic content (mg GAE L^{-1})	790.7	13	773.7	16	595.5	23	701.6	12	693.5	9	545.2	16	721.4	17	654.0	16	513.2	7
Antioxidant activity (% DPPHrem)	76.6	1	78.4	1	90.5	0	73.2	3	75.6	2	92.6	1	71.3	1	71.4	3	90.1	2
	14/09						21/09											
Berry weight (g)	1.9	5	1.7	2	1.9	3	2.0	2	1.8	4	1.9	3						
pH	3.2	1	3.1	1	3.1	0	3.1	1	3.1	0	3.2	2						
Titratable acidity (g tartaric acid L^{-1})	7.6	4	6.7	2	7.4	2	7.5	3	6.3	4	7.1	1						
Sugar content (g L^{-1})	176.8	4	180.8	3	174.5	4	181.9	4	186.4	4	170.0	4						
Phenolic content (mg GAE L^{-1})	683.3	11	609.8	6	501.6	21	694.1	14	627.5	16	522.0	12						
Antioxidant activity (% DPPHrem)	71.0	2	75.4	1	89.9	0	70.2	2	75.9	3	89.4	4						

* Gray color indicate maturity

Table S6. Physicochemical parameters of Touriga Nacional variety, through its maturation process, from 3 parcels with different characteristics, from 2010 to 2012.

	TN-SM1		**TN-SM2**		**TN-SM3**		**TN-SM1**		**TN-SM2**		**TN-SM3**		**TN-SM1**		**TN-SM2**		**TN-SM3**	
TOURIGA NACIONAL	**2010**																	
	24/08						31/08						07/09					
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Berry weight (g)	0.8	7	0.8	3	1.0	2	1.2	5	1.2	6	1.6	2	1.6	4	1.4	2	1.8	4
pH	2.4	1	2.3	0	2.5	0	2.6	1	2.7	0	2.8	0	2.9	0	2.9	0	2.9	0
Titratable acidity (g tartaric acid L^{-1})	27.6	0	27.8	0	28.7	0	16.2	0	11.4	0	10.7	1	5.9	1	5.9	1	5.4	2
Sugar content (g L^{-1})	37.5	1	44.2	4	40.8	4	94.6	3	139.4	1	140.5	1	138.8	2	215.3	1	168.3	1
Phenolic content (mg GAE L^{-1})	1610.2	1	1391.3	2	1312.5	0	1114.8	1	1144.3	0.9	771.6	1	962.5	1	1084.5	1	751.1	2
Antioxidant activity (% DPPHrem)	38.1	1	39.2	1	54.4	2	66.6	3	69.0	1	78.2	0	72.1	1	70.6	3	82.3	2
	14/09						21/09						28/09					
Berry weight (g)	1.4	2	1.5	3	1.8	2	1.8	2	1.6	3	1.9	2	1.8	2	1.5	3	2.0	1
pH	2.9	0	3.2	1	3.2	1	3.0	0	3.4	0	3.2	1	3.0	1	3.2	0	3.2	0
Titratable acidity (g tartaric acid L^{-1})	7.4	1	5.5	2	5.4	2	6.1	1	4.0	2	4.3	2	5.2	2	4.2	3	4.4	2
Sugar content (g L^{-1})	151.9	2	225.0	1	203.4	1	163.2	1	229.5	1	213.1	1	186.4	1	255.6	1	218.7	0
Phenolic content (mg GAE L^{-1})	832.2	1	1044.3	1	732.2	1	967.8	2	932.2	1	983.0	1	951.1	1	1460.2	2	999.6	1
Antioxidant activity (% DPPHrem)	80.0	2	67.7	1	82.6	1	79.1	1	71.5	2	75.5	3	72.4	2	51.7	1	69.9	2
	05/10						12/10						19/10					
Berry weight (g)	1.9	3	1.6	3	2.0	3	1.9	2	1.6	6	1.9	1	1.8	2	-	-	2.0	3
pH	3.1	0	3.2	0	3.3	0	3.2	0	3.3	2	3.3	1	3.2	0	-	-	3.4	0
Titratable acidity (g tartaric acid L^{-1})	4.3	2	4.3	2	3.8	2	4.4	3	4.2	3	4.3	2	4.2	4	-	-	3.7	1
Sugar content (g L^{-1})	193.1	3	246.8	3	190.2	3	200.1	2	254.2	6	199.4	3	198.1	2	-	-	200.4	3
Phenolic content (mg GAE L^{-1})	834.5	1	1323.9	1	765.5	1	747.3	2	1341.3	2	852.0	11	761.0	2	-	-	795.8	2
Antioxidant activity (% DPPHrem)	71.9	1	53.5	2	75.0	1	78.3	3	51.9	1	70.0	3	77.6	1	-	-	78.7	1
	2011																	
	11/08						18/08						25/08					
Berry weight (g)	1.3	2	1.2	2	1.4	3	1.5	1	1.1	5	1.4	2	1.5	2	1.1	1	1.5	6
pH	2.7	1	2.6	0	2.8	1	2.7	1	2.7	0	2.9	0	2.9	0	2.8	1	3.0	0
Titratable acidity (g tartaric acid L^{-1})	18.9	1	13.7	2	12.6	1	10.6	1	11.4	1	9.6	1	9.8	1	12.1	1	8.6	1
Sugar content (g L^{-1})	84.4	10	130.9	3	124.1	1	150.7	2	140.0	2	164.9	1	150.2	2	146.8	2	172.8	2

Phenolic content (mg GAE L^{-1})	1034.5	14	1550.4	8	1085.2	11	788.3	13	1366.3	5	692.8	15	767.8	11	1617.8	6	734.5	12
Antioxidant activity (% DPPHrem)	55.0	1	45.1	1	61.5	0	79.7	0	74.8	1	88.1	1	79.0	2	73.4	1	91.1	1
	01/09						08/09						15/09					
Berry weight (g)	1.6	2	1.3	3	1.6	3	1.6	5	1.2	1	1.6	2	1.6	3	1.2	2	1.6	3
pH	3.1	0	3.0	1	3.2	0	3.1	0	3.1	1	3.3	1	3.0	1	2.9	1	3.2	5
Titratable acidity (g tartaric acid L^{-1})	8.4	1	9.1	1	7.3	1	7.1	2	5.5	3	5.8	2	5.9	2	6.2	2	5.3	2
Sugar content (g L^{-1})	152.4	2	175.7	1	184.2	1	170.6	2	182.5	2	185.9	1	184.2	1	185.3	1	186.4	1
Phenolic content (mg GAE L^{-1})	1019.3	10	1111.0	12	737.5	15	630.7	21	1197.3	14	888.3	4	698.9	12	1081.4	8	742.0	7
Antioxidant activity (% DPPHrem)	89.4	0	74.7	1	88.2	2	85.6	1	72.1	1	86.1	2	79.1	0	57.6	0	84.3	1
	22/09						29/09						06/10					
Berry weight (g)	1.6	2	1.3	2	1.6	2	1.7	4	1.4	3	-	-	1.7	3	1.6	3	N/A**	N/A
pH	3.3	1	3.1	1	3.3	1	3.3	1	3.2	0	-	-	3.2	0	3.3	1	N/A	N/A
Titratable acidity (g tartaric acid L^{-1})	5.4	3	6.5	1	4.6	2	5.4	3	5.9	3	-	-	5.7	2	5.5	3	N/A	N/A
Sugar content (g L^{-1})	200.0	1	181.3	1	188.7	2	200.0	1	197.2	1	-	-	193.8	1	205.1	2	N/A	N/A
Phenolic content (mg GAE L^{-1})	834.5	21	1311.0	3	800.4	11	928.4	10	1414.0	9	-	-	948.9	13	1339.8	9	N/A	N/A
Antioxidant activity (% DPPHrem)	80.5	0	59.6	2	82.6	1	87.6	2	61.2	3	-	-	85.0	1	58.4	2	N/A	N/A
							2012											
	31/08						07/09						14/09					
Berry weight (g)	1.5	5	1.4	2	1.7	1	1.5	4	1.4	3	1.6	2	1.6	1	1.5	3	1.7	2
pH	2.8	1	2.6	0	2.9	1	2.8	1	2.7	1	2.9	1	3.0	1	3.2	0	3.0	1
Titratable acidity (g tartaric acid L^{-1})	20.8	1	22.5	1	19.0	1	10.8	2	11.6	3	10.7	3	6.2	5	6.5	5	7.1	4
Sugar content (g L^{-1})	112.8	2	111.1	2	115.6	4	151.3	3	143.9	2	147.3	4	164.3	2	172.8	2	160.4	3
Phenolic content (mg GAE L^{-1})	939.7	4	1231.6	7	775.1	12	930.9	11	1029.5	12	674.4	19	886.7	10	1022.0	9	639.0	12
Antioxidant activity (% DPPHrem)	83.1	2	80.9	0	84.6	0	84.2	1	76.8	2	91.0	1	86.3	0	74.3	0	90.6	2
	21/09						28/09						05/10					
Berry weight (g)	1.8	2	1.6	3	2.0	4	1.9	5	1.6	5	1.9	4	2.0	3	1.7	4	2.0	5
pH	3.3	0	3.2	0	3.2	0	3.3	1	3.3	0	3.4	1	3.2	1	3.3	0	3.3	0
Titratable acidity (g tartaric acid L^{-1})	6.1	3	5.6	3	6.9	2	5.8	8	5.4	7	6.4	2	5.8	2	5.4	2	6.1	7
Sugar content (g L^{-1})	181.3	2	192.7	3	179.1	4	185.9	4	194.4	3	182.5	4	187.6	3	195.5	2	184.7	2
Phenolic content (mg GAE L^{-1})	703.0	17	1097.6	10	542.4	25	640.4	11	1067.6	7	498.9	19	646.5	13	1084.6	8	501.6	14
Antioxidant activity (% DPPHrem)	83.1	3	71.0	1	88.0	1	81.7	1	69.9	3	86.6	3	81.2	1	67.8	1	86.3	1
	12/10																	
Berry weight (g)	-	-	1.7	5	-	-												
pH	-	-	3.3	1	-	-												

Titratable acidity (g tartaric acid L^{-1})	-	-	5.3	6	-	-
Sugar content (g L^{-1})	-	-	201.2	4	-	-
Phenolic content (mg GAE L^{-1})	-	-	1121.4	9	-	-
Antioxidant activity (% DPPHrem)	-	-	66.9	1	-	-

* Gray color indicates maturity. ** Grapes not available (overripe grapes).

Table S7. Physicochemical parameters of Sousão variety, through its maturation process, from one parcel, from 2010 to 2012.

SOUSÃO	**SO-SM1**													
	2010													
	20/08		27/08		03/09		10/09		17/09		24/09			
Physicochemical parameters	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)		
Berry weight (g)	1.0	4	1.1	9	1.4	3	1.5	3	1.5	3	1.6	5		
pH	2.4	1	2.5	2	2.7	1	2.9	1	3.1	0	3.1	0		
Titratable acidity (g tartaric acid L^{-1})	39.9	1	23.4	0	9.1	0	6.6	2	6.4	3	6.1	2		
Sugar content (g L^{-1})	45.9	4	106.0	2	188.7	1	193.8	1	207.4	2	206.3	1		
Phenolic content (mg GAE L^{-1})	1238.3	2	1222.3	1	1045.1	2	1211.7	3	1202.7	4	1432.2	4		
Antioxidant activity (% DPPHrem)	49.3	0	58.6	1	57.9	0	55.6	0	53.1	3	47.6	0		
	2011													
	08/08		16/08		22/08		29/08		05/09		12/09		19/09	
Berry weight (g)	1.3	3	1.6	3	1.5	5	1.6	2	1.7	3	1.8	3	1.6	3
pH	2.4	1	2.8	1	2.7	1	3.0	0	2.9	1	3.0	1	3.0	2
Titratable acidity (g tartaric acid L^{-1})	25.6	1	14.3	1	13.3	1	12.3	1	11.1	3	11.5	1	7.7	1
Sugar content (g L^{-1})	88.4	4	139.4	2	135.4	2	155.3	1	162.6	1	169.4	2	185.3	2
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-	957.2	13
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	-	-	73.0	4
	2012													
	28/08		04/09		11/09		18/09		25/09		02/10			
Berry weight (g)	1.5	2	1.5	4	1.4	2	1.7	6	1.7	3	1.7	3		
pH	2.5	1	2.7	1	2.9	0	2.9	0	3.0	1	3.0	1		
Titratable acidity (g tartaric acid L^{-1})	31.0	2	17.2	3	12.9	4	9.4	4	8.6	7	8.0	4		
Sugar content (g L^{-1})	94.1	3	136.6	3	150.2	4	160.9	3	171.7	3	177.9	4		
Phenolic content (mg GAE L^{-1})	-	-	-	-	-	-	-	-	-	-	1019.3	12		
Antioxidant activity (% DPPHrem)	-	-	-	-	-	-	-	-	-	-	72.1	2		

* Gray color indicate maturity

Table S8

Volatile components determined for *Vitis vinifera* L. cv. Arinto variety obtained from 3 parcels and 3 years of harvests, during maturation, at Bairrada Appellation.

		2010									
		18/08/2010		25/08/2010		01/09/2010		08/09/2010**		15/09/2010	
^{1}Dtr(s),^{2}Dtr(s)^a	Compound	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C_6 compounds											
194, 0.640	Hexanal	3414.0	5	3739.5	14	3875.7	4	5744.6	18	4459.3	7
206, 0.630	3-Hexenal	211.6	15	2478.0	14	886.4	11	1189.6	8	1543.5	10
230, 0.651	2-Hexenal	2864.9	16	5595.9	5	6576.9	6	1137.7	5	1140.4	8
242, 0.630	3-Hexen-1-ol	2543.1	2	2143.1	6	154.3	5	540.7	9	425.5	3
248, 1.076	2-Hexen-1-ol	804.6	8	850.1	13	626.6	10	1852.5	8	1390.7	10
266, 0.903	1-Hexanol	807.3	3	1424.3	7	1099.7	10	1571.3	12	1073.7	7
296, 0.930	2,4-Hexadienal	213.0	4	2535.9	14	2006.0	13	2630.2	7	3117.9	13
	Sub-Total	*10858.4*		*18766.7*		*15225.7*		*14666.6*		*13151.0*	
Aromatic alcohols											
420, 3.014	Benzyl Alcohol	59.2	11	84.5	5	99.3	6	70.7	11	117.4	9
446, 1.426	α,α-Dimethyl Benzyl alcohol	14.7	10	31.5	14	25.0	19	24.4	11	19.9	14
470, 1.960	2-Phenylethanol	163.0	16	79.9	11	186.8	14	243.2	3	224.1	14
	Sub-Total	*236.8*		*196.0*		*311.2*		*338.3*		*361.4*	
Monoterpenic compounds											
314, 0.440	a-Pinene	68.4	8	21.8	10	27.4	12	31.6	10	21.4	9
338, 0.480	Dehydroxylinalooloxide	53.3	12	210.8	13	85.4	7	152.9	2	74.7	6
344, 0.457	β-Pinene	113.0	9	219.2	12	88.9	4	59.7	8	66.6	12
356, 0.570	β-Myrcene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
392, 0.405	*m*-Cymene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
398, 0.476	Limonene	117.6	6	376.0	2	102.2	12	109.0	14	166.7	16
404, 0.476	1,8-Cineole	vt		177.7	16	144.9	14	91.3	17	161.0	15
416, 0.560	β-Ocimene	0.0	0	91.7	2	47.9	16	75.8	4	44.4	11
428, 0.678	Linalool oxide (isomer)	25.0	15	93.4	12	75.6	7	100.9	3	77.4	7
434, 0.727	Dihydromyrcenol	vt		596.2	4	623.5	6	526.8	8	469.9	16
440, 0.560	α-Terpinolene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
446, 0.700	Dihydrolinalool	0.0	0	17.6	19	13.6	17	12.4	14	25.0	13
452, 0.746	Linalool	69.0	10	802.2	7	1552.6	14	1180.4	2	1697.7	7
464, 0.600	Rose oxide (isomer 1)	0.0	0	25.1	19	30.2	6	16.7	16	25.6	11
464, 0.844	Fenchol	vt		53.3	8	46.1	10	28.8	21	33.7	9

470, 0.646	Hotrienol	680.0	11	530.6	9	668.6	6	960.7	3	216.0	15
476, 0.770	Rose oxide (isomer 2)	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
488, 0.690	Cosmene	0.0	0	43.2	5	36.6	18	36.6	18	38.8	4
512, 0.635	Nerol oxide	17.8	13	166.9	16	107.4	4	23.5	7	30.5	2
518, 0.834	Ocimenol	vt		179.2	16	284.3	6	196.5	8	231.6	2
524, 0.860	Borneol	0.0	0	36.8	18	61.8	14	33.8	7	33.8	7
530, 0.984	Menthol	vt		144.5	15	268.5	11	130.0	18	145.1	15
536, 0.715	Terpinen-4-ol	0.0	0	22.3	13	23.1	17	17.3	16	15.9	18
536, 1.269	Cymen-8-ol	0.0	0	34.2	14	27.9	10	28.1	10	16.8	11
542, 0.835	α-Terpineol	43.4	9	573.8	4	762.3	13	509.8	10	686.7	11
560, 0.850	Verbenone	0.0	0	39.9	18	48.2	17	50.2	19	32.4	17
566, 0.703	Menth-1-en-9-al	0.0	0	43.0	14	87.1	20	37.6	6	54.7	8
584, 0.873	Geraniol (isomer 1)	0.0	0	28.6	14	98.8	6	71.4	8	103.0	7
584, 0.943	β-Citronellol	vt		531.6	14	244.8	11	222.6	14	468.6	15
590, 0.737	Geraniol (isomer 2)	55.6	8	1314.2	11	874.3	18	587.8	3	866.8	8
596, 0.976	Citral (isomer 1)	0.0	0	0.0	0	0.0	0	52.2	18	83.4	12
602, 0.815	Carvone	0.0	0	29.6	13	84.7	15	20.0	7	24.0	5
626, 0.775	Citral (isomer 2)	0.0	0	91.3	15	65.9	7	92.9	5	174.7	12
	Sub-Total	*1243.2*		*6495.0*		*6582.7*		*5457.6*		*6086.9*	
C_{13} Norisoprenoids											
566, 0.532	*m/z* 159, 91, 131	16.6	17	391.0	13	76.1	16	54.5	13	39.0	8
602, 0.660	α-Ionene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
620, 0.595	Vitispirane	136.6	20	532.1	2	269.4	15	197.4	12	167.8	18
632, 0.517	Theaspirane (isomer 1)	723.5	7	320.5	5	80.1	12	134.8	8	60.8	15
644, 0.528	Theaspirane (isomer 2)	0.0	0	265.2	13	46.2	16	74.4	2	27.8	17
668, 0.790	TDN (Naphthalene, 1,2-dihydro-1,	0.0	0	30.3	18	21.1	9	22.4	4	22.4	4
674, 0.681	β-Damascenone (isomer 1)	0.0	0	129.4	9	80.6	5	51.3	9	58.6	7
680, 0.840	*m/z* 142, 157, 115	vt		1074.4	8	20.3	8	0.0	0	0.0	0
700, 0.702	β-Damascenone (isomer 2)	170.4	15	1074.4	8	1050.7	12	670.3	6	610.9	14
724, 0.750	Hydroxydihydroedulan	0.0	0	53.5	7	32.0	11	41.2	3	34.7	8
736, 0.648	Geranylacetone	201.6	4	50.9	4	272.9	19	177.8	17	408.2	19
742, 0.850	5,6-Epoxy-β-ionone	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
760, 0.868	3,4-Dehydro-β-ionone	0.0	0	13.0	15	11.3	9	11.5	18	11.9	12
778, 0.635	α-iso-methyl ionone	vt		747.8	15	11.3	3	14.3	10	0.0	0
784, 0.717	β-Ionone	0.0	0	28.3	14	48.0	18	37.5	4	28.1	9
900, 0.894	Methyl dihydrojasmonate	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
	Sub-Total	*1248.7*		*4710.7*		*2020.0*		*1487.2*		*1470.1*	
Sesquiterpenic compounds											
656, 0.583	Longipinene epoxide	vt		46.7	12	48.5	14	52.7	2	21.7	7

712, 0.508	Longifolene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
756, 0.630	Aromadendrene	107.6	8	42.2	19	42.2	19	39.8	15	53.0	10
790, 0.660	α-Farnesene	0.0	0	65.3	14	66.8	3	66.5	7	28.4	12
808, 0.630	Calamenene	0.0	0	46.7	14	6.1	5	0.0	0	0.0	0
832, 0.880	Nerolidol	vt		106.1	17	120.5	15	218.8	22	274.8	22
844, 0.810	Epiglobulol	0.0	0	111.9	15	95.5	4	67.4	19	65.9	11
886, 0.690	β-Eudesmol	vt		133.2	20	115.6	9	133.0	14	101.8	8
912, 0.654	*m/z* 119, 91, 191, 109	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
	Sub-Total	*107.6*		*552.1*		*495.2*		*578.2*		*545.6*	
	Total	*13694.7*		*30720.5*		*24634.8*		*22527.9*		*21615.1*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

vt - vestigial

AR-VA1															
2011															
01/08/2011		08/08/2011		16/08/2011		22/08/2011		29/08/2011		05/09/2011		12/09/2011		19/09/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
1413.4	20	3664.5	3	3353.1	3	5460.9	9	3042.5	7	3512.3	4	3912.2	15	3834.4	11
778.4	9	1067.1	14	854.4	16	1761.3	5	1193.9	14	1188.4	7	793.6	9	640.0	7
3705.7	12	6586.2	17	4809.4	11	9659.5	8	10915.5	5	13656.3	7	7484.3	11	9335.2	5
262.3	19	173.5	19	140.3	21	396.4	4	108.6	9	151.3	12	112.3	14	115.6	13
1214.2	17	1048.6	16	1179.7	21	1471.3	21	743.8	10	1338.6	22	868.2	8	752.6	7
1158.1	13	549.7	19	941.3	20	1528.6	18	767.0	15	1030.3	11	551.2	18	617.9	21
383.2	11	944.8	16	771.4	6	1696.8	19	1056.5	4	992.5	7	959.5	9	989.9	8
8915.3		*14034.3*		*12049.5*		*21974.9*		*17827.8*		*21869.7*		*14681.3*		*16285.6*	
0.0	0	vt		104.8	19	132.2	19	124.4	16	130.9	11	141.8	21	145.6	18
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
47.6	25	60.9	10	132.8	28	129.6	24	165.7	13	195.7	19	179.7	16	159.2	19
47.6		*60.9*		*237.6*		*261.8*		*290.1*		*326.6*		*321.5*		*304.7*	
156.8	19	156.0	22	125.1	22	22.0	132	13.1	9	12.4	15	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	24.1	20	19.2	13	14.4	16	14.4	1	21.8	11
63.8	3	53.8	6	67.4	16	70.8	7	66.5	6	60.7	4	61.1	7	74.8	19
0.0	0	0.0	0	0.0	0	54.2	19	62.1	15	32.7	20	32.1	4	44.2	20
0.0	0	0.0	0	0.0	0	24.4	21	11.6	9	11.5	10	9.0	20	11.0	17
26.0	9	14.6	22	26.0	12	38.2	8	61.7	4	57.0	19	76.0	15	97.6	7
52.0	7	32.7	17	52.1	10	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
47.3	8	47.4	18	64.4	10	24.7	24	28.3	9	15.4	2	27.7	11	19.1	14
0.0	0	0.0	0	0.0	0	246.5	18	187.3	11	77.0	6	59.5	5	50.0	4
12.0	33	16.5	21	17.5	26	28.4	13	22.5	14	24.7	10	20.7	9	28.7	15
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
417.4	14	282.6	19	436.5	12	516.9	11	545.3	12	541.2	16	669.0	7	515.9	13
0.0	0	12.9	22	38.8	17	17.3	19	18.7	17	23.3	21	19.0	20	20.3	8
0.0	0	25.2	19	34.1	14	36.7	13	0.0	0	0.0	0	0.0	0	0.0	0

638.6	13	352.0	8	389.1	18	257.3	15	216.1	9	198.4	7	159.4	21	179.7	18
15.9	29	28.0	11	53.4	11	42.0	14	15.2	15	12.4	12	13.4	10	16.6	6
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
77.7	6	50.4	12	49.7	7	53.0	10	42.2	10	24.1	15	26.3	16	15.5	30
71.0	10	56.1	7	39.4	20	52.1	9	44.2	18	50.1	8	39.8	9	23.1	9
20.9	33	12.0	20	19.6	15	20.3	15	13.0	24	11.9	6	11.9	6	11.2	14
41.2	12	26.1	17	34.9	6	38.5	2	32.6	15	35.0	17	29.4	10	28.2	7
0.0	0	0.0	0	0.0	0	13.6	19	12.2	20	11.9	15	14.1	18	14.5	20
18.5	15	18.3	15	16.6	14	22.9	12	17.3	7	13.9	17	15.7	17	15.7	5
vt		153.6	18	190.0	3	231.8	5	277.6	17	256.1	21	246.5	8	329.6	7
0.0	0	0.0	0	36.4	9	28.5	16	25.5	24	18.5	11	0.0	0	0.0	0
0.0	0	24.4	8	34.3	9	68.7	14	104.5	8	124.6	17	137.0	33	177.9	16
0.0	0	0.0	0	141.1	25	189.5	31	227.1	16	192.2	15	200.6	10	254.4	6
351.5	4	358.9	11	487.7	18	462.8	9	415.4	5	237.9	12	315.0	10	243.2	12
90.1	18	144.8	27	276.9	23	310.3	22	945.3	15	794.3	13	865.4	4	896.3	10
0.0	0	0.0	0	44.0	4	60.0	5	62.8	18	60.6	14	60.2	7	61.5	13
31.6	20	23.8	9	29.4	11	25.0	12	28.5	4	28.1	7	22.0	8	24.1	17
68.0	11	86.4	12	101.0	5	77.8	7	81.5	3	105.5	7	87.8	7	79.5	10
2200.3		*1976.6*		*2805.1*		*3058.1*		*3597.1*		*3045.8*		*3232.8*		*3254.3*	
193.4	23	147.4	18	18.4	18	11.6	24	43.4	10	16.2	16	23.1	11	0.0	0
19.0	12	16.9	25	10.9	21	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
199.4	25	173.9	21	158.0	16	130.3	19	140.6	22	145.5	7	165.9	11	144.0	22
71.7	11	88.9	10	85.1	21	61.7	20	36.5	3	16.0	16	17.6	11	22.6	6
64.1	9	63.1	13	50.9	12	32.7	11	38.9	17	17.3	12	13.9	5	16.6	17
38.2	14	15.6	12	50.4	12	36.8	17	vt		0.0	0	0.0	0	0.0	0
0.0	0	32.7	20	41.3	24	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
411.0	14	680.6	25	380.0	21	463.6	15	391.7	17	319.9	11	410.9	20	415.1	11
84.5	25	32.2	22	33.8	21	34.9	25	39.5	7	15.9	24	29.4	8	27.7	15
141.4	23	307.1	4	224.9	14	170.3	25	118.4	12	154.6	12	158.8	20	92.4	6
48.4	10	46.3	19	138.7	17	132.2	12	69.2	12	57.7	12	89.7	18	66.1	8
33.5	13	22.3	15	29.6	5	15.7	20	16.0	10	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
45.7	15	56.4	16	47.1	13	27.6	8	25.0	14	28.8	8	23.6	6	29.5	19
0.0	0	40.4	15	20.7	3	33.3	14	29.9	25	79.2	22	38.2	12	38.6	7
1350.2		*1723.9*		*1289.8*		*1150.8*		*949.0*		*851.1*		*971.0*		*852.6*	
18.6	13	43.5	11	36.3	24	75.3	9	71.4	8	51.2	13	29.5	7	48.1	17

vt		374.7	6	368.0	26	272.6	18	154.6	13	121.9	12	131.1	4	96.2	14
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	38.9	5	42.4	15	39.6	13	27.5	12	0.0	0
0.0	0	0.0	0	9.6	9	5.1	14	4.1	3	4.1	2	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	18.4	25	0.0	0	0.0	0
46.9	14	56.6	20	56.8	4	40.6	21	41.2	2	46.0	15	40.7	12	39.2	18
65.4		*474.8*		*470.7*		*432.6*		*313.7*		*281.2*		*228.7*		*183.5*	
12578.8		*18270.4*		*16852.7*		*26878.2*		*22977.8*		*26374.4*		*19435.4*		*20880.7*	

		2012											
26/09/2011**		27/08/2012		03/09/2012		11/09/2012		18/09/2012		25/09/2012		02/10/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2635.2	19	2499.2	19	2241.0	20	1970.3	19	2270.5	23	1776.2	19	1752.7	37
652.5	6	298.5	13	535.8	19	947.4	23	1421.9	21	2110.1	16	2023.3	28
10764.9	2	1423.5	29	3124.3	23	3453.7	19	2259.2	22	1797.8	6	2913.5	27
120.2	6	1205.6	2	1662.6	21	1179.7	18	630.5	18	404.6	21	336.8	17
867.5	10	442.5	17	605.9	8	892.5	14	3004.9	17	3048.8	5	3060.2	14
668.2	12	423.6	16	1349.8	31	1043.1	26	1290.9	25	3718.4	10	4346.5	15
858.8	6	338.8	16	514.1	16	973.8	12	1411.5	31	1243.4	18	1226.8	4
16567.2		*6631.7*		*10033.4*		*10460.5*		*12289.3*		*14099.3*		*15659.7*	
159.9	16	66.1	10	82.1	9	65.6	3	110.0	12	162.8	24	226.9	22
0.0	0	11.4	11	13.8	21	14.9	21	13.2	25	18.0	13	17.6	24
161.3	25	89.6	4	94.7	5	71.9	11	127.1	19	153.2	15	142.2	10
321.2		*167.2*		*190.7*		*152.4*		*250.2*		*334.1*		*386.7*	
0.0	0	46.0	12	149.6	23	157.1	11	116.1	21	107.1	27	214.9	16
12.0	16	0.0	0	24.6	25	33.3	22	19.2	12	13.7	16	26.4	17
54.3	18	44.1	17	60.8	16	70.4	30	41.9	14	41.2	20	48.8	14
39.0	9	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
12.4	21	39.4	25	43.1	25	43.0	16	41.0	28	33.3	6	19.3	12
87.4	3	81.7	13	144.1	29	180.3	21	120.6	24	173.5	22	224.4	20
0.0	0	vt		114.5	28	97.3	22	71.7	13	82.9	4	147.0	29
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
18.3	16	15.1	14	119.8	15	147.0	28	121.7	24	146.5	31	160.4	26
51.8	13	vt		134.1	30	152.6	33	124.9	29	149.5	23	168.1	19
21.3	20	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
513.6	9	183.7	29	599.3	13	367.5	11	176.5	18	368.2	11	329.2	12
18.2	15	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	49.8	14	65.1	15	63.9	4	68.7	4	54.5	20

181.4	9	290.9	14	460.9	20	336.3	13	184.8	20	233.8	21	275.9	11
16.3	20	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	15.2	14	19.3	20	21.7	26	31.1	13	60.1	3	42.7	13
13.5	26	vt		305.1	6	343.1	20	244.9	17	182.6	17	217.8	26
13.0	14	0.0	0	26.1	14	43.0	25	45.4	18	42.5	25	60.8	11
23.0	16	vt		206.3	28	195.5	32	237.6	14	172.3	32	247.8	16
12.5	11	0.0	0	14.6	19	29.9	20	21.8	15	29.0	27	17.2	19
15.1	11	0.0	0	26.9	18	23.8	26	15.7	10	20.0	15	18.1	29
333.7	5	23.4	30	252.5	29	251.7	23	200.5	22	199.0	30	270.2	16
0.0	0	0.0	0	38.3	16	22.1	28	11.7	17	15.4	24	10.2	9
159.1	17	0.0	0	42.3	11	51.2	8	42.0	14	56.6	23	39.3	25
229.7	17	vt		127.8	18	164.0	18	96.5	33	42.4	23	26.9	26
208.5	29	vt		509.1	17	383.2	11	284.1	24	278.2	20	283.5	7
797.4	8	108.2	22	268.2	23	274.0	6	247.4	16	253.0	14	258.0	17
51.4	21	0.0	0	35.7	17	27.3	20	20.9	21	41.9	4	47.9	12
27.0	19	0.0	0	26.3	18	83.6	17	72.6	9	48.3	9	54.6	22
70.3	6	vt		123.5	32	159.1	29	148.0	18	186.5	18	184.8	23
2980.4		*847.8*		*3922.6*		*3723.2*		*2802.7*		*3046.2*		*3448.6*	
0.0	0	164.5	18	286.8	8	264.0	25	206.6	27	123.5	25	148.1	17
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
114.0	11	128.2	16	304.3	9	219.5	15	189.8	31	136.5	22	164.4	41
14.7	11	72.9	19	165.3	18	121.1	17	152.4	31	93.5	8	105.4	17
18.5	17	vt	0	151.5	25	106.7	30	92.5	18	93.8	8	83.4	14
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	169.9	27	114.3	22	177.2	39	118.1	27	144.5	23
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
496.9	11	197.8	20	421.1	10	210.6	19	204.6	31	165.2	26	167.4	9
15.8	14	0.0	0	57.7	16	25.0	20	23.8	27	19.8	24	10.2	16
96.6	9	239.9	23	407.5	18	221.0	23	163.5	21	148.0	33	111.9	19
80.9	19	0.0	0	23.7	15	28.4	25	20.5	14	15.8	25	12.9	31
0.0	0	0.0	0	22.2	20	19.3	20	21.5	10	20.6	23	13.7	26
0.0	0	0.0	0	31.1	12	23.8	17	19.5	17	13.2	26	23.3	26
24.7	15	0.0	0	25.8	22	27.0	7	19.6	15	22.9	16	15.7	21
59.4	16	0.0	0	25.0	27	42.0	21	34.1	24	22.3	9	21.5	8
921.5		*803.3*		*2091.9*		*1422.6*		*1325.6*		*993.2*		*1022.6*	
50.0	16	14.6	21	16.0	27	79.9	13	66.0	18	95.5	10	49.1	5

110.4	15	vt		260.7	19	168.3	31	141.2	4	132.1	21	120.8	29
0.0	0	0.0	0	39.9	13	55.6	16	66.5	9	79.9	7	56.1	16
0.0	0	0.0	0	22.0	23	48.5	14	46.4	22	68.4	11	52.6	17
0.0	0	0.0	0	42.2	23	42.8	24	43.2	25	44.8	26	36.1	26
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	vt		57.0	15	43.1	28	38.8	18	36.2	8	39.6	12
0.0	0	0.0	0	0.0	0	34.7	16	37.9	27	42.5	28	22.5	2
51.7	14	32.9	24	36.3	14	32.4	14	37.2	32	53.4	23	67.7	20
212.1		*47.4*		*474.0*		*505.4*		*477.2*		*552.8*		*444.5*	
21002.5	*9.4*	*8497.4*		*16712.6*		*16264.2*		*17144.9*		*19025.6*		*20962.1*	

2010													
18/08/2010		25/08/2010		01/09/2010		08/09/2010		15/09/2010**		22/09/2010		01/08/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2530.1	13	3475.7	13	3748.5	10	4183.2	12	4405.7	4	4109.9	4	3114.3	12
412.6	17	1863.0	3	1902.3	8	2659.1	3	1940.8	3	1749.6	5	439.8	12
2758.1	8	1778.7	8	5533.8	8	4281.8	5	3496.4	12	3372.6	18	2626.0	22
1235.6	7	1547.0	7	171.4	13	689.4	5	199.3	5	149.4	12	449.0	14
653.8	5	1518.2	6	1182.9	16	1346.9	15	2105.7	8	1933.0	7	2381.0	7
2202.5	7	1100.3	9	1311.9	11	865.3	3	1784.1	13	1770.8	6	1366.6	11
218.5	18	2560.7	8	1962.9	5	4696.5	5	4259.0	5	3770.1	4	177.0	3
10011.1		*13843.5*		*15813.7*		*18722.1*		*18190.9*		*16855.4*		*10553.6*	
100.4	2	94.5	16	142.2	3	122.8	18	148.6	16	104.7	17	0.0	0
14.7	11	22.4	8	35.6	15	27.6	19	26.9	10	29.6	1	0.0	0
90.2	4	175.7	13	251.5	13	298.6	6	259.6	20	314.7	17	67.5	11
205.3		*292.7*		*429.2*		*449.0*		*435.2*		*449.0*		*67.5*	
98.2	13	28.5	6	17.5	16	16.5	13	20.6	8	19.3	14	275.2	4
93.1	16	273.8	13	183.9	16	89.3	8	173.5	22	138.6	10	0.0	0
61.4	9	182.7	16	185.0	11	167.2	16	174.8	7	160.8	12	70.9	1
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
112.5	5	340.1	12	512.9	15	176.9	15	427.1	4	94.8	8	26.7	17
0.0	0	295.6	13	144.3	20	49.4	15	62.3	3	32.6	10	61.0	3
0.0	0	64.1	6	75.2	15	59.3	7	51.5	5	136.7	9	0.0	0
114.3	26	77.7	15	124.0	13	115.7	12	99.9	15	72.9	18	73.9	8
vt		577.2	4	576.9	4	580.3	5	530.9	6	580.7	8	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	18.4	19
0.0	0	28.2	12	12.8	13	22.3	13	13.8	10	10.0	3	0.0	0
271.8	13	946.6	8	866.1	16	953.8	1	588.9	2	359.2	14	537.7	5
0.0	0	31.2	16	35.0	14	16.1	6	36.1	12	22.9	18	22.3	15
0.0	0	54.2	14	35.8	11	28.5	7	53.9	6	34.3	15	0.0	0

296.0	18	591.2	10	689.3	12	606.3	11	735.1	4	515.6	15	706.1	10
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	19.2	13
0.0	0	33.8	12	59.4	7	44.3	9	28.5	10	22.9	12	0.0	0
25.4	19	132.7	18	189.4	13	105.5	12	85.7	18	92.7	8	96.6	17
vt		148.8	17	290.6	11	328.5	4	306.4	9	335.3	12	144.8	28
0.0	0	73.9	5	13.9	18	24.1	12	22.2	8	43.8	8	26.7	17
vt		160.5	16	183.8	6	125.7	12	185.4	11	176.6	18	50.5	18
0.0	0	22.2	17	29.8	12	26.0	7	20.5	17	25.6	11	0.0	0
0.0	0	49.9	3	27.5	14	27.3	12	26.9	9	21.9	6	18.6	16
47.6	9	644.4	4	734.5	10	832.7	14	1052.2	7	788.0	7	vt	
0.0	0	44.8	7	64.6	16	38.4	18	46.5	20	41.3	19	0.0	0
0.0	0	69.2	11	158.2	12	112.0	15	115.0	15	230.8	7	0.0	0
vt		115.7	12	297.3	16	232.3	8	294.9	7	322.9	16	vt	
vt		763.7	9	714.6	9	586.6	14	231.5	9	263.2	14	407.7	22
43.3	14	1525.2	5	1580.0	17	1271.1	2	1178.0	22	1792.8	12	267.4	14
0.0	0	0.0	0	109.1	15	62.3	17	99.5	16	96.7	12	0.0	0
0.0	0	34.4	15	56.0	20	21.0	17	31.0	19	64.4	3	30.6	16
vt		167.7	3	199.3	25	121.4	20	150.0	18	158.5	15	73.9	6
1163.5		*7478.0*		*8166.9*		*6840.8*		*6842.6*		*6655.7*		*2928.5*	
36.2	12	229.5	14	322.8	4	51.6	20	42.2	11	40.2	17	156.6	6
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	24.9	23
154.3	26	439.6	10	332.0	4	156.5	16	156.4	19	180.0	10	189.1	12
1373.5	19	224.7	4	123.2	13	103.6	12	110.0	19	84.5	1	85.7	9
vt		136.3	11	91.4	16	44.6	13	43.2	12	39.3	7	91.3	6
0.0	0	20.7	11	24.1	11	22.5	13	18.4	15	11.3	12	21.5	12
vt		128.6	12	146.3	13	42.7	17	53.1	12	95.5	13	48.0	21
vt		1407.8	9	33.8	12	0.0	0	0.0	0	0.0	0	0.0	0
199.0	12	1441.1	13	1241.3	11	679.6	3	793.0	15	927.8	3	674.3	11
0.0	0	75.0	7	115.9	18	48.8	15	47.1	9	42.6	18	95.0	8
253.9	13	1178.5	1	563.1	7	567.6	17	344.7	7	174.5	7	174.8	12
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	93.8	8
0.0	0	47.9	9	29.2	17	9.0	8	12.5	12	0.0	0	27.9	9
vt		468.8	8	17.6	7	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	26.1	13	44.0	6	24.5	7	29.9	11	6.2	8	55.2	23
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	20.7	9
2016.9		*5824.6*		*3084.7*		*1751.0*		*1650.6*		*1601.8*		*1758.7*	
vt		41.0	9	27.1	14	57.0	3	50.2	10	27.6	10	16.1	7

0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	vt	
47.5	17	37.1	9	51.5	4	65.9	4	60.2	6	54.2	6	0.0	0
0.0	0	59.6	2	69.0	16	87.2	2	78.3	10	62.5	16	0.0	0
63.9	11	74.6	16	55.8	9	6.4	10	0.0	0	0.0	0	0.0	0
vt		108.4	10	113.5	10	307.9	10	315.6	11	285.4	2	0.0	0
0.0	0	92.6	3	104.7	5	59.0	12	51.9	20	0.0	0	0.0	0
vt		167.9	22	110.7	10	106.1	7	105.3	4	98.8	8	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	60.9	20
111.3		*581.1*		*532.3*		*689.6*		*661.5*		*528.5*		*77.0*	
13508.2		*28019.8*		*28026.9*		*28452.5*		*27780.7*		*26090.3*		*15385.4*	

GC×GC peak area x 10^4 (arbitrary units)

AR-VA2

2011

08/08/2011		16/08/2011		22/08/2011		29/08/2011		05/09/2011		12/09/2011		19/09/2011		26/09/2011**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2874.7	6	2866.8	3	3710.5	2	3466.2	6	4092.6	11	3132.9	2	4570.5	13	3631.0	6
1778.7	14	1074.8	9	741.4	14	676.3	5	857.2	5	1429.3	7	890.7	6	631.5	7
6264.0	9	7413.4	7	7930.8	14	10255.3	8	12745.6	23	9000.9	19	3024.2	19	8779.2	14
59.2	17	43.9	4	54.0	4	54.8	7	95.1	10	99.7	1	123.6	13	86.2	3
1108.0	15	2154.3	18	1097.5	4	453.9	9	1087.8	14	635.1	11	968.1	5	1050.4	3
749.7	7	1046.8	17	1387.1	22	333.4	11	1179.8	23	595.3	17	956.6	22	844.2	6
1437.2	18	1173.5	11	1288.1	10	721.6	16	852.6	3	1245.8	13	1081.6	7	940.5	8
14271.5		*15773.6*		*16209.4*		*15961.5*		*20910.7*		*16138.9*		*11615.4*		*15963.0*	
0.0	0	139.8	10	169.6	22	132.0	11	165.3	13	173.7	16	187.0	21	179.3	18
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
158.8	17	160.5	19	211.9	15	242.9	17	250.4	14	253.9	18	237.6	19	235.9	14
158.8		*300.2*		*381.5*		*374.9*		*415.7*		*427.6*		*424.6*		*415.2*	
205.8	23	144.3	20	12.8	13	13.1	17	10.0	5	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	26.4	8	24.9	7	22.2	6	26.9	6	9.2	10	13.0	15
71.7	8	81.8	1	86.1	11	73.0	14	82.8	5	75.9	1	47.4	10	54.2	10
0.0	0	0.0	0	52.4	3	60.1	6	34.2	10	42.6	14	36.6	15	41.4	17
0.0	0	0.0	0	27.8	16	13.9	14	13.4	25	12.9	18	8.1	18	13.4	6
19.7	17	25.6	13	69.4	4	72.2	8	66.1	7	82.7	4	67.2	12	104.2	7
59.7	7	66.6	11	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
93.4	5	124.2	19	31.9	12	33.0	9	72.4	2	17.8	17	11.5	24	21.9	10
0.0	0	0.0	0	259.9	18	240.8	16	104.2	6	78.0	9	77.2	5	66.6	8
21.1	22	24.5	19	35.1	9	22.7	12	14.0	6	28.2	8	18.9	13	24.2	12
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
607.2	4	862.5	23	901.0	10	807.4	16	633.7	14	904.1	5	707.3	10	691.3	10
31.3	21	48.6	8	32.9	10	21.7	15	23.0	21	21.2	8	24.6	16	22.2	15
27.9	10	41.9	18	44.3	15	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0

456.1	8	413.4	4	473.3	17	286.3	13	217.1	11	214.4	19	220.8	23	210.7	21
36.1	14	65.9	7	70.4	4	20.5	9	26.5	14	16.1	24	22.2	10	19.2	23
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
66.5	12	61.4	4	68.7	7	54.8	8	27.3	22	36.7	16	23.2	13	0.0	0
103.6	17	104.3	13	74.0	20	67.0	7	69.9	12	54.4	8	39.0	15	32.6	6
12.6	15	24.8	12	24.5	16	18.5	15	20.5	19	20.1	22	20.5	18	18.8	16
31.0	8	41.1	15	66.8	6	61.4	9	48.6	11	33.8	16	36.6	20	29.3	20
0.0	0	0.0	0	18.5	15	17.0	8	19.3	12	22.5	17	25.7	7	19.8	9
16.9	7	19.0	13	17.3	17	19.7	5	18.9	13	19.9	9	20.3	20	19.0	4
182.1	21	214.0	11	305.5	19	325.1	9	348.5	5	473.2	8	421.9	12	438.1	14
0.0	0	43.2	12	36.6	13	31.3	8	25.3	12	27.8	9	19.0	9	12.6	26
40.4	16	58.6	12	82.6	6	150.9	16	147.1	12	161.0	16	189.9	9	182.9	17
123.6	15	177.9	19	188.8	21	249.9	9	249.9	17	250.8	12	313.2	24	271.1	17
518.8	7	635.1	10	572.1	15	420.3	8	383.3	22	399.8	7	280.7	17	305.8	13
418.8	11	548.3	9	621.1	11	982.1	14	910.9	16	1017.7	9	1030.7	6	942.7	11
24.2	21	45.4	5	68.3	6	72.7	7	72.1	5	67.6	12	66.8	4	57.4	4
32.9	6	39.1	9	38.6	19	42.8	7	38.1	4	47.0	10	36.1	12	51.7	12
131.1	17	145.4	17	96.2	9	102.3	13	148.2	24	95.0	9	107.5	22	84.9	5
3332.5		*4056.6*		*4403.3*		*4305.3*		*3847.5*		*4248.0*		*3882.2*		*3749.1*	
112.7	17	71.3	11	28.4	11	16.8	16	10.1	7	18.9	22	12.2	13	12.8	17
25.2	20	81.1	8	11.1	19	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
275.8	13	135.4	17	160.6	28	159.5	11	153.7	3	129.3	26	100.6	12	144.8	14
138.3	16	80.3	10	79.7	12	31.8	16	17.7	16	19.5	23	12.2	12	14.2	9
58.5	8	42.3	11	35.1	16	22.6	13	27.3	20	33.1	17	13.8	22	21.2	23
30.2	8	12.6	16	81.3	8	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
149.8	10	165.5	20	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
802.6	9	675.8	15	616.5	7	530.5	15	629.4	11	574.7	14	618.7	9	582.9	11
71.4	15	29.1	22	35.2	21	22.8	22	15.9	12	26.8	17	17.9	22	19.1	18
217.3	22	180.0	15	291.3	6	104.2	12	138.2	24	170.9	11	131.6	21	130.5	12
60.2	11	179.1	12	138.1	14	85.2	11	89.6	8	111.5	13	90.4	1	153.3	16
23.8	6	48.5	7	20.7	2	46.6	21	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
76.2	21	46.5	10	32.0	7	27.1	21	25.7	8	28.3	18	31.5	12	29.2	9
25.3	9	13.4	20	38.2	13	74.7	8	38.3	14	31.7	7	40.7	10	73.7	5
2067.3		*1761.0*		*1568.4*		*1121.9*		*1145.8*		*1144.8*		*1069.6*		*1181.7*	
77.1	9	51.2	8	92.0	18	123.1	8	104.3	14	94.0	17	41.2	25	70.5	7

355.5	9	407.1	9	335.6	9	250.9	19	243.0	13	129.8	8	208.9	18	133.3	19
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	45.8	9	48.8	12	28.7	13	30.6	7	0.0	0	0.0	0
0.0	0	11.2	17	7.5	21	4.3	6	4.2	4	3.9	6	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	18.2	4	22.8	11	0.0	0	0.0	0	0.0	0
68.3	13	61.1	4	44.4	14	49.3	18	48.3	19	48.5	24	34.8	13	61.1	8
500.9		*530.6*		*525.3*		*494.6*		*451.3*		*306.9*		*284.9*		*264.9*	
20331.0		*22422.1*		*23087.8*		*22258.3*		*26771.0*		*22266.1*		*17276.7*		*21573.9*	

2012															
27/08/2012		03/09/2012		11/09/2012		18/09/2012		25/09/2012		02/10/2012**		18/08/2010		25/08/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
1459.4	14	2282.8	23	2124.4	23	2795.7	31	1943.5	28	1464.1	5	2516.2	11	3143.8	8
176.9	26	362.2	20	808.0	4	2746.5	21	1704.9	27	1733.1	8	239.6	7	2019.9	7
1761.9	10	2264.3	20	2519.3	16	1349.2	14	1597.3	5	2779.0	18	3522.0	2	6610.0	10
1307.8	11	1761.0	22	1455.5	8	392.9	23	237.6	24	144.9	12	2648.7	5	2035.6	4
360.6	7	521.1	15	2361.6	20	2893.3	27	3296.6	20	2485.5	21	544.7	4	2179.2	12
564.3	27	739.0	6	1219.8	23	1845.8	39	4338.8	10	3086.5	20	2526.2	8	933.6	5
201.8	5	361.6	15	392.7	13	2246.4	14	1207.5	6	1257.7	4	436.1	7	1967.2	15
5832.7		*8291.9*		*10881.3*		*14269.9*		*14326.3*		*12950.8*		*12433.5*		*18889.4*	
109.4	17	158.3	22	168.8	7	150.5	8	207.1	16	256.7	9	166.7	15	98.9	4
12.4	19	19.5	21	30.3	19	30.7	10	17.9	20	19.9	12	16.2	6	38.8	18
129.3	21	162.0	17	185.8	13	249.1	16	236.1	19	160.2	12	87.9	15	176.3	12
251.1		*339.8*		*384.8*		*430.3*		*461.1*		*436.9*		*270.9*		*314.0*	
60.6	11	180.5	23	202.8	35	212.2	28	222.5	23	252.5	11	52.7	18	17.3	18
0.0	0	55.9	9	82.9	7	65.0	12	66.6	28	64.9	7	115.4	8	143.3	11
73.0	12	84.5	9	62.5	15	71.0	15	57.3	15	64.8	20	125.7	16	140.5	15
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
50.2	10	79.0	8	68.8	12	59.5	8	29.0	28	20.8	31	0.0	0	0.0	0
176.2	22	233.3	16	273.4	13	288.3	13	258.6	28	277.0	21	121.4	16	267.6	17
vt		148.4	16	166.7	19	279.8	15	213.9	22	226.1	16	vt		278.4	14
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	46.1	16
24.6	14	142.0	3	252.8	17	234.2	23	222.0	17	282.4	13	23.8	12	66.8	8
vt		176.4	13	178.7	26	169.2	24	278.7	13	185.4	12	vt		582.5	2
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	31.1	16
175.1	14	925.2	6	600.3	12	608.6	6	567.3	17	539.4	3	239.4	14	1060.4	11
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	31.0	5
0.0	0	66.6	10	115.1	17	61.2	13	75.2	16	75.6	16	vt		60.7	4

335.8	20	546.8	11	533.4	10	527.1	9	492.7	9	481.0	10	313.9	16	574.3	11
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	31.0	6
27.1	10	42.0	17	34.3	16	57.3	9	97.8	21	82.5	17	20.4	7	113.9	17
vt		366.4	16	314.8	20	296.0	25	271.5	21	304.7	14	vt		198.3	9
0.0	0	33.1	24	54.8	16	66.8	18	74.9	26	69.9	33	0.0	0	34.8	3
vt		315.8	19	288.9	25	345.7	4	318.4	20	287.4	5	vt		207.2	2
0.0	0	28.0	9	31.6	9	45.4	17	59.2	29	34.8	18	0.0	0	20.0	5
0.0	0	23.1	10	45.1	9	18.3	25	14.5	24	41.6	28	0.0	0	25.3	18
38.2	10	293.8	17	319.3	21	283.7	16	287.4	18	323.8	22	175.5	18	682.9	6
0.0	0	47.7	16	19.4	17	25.9	27	35.2	33	41.3	30	0.0	0	36.9	11
0.0	0	39.9	6	62.4	10	41.0	16	61.5	10	39.6	9	0.0	0	68.8	8
vt		218.5	21	242.5	33	223.7	26	205.8	15	168.0	19	vt		164.8	1
vt		760.3	11	523.7	7	599.3	15	293.0	13	329.7	20	vt		960.4	2
175.4	15	397.2	30	291.9	5	214.5	16	224.5	20	319.4	27	35.8	14	1536.0	9
0.0	0	44.7	11	45.1	13	44.6	27	57.6	22	51.3	15	0.0	0	0.0	0
0.0	0	32.7	25	122.7	19	74.2	7	47.2	18	50.7	16	0.0	0	83.0	14
vt		209.4	28	232.4	14	186.9	21	152.9	16	224.2	11	vt		108.0	13
1136.2		*5491.3*		*5166.3*		*5099.3*		*4685.2*		*4838.7*		*1224.0*		*7571.0*	
226.9	29	489.4	12	373.2	9	320.3	18	239.5	16	287.4	12	25.5	12	412.1	17
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
149.4	21	307.8	16	264.9	21	245.4	20	180.4	21	196.8	22	320.1	5	462.2	9
58.4	31	263.0	25	200.7	18	189.7	26	156.7	28	95.7	21	1018.5	14	340.1	4
0.0	0	198.0	32	150.4	16	138.2	33	169.1	15	84.4	10	vt		267.7	5
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	17.9	10
vt	0	146.7	33	175.1	35	176.6	37	184.3	29	43.0	8	44.3	2	132.1	11
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	vt		1176.1	5
161.1	8	505.3	12	346.3	12	297.8	22	270.5	18	274.4	13	252.3	12	1176.1	5
0.0	0	69.9	8	30.0	29	21.8	12	22.2	12	26.5	14	0.0	0	74.5	7
172.7	27	524.5	8	319.5	23	308.9	15	241.6	20	284.3	21	252.0	17	1306.7	1
0.0	0	42.4	16	30.5	22	14.7	24	27.8	11	26.9	18	0.0	0	0.0	0
0.0	0	32.8	12	20.8	30	19.8	24	25.3	9	22.2	23	0.0	0	51.4	2
0.0	0	39.1	7	23.5	7	29.9	14	30.4	15	45.5	24	vt		621.3	17
vt		34.8	17	27.4	20	21.8	27	32.8	12	33.1	31	0.0	0	21.9	18
35.0	10	54.8	11	36.3	27	29.3	10	38.5	19	39.6	10	0.0	0	0.0	0
803.5		*2708.4*		*1998.7*		*1814.2*		*1619.2*		*1460.0*		*1912.8*		*6060.1*	
26.5	11	142.4	18	98.9	18	95.5	15	90.9	21	89.4	5	vt		36.3	9

vt		316.9	15	321.7	16	240.4	20	216.4	26	131.9	30	0.0	0	0.0	0
0.0	0	70.9	10	67.2	11	116.7	15	114.5	13	84.3	12	79.8	17	55.7	14
vt		90.4	5	79.7	12	84.4	6	86.7	14	64.0	17	0.0	0	6.3	7
0.0	0	83.5	8	51.3	24	75.9	14	73.2	14	81.8	19	0.0	0	42.0	10
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	vt		109.0	16
vt		93.6	13	81.9	9	49.5	13	44.5	19	47.0	10	0.0	0	85.7	17
0.0	0	0.0	0	42.1	30	41.6	9	43.6	13	41.6	22	vt		140.8	10
41.8	20	48.8	14	34.6	21	163.5	14	104.6	21	78.3	12	0.0	0	0.0	0
68.3		*846.5*		*777.5*		*867.5*		*774.4*		*618.4*		*79.8*		*475.9*	
8091.8		*17678.1*		*19208.6*		*22481.1*		*21866.2*		*20304.7*		*15920.9*		*33310.4*	

AR-SM1																
2010						**2011**									
01/09/2010		08/09/2010**		15/09/2010		01/08/2011		08/08/2011		16/08/2011		22/08/2011		29/08/2011**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3538.7	10	4331.4	13	5721.1	3	2722.6	10	2657.0	14	2610.1	1	4306.4	4	2260.4	11
1824.8	15	2664.4	5	1841.0	10	147.5	21	579.7	9	1248.1	5	1509.7	12	860.9	11
4289.6	10	1940.5	13	3411.0	13	1508.7	20	4188.7	23	6989.3	11	9237.1	7	10947.3	15
165.9	10	531.8	14	318.4	10	275.7	20	343.8	19	53.6	21	320.6	6	450.7	20
1353.4	16	1448.0	15	1422.0	9	798.3	6	1216.5	20	1314.4	16	1257.6	16	953.9	19
1478.7	6	1557.7	5	1792.3	8	537.1	15	873.2	18	1145.6	7	1132.3	18	1107.6	16
2072.4	7	5053.9	5	3717.3	2	58.3	13	395.2	5	1280.7	7	1794.9	15	727.9	22
14723.7		*17527.7*		*18223.1*		*6048.1*		*10254.2*		*14641.9*		*19558.7*		*17308.8*	
149.3	11	126.6	12	136.7	7	0.0	0	0.0	0	118.4	7	131.0	16	168.6	25
28.5	13	21.1	14	27.8	6	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
264.1	17	319.4	18	326.9	11	59.7	13	81.5	5	141.9	14	203.3	9	182.1	24
441.9		*467.1*		*491.4*		*59.7*		*81.5*		*260.3*		*334.2*		*350.7*	
30.9	15	17.0	8	12.2	8	176.1	18	163.0	16	113.2	17	9.0	8	10.6	5
166.9	15	149.7	16	105.0	5	0.0	0	0.0	0	0.0	0	25.4	13	22.7	21
180.2	10	84.2	3	126.8	17	71.9	3	59.5	10	79.0	8	77.6	15	67.1	3
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	50.9	7	55.7	7
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	27.7	8	14.4	20
562.6	3	129.9	16	524.8	9	23.0	23	17.2	16	23.1	12	66.1	12	62.8	4
160.6	16	62.6	4	645.3	6	62.1	5	58.0	8	60.8	22	0.0	0	0.0	0
67.3	9	30.6	9	70.5	7	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
114.2	10	120.2	17	121.6	4	72.3	10	58.4	20	106.1	15	32.7	16	32.7	22
541.7	6	436.1	7	592.8	8	0.0	0	0.0	0	0.0	0	247.7	15	199.1	19
0.0	0	0.0	0	0.0	0	13.7	21	13.7	20	20.2	4	22.1	18	30.9	14
16.5	16	12.2	16	27.3	9	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
1643.8	4	1499.2	18	667.4	12	508.2	14	496.6	4	566.2	21	597.6	14	805.2	8
28.5	3	12.6	9	28.8	17	23.2	10	23.4	9	47.6	17	30.9	9	23.4	11
36.5	15	24.2	13	32.0	2	vt		25.5	17	36.2	5	43.0	7	0.0	0

597.6	1	1224.0	3	909.0	7	606.3	20	428.2	9	415.9	13	450.2	16	275.5	13
0.0	0	0.0	0	0.0	0	20.5	3	28.4	14	63.5	8	67.2	4	21.5	12
53.8	5	53.8	5	40.4	6	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
128.5	10	138.8	1	99.3	17	96.3	8	63.7	19	57.0	22	58.2	9	51.2	9
217.5	11	137.1	11	335.9	6	70.5	5	82.8	20	63.0	22	57.9	17	57.5	10
23.3	14	23.3	14	45.4	6	24.1	21	11.4	6	21.2	11	25.0	10	15.8	16
192.7	18	96.5	14	154.1	15	40.7	8	28.7	17	37.3	20	58.0	4	55.6	10
25.9	9	27.3	13	18.0	11	0.0	0	0.0	0	0.0	0	16.2	24	16.0	12
27.2	16	18.6	16	24.5	12	20.7	22	19.5	26	20.6	19	25.8	9	18.9	9
630.8	6	962.0	5	1173.2	18	vt		161.5	5	204.5	8	259.2	17	319.3	14
48.9	15	48.7	4	45.3	19	0.0	0	0.0	0	39.5	19	27.5	16	23.8	10
195.0	6	57.8	13	199.7	18	0.0	0	27.6	11	47.8	17	73.7	5	119.0	11
339.7	15	274.9	2	182.3	19	0.0	0	0.0	0	149.1	27	199.2	13	229.7	15
541.1	8	363.6	3	457.9	17	359.5	23	333.7	18	531.0	20	539.9	23	386.3	13
1324.3	9	1197.5	16	1401.8	10	89.4	5	292.5	27	426.2	18	455.5	17	975.7	9
149.0	3	60.4	3	96.0	1	0.0	0	0.0	0	45.5	19	68.3	9	69.3	10
54.8	17	23.3	12	26.9	14	26.3	24	26.4	21	38.5	16	39.2	15	44.0	23
113.4	19	131.2	16	198.6	11	67.4	5	87.0	5	120.3	14	89.9	8	90.9	7
8213.5		*7417.2*		*8362.8*		*2372.3*		*2506.7*		*3333.1*		*3741.8*		*4094.7*	
226.1	115	48.4	19	46.4	16	166.4	6	167.2	13	12.3	14	25.6	17	25.1	18
0.0	0	0.0	0	0.0	0	16.5	17	13.8	7	19.1	6	0.0	0	0.0	0
386.7	2	135.3	14	150.3	17	196.7	23	267.5	20	149.6	4	133.7	12	169.0	19
150.2	2	238.4	12	138.9	19	114.7	15	108.4	13	88.3	14	81.7	17	45.2	11
104.8	14	104.8	18	53.7	3	90.4	19	83.7	8	50.7	19	34.4	18	26.2	14
30.4	7	32.1	10	32.1	10	30.7	3	32.3	11	13.8	15	69.3	10	0.0	0
161.8	12	46.9	11	53.2	10	0.0	0	35.4	18	148.0	13	0.0	0	0.0	0
41.7	15	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
1177.5	11	711.5	5	693.3	7	595.5	11	778.4	7	628.6	12	582.9	10	488.4	17
167.7	7	41.6	14	43.9	7	98.9	13	68.4	21	43.7	12	37.2	16	19.1	9
634.1	2	305.2	14	520.6	16	140.1	12	223.5	20	178.2	18	283.5	16	116.1	17
0.0	0	0.0	0	0.0	0	47.4	11	51.0	19	224.5	11	144.8	23	82.1	13
29.8	11	26.2	14	27.8	15	36.4	20	22.1	22	31.2	7	13.3	20	36.1	8
18.6	3	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
54.6	6	26.3	19	24.4	8	53.6	7	48.2	9	42.9	8	30.2	5	29.0	13
0.0	0	0.0	0	0.0	0	22.7	24	22.3	17	17.0	22	25.5	19	41.1	17
3184.0		*1716.6*		*1784.7*		*1610.0*		*1922.1*		*1647.9*		*1462.2*		*1077.4*	
48.8	13	55.9	6	26.4	19	16.6	6	51.5	12	50.7	6	105.6	10	127.8	18

0.0	0	0.0	0	0.0	0	vt		418.2	21	392.0	12	266.9	9	218.8	22
109.5	12	183.3	11	178.6	18	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
94.9	6	93.5	3	97.1	7	0.0	0	0.0	0	vt		41.4	23	46.4	9
46.8	1	5.6	8	0.0	0	0.0	0	0.0	0	11.1	12	6.9	4	3.9	4
136.1	4	264.9	12	300.4	14	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
98.3	2	37.2	10	46.3	4	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
133.2	17	102.0	4	95.2	10	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	59.1	7	79.9	20	59.9	3	47.2	21	41.8	3
667.7		*742.4*		*744.0*		*75.7*		*549.7*		*513.6*		*468.0*		*438.8*	
27230.8		*27871.1*		*29606.0*		*10165.9*		*15314.1*		*20396.8*		*25565.0*		*23270.4*	

		2012											
05/09/2011		27/08/2012		03/09/2012		11/09/2012		18/09/2012		25/09/2012		02/10/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3314.1	19	1601.3	20	2582.5	19	1809.0	10	2132.9	17	1929.2	17	1541.6	5
1067.1	17	226.7	29	426.8	16	1113.7	12	1886.1	8	2227.6	9	1345.1	23
9939.0	7	1683.7	10	1990.3	9	1580.9	21	2421.5	19	2032.1	17	1775.7	19
97.6	3	929.5	18	1246.8	22	1328.1	21	364.4	17	231.0	25	89.6	14
731.4	14	442.1	24	587.2	7	718.4	22	3262.0	26	2751.7	9	3268.1	16
753.5	11	451.2	13	526.3	15	2202.3	20	1038.1	19	3418.3	9	4399.3	18
1106.9	14	363.3	17	471.8	12	432.3	12	1845.0	20	1411.8	17	876.0	14
17009.6		*5697.8*		*7831.7*		*9184.7*		*12949.9*		*14001.8*		*13295.5*	
172.8	22	124.0	20	91.1	8	124.6	17	147.3	24	163.8	18	239.3	29
0.0	0	15.3	13	20.0	14	26.6	20	21.9	16	21.3	27	19.6	17
221.6	15	157.7	17	147.6	28	138.4	26	208.7	25	196.3	23	193.8	17
394.3		*297.1*		*258.6*		*289.6*		*377.8*		*381.4*		*452.7*	
12.6	20	53.7	19	215.4	21	226.7	31	179.9	25	187.5	25	226.1	10
18.1	10	0.0	0	40.6	8	67.9	22	57.3	10	68.7	4	46.7	18
81.4	11	44.2	30	73.2	27	66.3	16	57.0	17	67.7	19	57.0	15
32.0	11	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
14.4	14	43.0	23	63.3	7	62.4	8	50.5	7	45.8	21	31.8	4
66.4	3	168.3	17	211.2	18	263.4	11	215.1	19	242.5	19	290.2	7
0.0	0	vt		147.6	28	154.2	17	122.7	21	165.0	15	199.7	15
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
54.1	8	26.3	20	178.5	23	233.4	29	226.5	25	220.5	27	274.8	21
92.3	10	vt		146.9	12	210.0	27	180.5	16	209.4	31	215.5	14
25.1	17	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
685.4	24	186.1	23	810.1	11	494.5	7	421.7	5	434.8	19	526.1	4
18.1	13	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	79.5	27	97.5	8	63.6	14	75.3	29	54.2	29

213.2	15	318.2	8	444.4	17	366.9	10	342.8	17	399.3	7	462.8	12
27.8	11	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
29.0	13	27.1	17	34.2	12	29.7	11	33.8	19	42.3	13	42.4	26
62.1	2	vt		344.3	13	262.3	18	164.2	26	214.9	22	258.2	23
17.0	6	0.0	0	30.8	12	66.9	15	70.8	14	66.2	14	58.6	24
44.5	23	vt		281.0	24	283.2	20	279.2	23	260.5	15	207.2	23
12.2	13	0.0	0	20.5	12	31.9	18	26.2	13	19.2	21	24.7	17
16.0	11	0.0	0	28.1	7	32.0	14	20.7	13	41.6	15	42.0	19
327.5	16	25.7	18	283.8	27	263.2	16	288.1	15	258.5	19	270.4	18
20.1	10	0.0	0	48.2	12	19.0	23	28.6	26	65.0	17	29.1	20
133.1	10	0.0	0	34.6	14	59.0	13	41.4	11	49.7	7	42.3	6
209.0	15	vt		222.7	23	231.8	28	210.3	28	217.7	17	175.0	19
332.4	21	vt		647.3	14	333.5	10	366.2	11	312.3	18	295.4	15
814.6	15	193.4	27	346.0	23	283.0	29	149.1	6	190.9	11	241.5	18
71.0	4	0.0	0	45.4	6	36.0	21	24.5	19	46.2	4	45.5	26
39.4	4	0.0	0	31.2	23	108.3	20	77.2	16	52.0	24	54.2	24
117.1	13	vt		231.9	26	230.2	23	193.3	17	224.0	19	192.2	24
3585.6		*1086.1*		*5040.7*		*4513.2*		*3891.5*		*4177.5*		*4363.4*	
72.7	19	116.0	16	437.9	24	327.1	12	268.3	10	248.5	12	239.5	8
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
131.0	24	130.2	14	341.8	22	265.8	20	199.6	27	162.6	4	177.9	25
30.9	25	43.7	27	236.1	20	182.4	19	180.2	26	168.3	22	38.1	5
26.5	16	vt		161.9	21	133.5	33	114.9	28	103.8	10	95.7	9
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	vt		141.8	23	128.7	27	176.4	31	167.4	22	44.5	15
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
494.3	11	262.4	22	471.3	12	288.0	25	283.2	23	258.4	21	261.8	10
26.9	5	0.0	0	65.0	19	32.0	17	20.0	4	20.1	17	29.8	18
119.7	20	237.8	23	533.7	10	243.1	26	188.8	23	214.3	12	227.0	19
69.0	11	0.0	0	41.6	20	31.6	11	11.5	16	26.1	9	30.2	11
0.0	0	0.0	0	36.1	12	19.6	16	19.8	26	26.3	21	24.3	19
0.0	0	0.0	0	25.0	22	31.2	16	28.9	17	32.1	20	47.6	20
28.7	6	0.0	0	24.4	14	25.1	22	20.1	29	26.2	13	30.9	10
57.7	17	25.3	28	37.6	15	47.8	22	26.6	14	39.3	23	43.6	23
1057.2		*815.4*		*2554.2*		*1756.0*		*1538.5*		*1493.3*		*1291.1*	
59.3	17	24.4	18	131.8	29	102.5	27	112.5	18	96.6	14	84.9	10

250.9	10	vt		245.1	14	199.3	22	192.2	22	178.0	29	154.4	9
0.0	0	0.0	0	84.4	8	66.5	15	89.8	25	92.6	15	75.5	10
40.0	5	vt		56.4	21	70.9	21	85.9	13	85.0	7	69.8	7
4.7	21	0.0	0	67.7	10	68.7	11	62.7	13	73.8	16	81.3	15
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	vt		76.8	11	51.7	20	54.7	22	41.7	23	42.9	22
13.5	12	0.0	0	0.0	0	34.6	23	42.3	9	44.2	14	33.5	23
51.7	22	63.0	10	49.1	12	35.1	24	93.6	17	84.1	10	74.1	13
420.1		*87.4*		*711.3*		*629.4*		*733.6*		*696.0*		*616.4*	
22466.9		*7983.7*		*16396.6*		*16372.9*		*19491.3*		*20750.0*		*20019.0*	

Table S9

Volatile components determined for *Vitis vinifera* L. cv. Bical variety obtained from 3 parcels and 3 years of harvests, during maturation, at Bairrada Appellation.

		2010									
		02/08/2010		09/08/2010		16/08/2010		23/08/2010		30/08/2010	
^{1}Dtr(s),^{2}Dtr(s)^a	Compound	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C6 compounds											
194, 0.640	Hexanal	2603.9	15	3120.4	20	4052.6	12	4052.6	12	4307.8	16
206, 0.630	3-Hexenal	3909.3	19	2194.3	14	1932.9	5	2172.9	3	1113.6	18
230, 0.651	2-Hexenal	0.0	0	4541.9	9	5774.7	6	7396.7	12	5357.1	7
242, 0.630	3-Hexen-1-ol	697.0	14	1979.4	13	797.4	4	834.0	2	1220.0	9
248, 1.076	2-Hexen-1-ol	1510.6	14	4355.2	7	4249.9	4	1640.4	10	4399.5	6
266, 0.903	1-Hexanol	1777.8	9	4188.4	2	3724.6	7	3614.3	15	2772.7	9
296, 0.930	2,4-Hexadienal	635.6	15	2267.8	17	3223.4	10	4048.9	6	1331.4	15
	Sub-Total	*11134.2*		*22647.3*		*23755.5*		*23759.8*		*20502.1*	
Aromatic alcohols											
420, 3.014	Benzyl Alcohol	174.7	12	166.3	18	257.2	16	363.7	11	383.5	11
446, 1.426	α,α-Dimethyl Benzyl	0.0	0	21.5	12	20.8	13	24.8	3	18.4	20
470, 1.960	2-Phenylethanol	32.0	12	192.2	9	343.4	7	317.8	5	323.2	11
	Sub-Total	*206.8*		*379.9*		*621.4*		*706.4*		*725.1*	
Monoterpenic compounds											
314, 0.440	α-Pinene	20.3	18	16.9	17	11.2	19	18.9	5	13.6	16
344, 0.457	β-Pinene*	11.6	14	13.3	13	13.3	1	10.7	9	12.2	16
392, 0.405	*m*-Cymene	20.2	13	20.9	6	18.1	3	19.3	15	18.9	9
398, 0.476	Limonene	31.1	10	57.5	8	37.2	8	68.3	9	174.4	12
404, 0.476	1,8-Cineole	vt		267.2	20	137.3	15	40.4	2	42.1	2
428, 0.678	Linalool oxide (isome	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
434, 0.727	Dihydromyrcenol	vt		410.0	12	370.4	16	447.1	10	486.6	9
446, 0.700	Dihydrolinalool	0.0	0	0.0	0	0.0	0	12.5	16	13.2	4
452, 0.746	Linalool *	20.3	9	137.5	8	444.8	11	121.6	2	125.9	16
464, 0.844	Fenchol (exo)	0.0	0	0.0	0	0.0	0	38.8	18	34.3	5

470, 0.646	Hotrienol	163.1	25	445.3	11	315.2	16	368.0	12	259.7	9
494, 1.050	Pinocarveol	vt		44.2	18	44.2	18	44.2	18	0.0	0
512, 0.635	Nerol oxide	vt		146.4	10	54.6	5	42.7	5	15.0	16
518, 0.834	Ocimenol	vt		65.7	9	138.0	19	216.8	12	206.4	10
524, 0.860	Borneol	0.0	0	0.0	0	0.0	0	28.5	9	20.5	5
530, 0.984	Menthol*	vt		182.2	16	150.5	9	141.4	20	194.0	18
536, 0.715	Terpinen-4-ol	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
536, 1.269	Cymen-8-ol	0.0	0	15.9	16	14.9	6	26.2	7	23.7	14
542, 0.835	α-Terpineol	vt		264.4	12	164.4	13	391.7	18	400.9	19
560, 0.850	Verbenone	0.0	0	0.0	0	0.0	0	66.4	20	68.3	12
566, 0.703	Menth-1-en-9-al	vt		52.0	13	21.0	16	44.4	10	56.9	16
584, 0.873	Geraniol (isomer 1) *	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
584, 0.943	β-Citronellol*	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
590, 0.737	Geraniol (isomer 2) *	45.3	3	114.7	17	139.0	20	145.8	11	71.0	15
596, 0.976	Citral (isomer 1)	0.0	0	0.0	0	53.0	15	53.7	4	68.2	4
602, 0.815	Carvone *	vt		65.6	6	70.9	13	76.7	15	24.4	11
626, 0.775	Citral (isomer 2)	20.6	8	20.6	8	20.6	8	59.3	15	59.3	15
	Sub-Total	*332.6*		*2340.3*		*2218.4*		*2483.4*		*2389.3*	
C13 Norisoprenoids											
566, 0.532	*m/z* 159, 91, 131	103.8	13	88.7	1	32.9	12	37.3	4	26.6	12
620, 0.595	Vitispirane	213.0	17	125.3	10	105.1	16	77.9	6	16.4	3
632, 0.517	Theaspirane (isomer	145.6	25	226.5	15	128.8	9	29.8	7	32.0	20
644, 0.528	Theaspirane (isomer	0.0	0	119.4	16	31.3	16	12.4	7	9.0	12
674, 0.681	β-Damascenone (iso	156.2	18	55.4	15	102.4	6	38.1	6	12.4	7
700, 0.702	β-Damascenone (iso	vt		722.8	12	370.2	6	762.4	6	468.3	2
724, 0.750	Hydroxydihydroedula	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
736, 0.648	Geranylacetone *	361.9	13	222.1	16	312.1	4	166.2	9	267.2	15
742, 0.850	5,6-Epoxy-β-ionone	0.0	0	0.0	0	0.0	0	14.8	9	14.8	9
760, 0.868	3,4-Dehydro-β-ionone	vt		18.1	10	8.8	2	15.4	7	15.4	7
778, 0.635	α-iso-methyl ionone	24.0	18	18.7	10	19.7	5	22.0	6	22.0	6
784, 0.717	β-Ionone*	0.0	0	78.7	19	60.1	15	85.6	4	56.4	5
900, 0.894	Methyl dihydrojasmo	0.0	0	41.1	2	14.3	24	35.3	8	43.5	8
	Sub-Total	*1004.5*		*1716.7*		*1185.8*		*1297.1*		*984.1*	

Sesquiterpenic compounds											
656, 0.583	Longipinene epoxide	22.2	10	42.8	5	43.5	11	33.7	9	0.0	0
680, 0.469	α-Copaene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
712, 0.508	Longifolene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
756, 0.630	Aromadendrene	32.0	13	45.6	5	48.2	16	36.4	10	13.8	15
790, 0.660	α-Farnesene	vt		41.0	5	30.9	3	23.7	18	16.5	19
808, 0.630	Calamenene	31.3	3	34.0	1	34.8	4	5.6	9	14.8	20
826, 0.629	α-Calacorene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
832, 0.880	Nerolidol	vt		82.1	9	100.6	3	76.2	3	72.1	4
850, 0.751	Globulol	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
886, 0.690	β -Eudesmol	vt		101.1	2	109.9	8	139.3	20	107.7	9
912, 0.654	*m/z* 119, 91, 191, 10	vt		118.5	9	106.5	3	310.3	3	345.0	14
	Sub-Total	*85.5*		*465.0*		*474.4*		*625.2*		*569.9*	
Diterpenoid											
1116, 0.929	Phytol	0.0	0	0.0	0	0.0	0	32.5	6	39.8	5
	Total	*12763.6*		*27549.3*		*28255.5*		*28904.3*		*25210.2*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

vt - vestigial

BI-VA1															
				2011											
06/09/2010**		13/09/2010		27/07/2011		03/08/2011		10/08/2011		17/08/2011**		16/08/2012			
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)		
2621.4	16	5305.0	18	3554.6	22	2313.1	12	5880.8	14	4392.6	14	2317.3	14		
1694.3	9	1194.6	17	586.1	14	976.4	11	1239.6	22	733.9	6	1226.4	11		
10260.3	8	5594.6	19	2476.4	9	6458.7	13	6881.4	17	8847.4	2	1500.1	5		
668.6	10	326.6	4	1311.8	19	1991.5	18	503.1	5	381.5	18	503.1	9		
2818.1	9	3972.0	14	3300.4	13	2486.6	7	2162.8	13	2450.8	21	1420.2	8		
1957.5	6	2757.6	2	2229.2	16	2729.8	22	1155.3	21	2837.4	29	1182.3	15		
2220.2	10	1907.8	17	385.3	13	610.9	21	945.0	16	1040.8	10	391.0	5		
22240.4		*21058.1*		*13843.8*		*17566.9*		*18767.9*		*20684.4*		*8540.3*			
533.5	8	417.3	11	147.8	13	132.0	23	181.9	14	176.5	17	126.8	13		
0.0	0	20.1	7					vt		vt		vt			
174.3	9	245.0	3	142.0	5	124.1	19	217.1	8	252.9	23	143.4	10		
707.8		*682.4*		*289.8*		*256.1*		*399.0*		*429.4*		*270.2*			
15.4	18	13.9	7	16.0	12	16.5	14	6.7	5	15.4	21	18.1	14		
16.4	15	10.5	13	24.5	13	14.9	5	7.9	5	44.2	7	14.6	18		
13.6	16	18.3	20	0.0	0	0.0	0	0.0	0	18.6	8	10.2	20		
142.1	3	44.9	10	29.1	15	12.3	21	9.7	24	51.3	4	41.5	9		
30.9	4	0.0	0	49.2	5	40.1	15	55.2	8	82.5	4	0.0	0		
0.0	0	0.0	0	58.7	8	47.9	19	50.2	15	0.0	0	13.9	23		
454.0	5	388.3	6	79.2	3	31.2	8	39.9	5	109.5	12	22.2	23		
15.2	12	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0		
49.5	14	142.9	16	87.2	8	103.8	13	95.7	17	100.6	18	26.6	25		
21.0	9	25.7	19	36.7	20	55.4	7	66.2	6	0.0	0	21.9	21		

106.0	9	253.4	6	104.8	21	153.2	21	116.3	10	109.2	14	160.4	13
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
49.6	2	32.3	6	36.7	14	44.6	7	28.7	33	17.5	11	vt	
148.0	20	115.2	6	0.0	0	0.0	0	0.0	0	0.0	0	vt	
43.3	7	0.0	0	70.7	22	62.9	10	40.6	8	46.4	2	0.0	0
152.5	32	128.2	5	9.9	7	28.8	14	39.2	6	56.9	4	vt	
9.7	2	24.7	10	0.0	0	0.0	0	0.0	0	29.3	21	0.0	0
23.7	14	37.2	14	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
368.5	6	286.3	5	0.0	0	0.0	0	0.0	0	39.6	22	vt	
25.6	8	47.1	9	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
28.4	14	75.5	6	0.0	0	0.0	0	0.0	0	0.0	0	vt	
0.0	0	0.0	0	vt		98.3	13	130.1	4	89.7	8	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	46.5	7	18.2	13	0.0	0
134.2	12	113.9	10	7.7	7	82.8	7	50.8	14	77.1	12	30.8	4
42.2	14	72.8	15	0.0	0	0.0	0	0.0	0	29.6	9	0.0	0
15.0	16	0.0	0	vt		23.2	16	35.5	8	0.0	0	vt	
24.3	15	56.4	6	0.0	0	0.0	0	0.0	0	60.9	14	27.5	17
1929.0		*1887.4*		*610.4*		*815.9*		*819.2*		*996.5*		*387.5*	

13.0	18	27.0	14	70.5	2	97.7	6	27.9	7	20.6	12	120.4	10
45.9	7	27.3	9	124.6	25	166.9	18	59.5	7	58.9	11	201.7	5
13.6	16	0.0	0	57.3	18	44.4	19	42.8	14	12.8	25	147.3	6
3.3	11	0.0	0	36.4	14	27.5	19	8.2	8	10.9	13	43.3	9
0.0	0	0.0	0	381.4	5	398.4	9	209.3	16	29.6	4	147.9	15
383.7	7	262.7	10	0.0	0	0.0	0	0.0	0	0.0	0	219.4	16
0.0	0	0.0	0	30.1	21	55.2	20	21.7	20	25.0	10	65.2	5
331.2	20	258.1	12	0.0	0	0.0	0	0.0	0	0.0	0	307.4	8
17.8	10	9.2	3	128.1	14	178.1	16	75.5	20	138.4	21	0.0	0
13.2	5	13.8	10	0.0	0	0.0	0	0.0	0	12.3	13	23.7	17
15.7	15	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	15.1	26
19.0	8	43.9	11	45.5	11	24.9	21	31.1	24	14.3	15	38.2	6
33.3	6	0.0	0	16.5	21	21.0	15	43.5	13	18.0	9	15.7	10
889.7		*641.9*		*890.5*		*1014.2*		*519.4*		*340.9*		*1345.4*	

0.0	0	0.0	0	50.8	8	100.0	3	60.3	14	50.4	18	30.6	19
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	vt	
45.5	17	18.6	15	0.0	0	vt		17.4	8	25.9	7	16.8	24
32.9	9	31.9	7	0.0	0	vt		18.4	18	22.8	14	0.0	0
17.4	3	15.4	9	0.0	0	0.0	0	0.0	0	0.0	0	vt	
0.0	0	0.0	0	12.7	15	9.6	12	17.4	4	46.9	9	vt	
60.5	12	65.4	4	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
85.2	3	72.3	3	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
64.1	8	101.4	6	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
261.5	9	109.5	9	0.0	0	vt		vt		45.7	5	vt	
567.1		*414.6*		*63.4*		*109.6*		*113.5*		*191.7*		*47.4*	
42.6	2	41.1	5	0.0	0	0.0	0	vt		vt		0.0	0
26376.6		*24725.5*		*15697.931*		*19762.5322*		*20618.973*		*22642.948*		*10590.793*	

2012													
23/08/2012		30/08/2012		06/09/2012**		13/09/2012		02/08/2010		09/08/2010		16/08/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
1940.4	3	2603.0	9	2181.5	19	2502.6	10	4450.8	8	4425.1	8	3513.7	7
1237.9	8	1566.1	16	2011.5	21	2300.7	16	3164.8	10	2194.1	13	1518.1	16
1794.5	19	1834.1	13	2042.5	13	2001.7	19	3896.1	6	3853.1	6	4764.5	11
525.2	13	523.9	7	537.9	16	522.6	6	661.6	11	2350.8	17	1106.2	11
1417.3	25	1526.8	16	1602.3	16	1315.7	25	2216.0	7	4364.1	6	4623.9	19
2019.0	12	2243.5	8	1377.2	11	1013.3	14	2174.3	15	3473.2	9	2273.1	7
418.4	20	536.0	6	538.2	15	576.2	3	1335.4	6	2704.3	15	2750.3	14
9352.7		*10833.4*		*10291.1*		*10232.9*		*17899.0*		*23364.6*		*20549.8*	
193.6	7	216.1	23	240.6	8	261.3	4	154.3	10	242.0	10	333.9	7
16.9	12	23.7	14	21.7	7	20.9	9	0.0	0	26.6	5	23.2	10
175.0	21	172.1	18	244.9	6	231.0	6	135.8	18	268.3	14	331.5	8
385.5		*411.9*		*507.1*		*513.2*		*290.1*		*536.9*		*688.7*	
21.0	15	13.9	19	10.3	7	10.5	11	34.9	14	23.0	18	18.0	7
16.9	14	20.6	15	27.1	15	13.4	21	14.3	17	16.4	4	20.5	13
21.3	12	21.3	13	24.0	16	19.7	16	20.4	11	19.3	15	14.8	14
103.1	14	111.9	18	124.9	8	108.5	25	41.3	14	41.7	11	38.7	17
47.2	11	56.5	12	105.2	13	74.9	3	vt		548.1	7	1123.4	10
12.8	10	11.9	25	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
21.0	21	32.6	18	38.9	17	21.4	20	vt		562.1	3	565.4	5
0.0	0	0.0	0	0.0	0	0.0	0	vt		20.5	17	12.8	20
77.1	8	84.4	5	129.2	27	100.5	8	vt		120.4	20	106.4	6
37.1	7	27.1	19	31.0	11	27.1	19	vt		37.2	12	31.9	8

178.7	21	131.2	22	96.4	9	62.8	11	532.4	4	408.0	1	435.0	7
20.8	16	27.8	20	0.0	0	0.0	0	0.0	0	41.9	4	35.3	12
143.5	19	153.9	19	59.6	24	43.1	24	vt		210.7	12	80.2	3
73.5	15	95.4	14	80.9	9	96.7	20	vt		186.2	11	205.4	11
0.0	0	0.0	0	14.6	23	12.1	21	vt		24.6	4	24.5	7
111.0	29	126.3	18	124.5	20	79.2	17	vt		204.3	11	177.2	7
0.0	0	0.0	0	13.8	24	14.3	10	0.0	0	19.0	6	17.6	16
0.0	0	11.0	15	9.3	16	10.5	12	0.0	0	23.0	9	20.0	13
130.5	23	127.3	22	130.8	25	80.1	5	vt		420.5	4	385.8	7
vt		16.3	27	14.9	16	14.1	15	vt		45.5	9	41.5	10
19.3	29	17.3	24	14.6	27	12.1	20	vt		34.9	3	43.9	8
11.3	15	13.2	14	36.6	17	27.5	15	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
188.2	16	113.3	10	156.7	17	126.7	22	22.7	9	123.9	11	55.3	6
16.4	25	16.7	5	23.4	15	13.5	16	vt		23.0	13	23.0	13
36.6	15	37.9	10	21.2	28	20.9	14	vt		40.9	8	81.3	4
28.1	18	29.8	8	41.2	19	29.2	12	21.5	13	30.5	6	30.5	6
1315.3		*1297.8*		*1329.2*		*1018.9*		*687.4*		*3225.7*		*3588.3*	

220.1	4	130.4	17	95.5	13	78.6	5	62.9	16	71.8	2	86.5	5
233.5	14	111.2	10	94.6	7	69.0	5	138.8	15	130.6	13	165.7	13
158.5	19	96.5	5	65.8	12	57.1	8	179.9	19	200.8	6	209.6	12
88.1	6	78.0	5	52.7	15	32.6	18	vt		118.4	4	39.4	10
159.7	15	119.2	18	77.1	6	63.1	6	613.4	2	190.3	14	167.4	14
234.7	6	174.0	12	124.2	22	110.5	18	vt		899.8	6	693.8	9
92.4	11	68.4	7	50.6	17	34.6	8	0.0	0	0.0	0	0.0	0
489.7	12	161.7	26	112.2	20	98.9	12	119.1	14	254.4	10	381.6	6
0.0	0	0.0	0	8.2	5	7.2	5	0.0	0	0.0	0	0.0	0
69.7	10	47.8	10	41.0	10	28.7	19	vt		17.6	10	12.0	16
18.7	23	16.3	12	15.2	17	9.9	4	39.9	10	19.5	10	23.3	7
22.1	14	14.8	16	10.7	18	8.1	7	vt		99.9	5	44.3	4
25.0	8	18.3	23	15.4	6	10.7	10	vt	0	44.2	6	18.5	22
1812.2		*1036.7*		*763.2*		*608.9*		*1154.0*		*2047.3*		*1842.4*	

37.9	24	42.0	5	38.0	12	32.3	21	29.7	7	43.1	19	29.9	10
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
28.1	5	18.5	15	24.0	7	24.9	14	0.0	0	0.0	0	0.0	0
32.5	12	33.3	13	26.3	20	27.8	11	42.1	7	61.6	19	48.3	12
12.8	27	18.4	19	20.7	5	20.2	13	vt		44.0	14	41.6	13
23.4	10	32.9	15	31.2	19	33.5	11	35.0	7	39.7	17	39.7	17
36.8	17	49.0	20	37.2	15	34.2	2	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	vt		99.4	19	154.9	17
0.0	0	0.0	0	58.2	8	60.0	9	0.0	0	0.0	0	0.0	0
0.0	0	23.6	14	26.8	19	28.8	24	vt		93.8	6	109.9	19
12.6	17	16.9	17	12.4	16	16.6	21	vt		124.4	18	98.9	4
183.9		*234.6*		*274.7*		*278.3*		*106.9*		*506.0*		*523.2*	
0.0	0	26.4	19	35.0	7	34.6	6	0.0	0	0.0	0	0.0	0
13049.593		*13840.702*		*13200.334*		*12686.756*		*20137.4*		*29680.5*		*27192.2*	

										GC×GC peak area x 10^4 (arbitrary units)			
													BI-VA2
2010													
23/08/2010		30/08/2010		06/09/2010**		13/09/2010		27/07/2011		03/08/2011		10/08/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4387.1	17	4297.1	13	3135.5	11	5224.9	14	4217.2	13	5019.2	13	5366.3	6
5036.6	12	1609.9	10	1782.0	6	1066.8	7	259.4	25	653.3	9	589.3	7
1680.4	14	6746.8	4	9071.3	10	8625.2	12	4528.7	13	6739.2	18	6781.3	10
1316.2	8	752.2	19	926.8	11	563.7	6	1804.5	11	1757.1	15	213.1	18
4245.8	4	4031.8	5	3330.8	6	3902.8	13	4602.7	24	2230.0	24	1638.3	23
2003.4	7	1549.7	16	3022.9	2	752.6	5	2355.4	15	2136.9	14	1722.1	19
4289.1	0	2141.8	15	1844.3	3	1339.6	22	339.3	20	764.0	5	469.8	16
22958.5		*21129.3*		*23113.7*		*21475.6*		*18107.2*		*19299.7*		*16780.2*	
331.7	7	392.6	6	441.3	6	421.1	11	238.3	4	152.5	26	347.3	6
24.8	5	20.1	12	37.1	6	22.3	9						
399.8	9	374.4	9	399.1	4	317.3	9	179.2	23	335.4	18	362.4	15
756.2		*787.1*		*877.5*		*760.8*		*417.5*		*487.8*		*709.7*	
15.8	19	12.1	18	17.3	11	11.8	13	29.4	5	11.6	5	8.3	12
18.6	12	12.0	19	22.3	10	15.3	17	50.8	7	17.7	11	11.9	13
19.5	14	9.0	17	26.9	11	19.4	6	0.0	0	0.0	0	0.0	0
234.4	3	195.4	22	169.5	1	52.2	20	26.8	11	13.0	23	16.5	23
624.5	4	645.3	2	505.2	11	0.0	0	55.4	22	31.8	22	58.3	5
0.0	0	0.0	0	0.0	0	0.0	0	71.7	4	50.7	12	39.6	6
635.0	4	590.7	7	632.1	7	580.4	14	80.0	4	38.3	12	38.1	4
12.8	20	13.2	1	19.1	7	0.0	0	0.0	0	0.0	0	0.0	0
119.7	13	188.3	5	144.6	11	121.7	13	114.1	19	107.8	15	105.7	15
27.3	9	20.7	18	26.0	10	26.4	12	40.3	4	73.9	15	42.9	11

568.5	1	432.2	13	279.0	16	262.0	11	144.9	29	228.9	11	170.5	20
35.3	12	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
24.3	16	19.8	4	27.3	14	27.4	8	50.6	15	23.0	15	17.1	10
186.5	5	182.5	24	245.0	15	158.1	12	0.0	0	0.0	0	0.0	0
10.9	11	10.9	11	21.2	5	0.0	0	78.8	19	66.0	13	68.2	18
166.2	7	160.2	19	286.8	10	188.6	19	14.1	30	34.9	23	35.9	11
17.6	16	18.3	5	27.4	11	13.1	11	0.0	0	0.0	0	0.0	0
18.6	8	21.6	14	29.5	4	24.7	10	0.0	0	0.0	0	0.0	0
421.2	1	499.6	4	590.1	13	440.7	17	0.0	0	0.0	0	0.0	0
36.5	9	33.9	10	34.7	12	40.7	11	0.0	0	0.0	0	0.0	0
55.8	18	27.9	1	85.5	5	86.0	5	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	vt		285.6	13	285.8	17
0.0	0	0.0	0	0.0	0	0.0	0	24.2	14	34.4	8	67.6	13
241.6	19	278.9	8	186.3	8	176.2	22	25.6	26	49.3	9	61.5	7
23.0	13	73.6	3	37.0	8	51.1	14	0.0	0	0.0	0	0.0	0
17.1	3	31.6	14	30.9	19	0.0	0	26.9	18	48.8	5	28.3	19
37.9	13	41.2	17	31.7	3	60.4	3	0.0	0	0.0	0	0.0	0
3568.7		*3518.9*		*3475.4*		*2356.4*		*833.4*		*1115.8*		*1056.2*	

28.8	5	15.2	9	31.1	12	33.1	14	75.4	8	104.3	12	20.5	12
116.4	6	122.6	1	48.4	16	83.2	6	143.2	22	253.8	17	71.3	5
27.7	13	25.8	16	19.8	13	0.0	0	81.3	5	62.7	9	51.5	11
12.2	12	31.9	5	5.1	1	0.0	0	44.0	23	30.9	7	15.7	30
38.0	4	12.2	12	39.4	6	0.0	0	617.4	7	614.6	13	333.7	12
701.2	5	414.5	20	458.2	3	199.3	11	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	43.3	22	68.7	10	23.1	23
379.5	13	347.5	2	268.1	9	313.0	16	0.0	0	0.0	0	0.0	0
11.8	11	59.8	3	6.3	4	9.8	14	199.3	18	258.6	15	103.1	7
10.9	9	10.9	9	7.1	2	13.2	14	0.0	0	0.0	0	0.0	0
22.6	5	18.4	5	21.9	9	0.0	0	0.0	0	0.0	0	0.0	0
59.8	3	39.0	12	30.9	5	34.2	5	67.6	24	64.2	14	34.8	11
35.0	1	53.7	3	83.0	9	0.0	0	37.8	19	31.7	18	49.8	17
1444.0		*1151.5*		*1019.3*		*685.6*		*1309.4*		*1489.4*		*703.5*	

19.7	12	19.5	4	30.8	5	27.5	16	79.2	8	78.7	14	68.7	13
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
35.6	4	23.4	20	47.0	5	52.2	15	0.0	0	0.0	0	0.0	0
31.8	9	31.7	4	28.2	1	105.9	5	vt	0	10.3	10	28.4	8
5.9	4	18.9	3	11.9	21	8.2	2	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	13.7	22	27.7	21	7.8	11
86.1	10	80.1	12	60.1	14	96.5	7	0.0	0	0.0	0	0.0	0
0.0	0	220.6	10	167.5	22	0.0	0	0.0	0	0.0	0	0.0	0
114.4	12	108.2	15	113.9	5	130.3	17	0.0	0	0.0	0	0.0	0
356.4	15	319.5	6	333.2	8	103.7	22	vt		vt		30.4	0
649.9		*821.9*		*792.6*		*524.3*		*92.9*		*116.7*		*135.3*	
62.2	3	72.2	2	67.5	4	72.1	5	0.0	0	0.0	0	0.0	0
29439.5		*27480.8*		*29345.9*		*25874.8*		*20760.39*		*22509.409*		*19384.934*	

2011												**2012**	
17/08/2011		24/08/2011		31/08/2011**		07/09/2011		16/08/2012		23/08/2012		30/08/2012	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4335.3	10	3192.2	18	3456.7	15	3701.0	7	2199.0	24	2235.1	22	2608.8	11
400.7	22	1775.4	24	489.0	12	444.3	13	1112.8	11	1546.8	21	1673.1	18
7597.1	14	6633.2	5	8163.5	10	5691.6	13	1392.3	11	1461.8	29	1710.5	9
372.5	8	360.1	17	266.0	16	160.1	25	368.1	9	431.2	14	527.5	6
2200.4	23	2410.4	15	2016.6	6	2050.8	6	1447.0	24	1392.9	13	1606.0	17
2787.8	15	1894.3	23	1839.7	15	1943.5	22	1218.7	19	1506.4	9	1972.9	10
581.0	13	762.3	6	968.4	20	484.6	19	880.3	19	486.3	21	543.3	5
18274.8		*17027.9*		*17199.9*		*14475.8*		*8618.3*		*9060.6*		*10642.1*	
403.5	16	323.4	11	343.0	19	419.0	10	140.7	20	291.3	17	313.9	6
vt		vt		vt		vt		0.0	0	19.2	12	27.2	14
359.8	8	344.5	18	314.2	25	364.9	4	155.7	16	237.0	15	279.1	7
763.2		*667.9*		*657.2*		*783.9*		*296.4*		*547.5*		*620.2*	
31.5	4	0.0	0	0.0	0	0.0	0	20.0	7	24.6	15	32.2	3
63.8	2	14.7	13	16.4	19	11.7	17	15.9	18	19.6	22	20.5	22
19.9	11	9.4	15	14.1	22	6.6	12	16.0	14	29.4	21	25.6	11
43.3	5	49.9	4	50.0	9	58.1	21	55.6	10	169.0	16	225.4	21
88.9	19	33.0	14	61.2	6	20.5	7	vt		62.3	4	105.4	16
0.0	0	0.0	0	0.0	0	0.0	0	25.9	12	21.8	16	23.5	15
162.7	13	40.3	11	46.8	22	50.3	7	32.9	26	36.5	7	52.9	3
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
88.0	20	140.4	15	115.4	21	134.2	14	29.2	21	92.8	9	110.2	18
39.6	18	0.0	0	39.8	9	0.0	0	29.2	8	49.0	20	41.3	9

148.8	6	171.5	15	103.3	17	167.3	20	203.7	6	270.1	21	252.8	18
0.0	0	0.0	0	0.0	0	0.0	0	vt		35.7	21	40.0	18
28.0	10	27.9	14	28.9	9	22.9	8	vt		247.2	9	223.4	25
0.0	0	0.0	0	0.0	0	0.0	0	vt		189.8	16	169.2	14
44.7	16	20.6	16	15.0	1	17.0	12	vt		23.1	24	28.3	19
38.9	3	42.5	23	56.7	21	45.9	7	vt		173.8	31	191.2	19
41.9	4	0.0	0	48.2	14	0.0	0	0.0	0	12.0	21	15.0	24
18.4	4	0.0	0	0.0	0	0.0	0	vt		17.2	26	20.0	18
58.4	2	40.2	15	49.0	15	37.6	14	vt		184.4	12	183.8	15
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	21.6	13	30.1	25
0.0	0	0.0	0	0.0	0	0.0	0	vt		27.2	12	32.0	10
221.8	13	281.0	16	277.6	6	213.8	12	vt		29.3	8	28.4	14
20.6	11	18.9	12	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
95.2	7	90.2	18	91.0	16	75.2	10	50.6	11	202.9	20	214.0	10
32.5	10	30.4	13	29.9	6	19.6	15	0.0	0	19.5	28	20.5	28
0.0	0	0.0	0	0.0	0	0.0	0	vt		17.1	20	46.5	8
52.8	15	38.0	10	68.0	7	59.6	9	52.5	23	36.6	17	44.3	12
1339.8		*1049.0*		*1111.3*		*940.1*		*531.5*		*2012.4*		*2176.4*	

19.2	13	18.3	18	11.1	14	11.8	16	188.7	11	198.9	13	148.0	13
51.9	18	68.6	16	79.2	9	36.5	22	242.4	20	243.7	19	145.7	23
14.9	12	11.9	18	0.0	0	0.0	0	157.8	17	209.3	12	151.2	15
6.7	15	8.3	13	0.0	0	0.0	0	67.4	12	98.4	13	88.7	2
20.0	9	20.7	9	28.0	11	19.7	9	165.8	5	212.1	20	148.6	12
172.3	24	179.7	12	194.8	14	190.2	26	290.0	18	291.7	14	166.4	25
14.2	8	16.8	26	17.9	9	15.2	20	73.0	5	103.0	12	84.4	4
0.0	0	0.0	0	0.0	0	0.0	0	459.8	6	519.9	7	224.9	19
125.2	8	89.4	21	97.2	12	147.5	8	0.0	0	0.0	0	0.0	0
12.8	15	7.9	10	7.2	2	8.0	6	27.4	17	105.8	13	92.9	4
0.0	0	0.0	0	0.0	0	0.0	0	16.2	25	26.8	6	18.0	21
9.6	18	35.0	11	36.8	22	25.6	12	55.1	14	53.3	4	29.9	14
18.6	13	18.3	14	12.5	14	12.4	18	16.3	11	27.5	9	23.0	10
465.4		*474.9*		*484.8*		*466.8*		*1760.1*		*2090.3*		*1321.6*	

49.3	15	47.3	19	40.6	9	50.5	25	45.4	6	51.1	7	61.1	9
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
158.0	17	97.5	17	92.5	7	91.3	10	vt		41.9	15	45.0	15
0.0	0	0.0	0	0.0	0	0.0	0	20.5	22	45.8	4	41.9	7
47.0	11	60.9	10	53.0	12	65.5	10	0.0	0	13.4	21	21.1	22
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	25.8	7	31.9	15
4.1	18	23.2	12	19.5	8	16.0	15	vt		38.8	2	46.1	17
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	vt		44.4	11	51.0	11
0.0	0	30.0	2	31.4	23	25.3	19	0.0	0	10.5	8	25.9	21
35.1	8	114.7	20	100.2	12	96.6	16	vt		12.8	16	19.3	21
293.4		*373.6*		*337.3*		*345.2*		*65.9*		*284.6*		*343.3*	
0.0	0	vt		vt		vt		0.0	0	0.0	0	39.3	10
21136.567		*19593.255*		*19790.373*		*17011.938*		*11272.172*		*13995.326*		*15142.983*	

				2010									
06/09/2012**		13/09/2012		02/08/2010		09/08/2010		16/08/2010		23/08/2010		30/08/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2072.2	3	2353.9	10	4472.6	11	4214.5	10	4085.5	3	3943.2	5	5488.7	3
1833.6	19	1819.8	14	2766.5	12	1379.7	5	1728.6	13	2399.8	7	1681.1	11
1128.5	7	1556.4	12	2692.4	20	2786.6	19	5898.6	2	6362.2	16	5868.4	2
535.7	9	529.9	6	1024.0	6	2181.8	17	1163.8	6	1197.2	15	536.4	9
1184.6	11	1562.9	13	1205.6	15	4521.3	11	2527.9	9	3810.7	7	3460.1	9
1419.8	5	1563.2	18	2094.5	9	2300.6	16	4277.9	3	3597.3	11	1996.6	10
523.7	16	570.0	4	968.4	2	2488.4	15	3234.9	11	4023.1	2	1853.9	13
8698.1		*9956.2*		*15224.0*		*19873.0*		*22917.2*		*25333.5*		*20885.1*	
366.9	12	349.1	10	120.3	9	264.9	1	311.1	4	332.6	5	350.8	5
30.3	11	25.0	11	vt		22.1	5	23.8	19	19.6	6	22.6	9
296.4	9	322.7	6	105.1	7	229.0	6	300.8	10	406.3	2	396.1	10
693.6		*696.8*		*225.4*		*516.0*		*635.6*		*758.5*		*769.5*	
23.3	22	23.3	15	34.4	17	15.4	6	14.7	20	18.6	2	13.6	13
42.5	15	25.3	10	17.2	18	16.2	3	17.6	3	17.4	13	13.3	13
33.0	15	30.9	14	16.8	20	13.1	7	18.6	5	18.1	15	6.3	5
161.7	12	191.8	19	51.4	9	47.3	10	242.9	10	262.9	2	175.6	7
200.8	17	130.2	25	vt		562.5	10	629.9	3	609.9	3	623.2	2
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
45.1	11	30.1	9	vt		523.9	10	572.4	11	609.1	7	624.4	3
0.0	0	0.0	0	0.0	0	11.8	12	11.1	1	11.1	1	11.6	12
239.9	16	160.4	8	13.9	14	106.2	11	139.2	13	116.0	13	131.2	13
34.4	13	34.1	18	vt		46.4	7	30.8	10	36.2	11	29.4	15

199.8	27	183.1	17	151.9	15	463.2	14	513.3	6	572.4	6	468.9	9
0.0	0	0.0	0	0.0	0	37.5	11	32.9	11	32.9	11	0.0	0
83.8	19	58.7	8	vt		62.5	17	28.6	5	21.5	5	21.5	5
195.8	12	150.1	13	vt		207.7	15	162.5	9	167.7	12	183.1	11
23.4	18	23.3	14	vt		33.4	15	11.9	14	21.1	12	21.1	12
162.9	27	190.6	22	vt		219.6	19	223.6	10	144.1	8	163.7	11
26.9	18	19.7	25	0.0	0	15.6	14	15.8	16	15.8	16	11.5	8
18.9	17	12.1	24	0.0	0	21.0	7	28.1	18	28.9	11	25.6	10
253.2	3	182.1	24	vt		421.2	4	434.2	5	432.9	8	448.9	9
40.9	7	27.3	11	vt		51.0	11	31.4	13	38.0	18	28.5	18
32.4	14	28.0	12	vt		37.0	10	21.7	17	42.1	14	40.5	10
43.4	17	34.6	12	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
190.3	23	221.2	18	40.8	5	151.9	12	257.9	10	239.1	13	288.1	1
29.8	16	28.2	13	0.0	0	45.1	19	109.1	12	59.6	14	55.9	6
44.7	29	38.2	14	vt		52.8	7	41.3	17	17.2	13	24.0	4
48.2	10	37.3	22	20.9	12	34.4	17	46.4	13	50.1	11	42.1	5
2175.2		*1860.6*		*347.4*		*3196.8*		*3636.0*		*3582.9*		*3451.9*	

128.6	18	84.8	5	65.7	7	73.9	4	80.9	2	23.8	6	23.8	6
119.8	20	104.4	20	159.7	10	120.1	17	134.3	4	147.0	11	128.4	4
81.2	10	68.3	5	182.7	11	201.3	5	224.0	4	30.4	3	21.5	8
73.8	12	48.4	12	0.0	0	125.3	4	19.1	1	11.4	12	30.4	3
101.3	7	82.3	3	670.2	8	183.2	1	182.5	11	39.3	4	11.4	12
139.4	21	101.2	15	0.0	0	914.8	1	710.7	8	720.0	2	444.6	13
73.3	11	59.6	9	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
142.8	16	121.0	17	122.5	12	275.3	5	221.0	2	383.6	4	333.5	3
18.5	4	10.9	14	0.0	0	0.0	0	0.0	0	19.2	6	19.2	6
49.9	15	39.8	19	0.0	0	18.7	6	13.6	15	16.3	7	16.3	7
18.1	26	11.9	15	31.7	14	20.2	7	21.8	5	26.0	7	16.6	17
16.9	38	10.8	21	0.0	0	116.3	11	29.0	17	63.5	6	25.7	16
15.9	17	11.6	20	0.0	0	46.5	9	27.0	4	34.8	2	39.7	8
979.5		*755.0*		*1232.6*		*2095.4*		*1663.9*		*1515.2*		*1111.2*	

42.2	8	41.4	16	17.0	10	26.5	13	26.8	12	20.7	7	20.7	7
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
44.2	10	42.4	8	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
45.6	9	41.5	11	20.6	13	45.8	3	44.8	11	39.4	2	41.0	3
37.5	12	42.1	7	vt	0	37.4	17	43.9	14	28.5	8	28.4	14
33.3	12	35.9	9	32.3	4	34.3	6	34.3	6	6.0	3	6.9	5
44.4	21	48.7	10	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	vt		112.2	8	120.6	19	76.3	5	56.4	13
77.4	6	73.2	6	0.0	0	0.0	0	0.0	0	0.0	0	108.6	10
32.3	5	36.0	9	vt		110.6	6	109.9	8	103.3	1	92.6	10
21.5	17	21.6	23	vt		108.6	4	121.7	18	356.5	13	330.2	7
378.4		*382.8*		*69.9*		*475.3*		*502.1*		*630.6*		*684.8*	
52.6	16	55.7	10	0.0	0	0.0	0	0.0	0	64.1	7	70.7	2
12977.364		*13707.052*		*17099.3*		*26156.5*		*29354.8*		*31884.8*		*26973.3*	

BI-SM1													
				2011									
06/09/2010**		13/09/2010		27/07/2011		03/08/2011		10/08/2011**		16/08/2012		23/08/2012	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4460.6	6	4300.3	6	3396.5	16	2996.9	4	4911.1	14	2419.9	1	2240.2	16
866.3	2	1014.7	9	537.6	17	584.8	7	908.6	18	1074.3	8	1061.8	20
8201.5	7	6915.1	10	3914.4	10	4621.2	18	5043.7	20	1752.1	21	1334.3	22
885.0	2	391.2	6	1398.2	19	1645.6	21	177.9	24	546.0	12	400.5	7
3034.3	7	3884.0	18	2552.7	8	2220.3	15	1659.3	19	1748.5	13	1339.7	20
2608.0	11	1671.2	14	4261.4	6	1956.4	17	1119.0	6	1625.7	24	1596.0	23
1212.9	7	1411.3	13	367.6	4	531.0	9	920.8	15	272.6	20	525.3	10
21268.7		*19587.9*		*16428.3*		*14556.2*		*14740.4*		*9439.2*		*8497.8*	
467.6	7	441.4	10	179.8	14	274.3	20	397.9	20	187.4	26	277.1	21
31.1	12	26.1	18			vt		vt		vt		21.6	13
371.3	5	302.3	8	379.2	25	348.4	13	356.4	16	158.5	3	203.1	18
869.9		*769.8*		*559.0*		*622.7*		*754.2*		*346.0*		*501.8*	
29.3	18	12.6	16	53.2	17	17.2	2	11.1	15	20.5	13	25.0	13
22.3	8	13.5	5	54.3	17	15.2	9	13.7	19	16.7	16	29.5	8
29.3	7	13.0	9	0.0	0	0.0	0	0.0	0	16.1	9	26.9	20
169.2	7	73.0	16	40.6	10	17.5	22	11.6	9	58.6	9	217.8	25
549.1	16	vt		126.8	17	59.6	15	109.0	9	0.0	0	93.5	6
0.0	0	0.0	0	92.0	8	48.2	9	39.2	21	27.9	16	24.3	17
640.3	7	545.3	11	57.6	9	30.8	6	43.3	13	45.5	14	54.4	7
13.1	8	10.6	14	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
130.8	8	156.6	16	110.2	11	179.3	21	224.7	10	34.0	8	90.2	12
35.2	3	25.9	13	29.1	8	88.0	7	49.1	5	40.2	11	52.2	8

23.6	2	233.5	13	166.7	17	238.3	13	170.6	20	207.8	23	429.7	14		
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	36.5	22		
33.5	3	28.8	9	63.3	6	17.3	18	37.3	16	vt		259.9	11		
200.4	8	145.0	4	0.0	0	0.0	0	0.0	0	vt		163.0	31		
23.4	12	0.0	0	73.5	4	62.5	2	99.4	19	0.0	0	30.7	13		
356.8	10	179.7	16	40.7	13	41.9	7	40.8	20	vt		119.2	18		
26.4	9	10.3	5	0.0	0	0.0	0	0.0	0	0.0	0	36.9	19		
21.9	18	13.0	20	0.0	0	0.0	0	0.0	0	0.0	0	25.4	17		
541.3	3	349.7	8	0.0	0	0.0	0	0.0	0	vt		184.3	14		
39.7	6	28.7	12	0.0	0	0.0	0	0.0	0	vt		38.8	19		
30.9	8	77.9	14	0.0	0	0.0	0	0.0	0	vt		48.1	15		
0.0	0	0.0	0	vt		256.0	32	235.5	9	vt		31.4	22		
0.0	0	0.0	0	39.8	16	61.6	10	59.3	6	0.0	0	0.0	0		
169.0	14	202.0	13	30.8	7	60.0	21	72.1	16	47.5	11	201.1	9		
56.7	5	130.7	16	0.0	0	0.0	0	23.4	16	0.0	0	17.1	11		
28.0	7	0.0	0	34.0	5	32.0	22	37.6	23	vt		31.7	19		
31.8	5	47.6	8	38.8	2	61.2	12	71.7	19	71.7	21	41.1	9		
3201.9		*2297.3*		*1051.6*		*1286.8*		*1349.1*		*586.5*		*2308.6*			

23.8	6	38.8	13	82.4	6	111.6	14	24.0	24	188.0	16	188.9	22
36.2	4	59.5	8	145.7	16	271.1	16	80.2	18	249.8	5	238.0	15
24.3	9	0.0	0	84.2	8	68.3	16	58.6	8	151.2	16	200.9	4
30.4	3	0.0	0	49.9	15	11.2	14	19.5	19	71.7	8	97.6	6
11.4	12	0.0	0	675.1	6	660.1	7	461.6	10	202.8	11	258.4	12
395.4	6	254.9	3	0.0	0	0.0	0	0.0	0	325.6	10	327.5	17
0.0	0	0.0	0	48.7	17	71.1	8	24.3	17	82.0	19	128.8	22
341.3	2	315.4	4	0.0	0	0.0	0	0.0	0	510.2	5	577.8	8
19.2	6	0.0	0	228.6	14	263.7	12	120.7	18	0.0	0	0.0	0
16.3	7	0.0	0	0.0	0	0.0	0	0.0	0	41.5	17	112.0	25
19.6	16	0.0	0	0.0	0	0.0	0	0.0	0	17.7	11	24.2	19
21.8	12	0.0	0	70.2	22	80.5	2	39.0	20	65.8	13	57.5	10
110.8	5	0.0	0	35.0	14	35.1	10	56.0	9	17.4	10	28.0	12
1050.5		*668.6*		*1419.8*		*1572.5*		*883.8*		*1923.8*		*2239.6*	

29.0	6	0.0	0	88.8	8	98.5	8	79.2	8	52.7	7	56.0	6
0.0	0	0.0	0	0.0	0	0.0	0	21.6	3	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	vt		45.2	11
38.4	6	39.0	13	0.0	0	0.0	0	0.0	0	45.6	15	49.3	9
33.6	6	75.4	5	vt		vt		31.2	5	0.0	0	14.3	22
7.3	8	6.1	11	0.0	0	0.0	0	0.0	0	vt		28.6	14
0.0	0	0.0	0	21.0	14	28.9	8	8.1	11	vt		41.8	6
68.2	3	85.6	15	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
98.3	18	65.4	6	0.0	0	0.0	0	0.0	0	vt		44.6	20
114.4	10	84.2	5	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
326.1	3	100.2	20	0.0	0	0.0	0	0.0	0	vt		13.8	11
715.3		*456.0*		*109.8*		*127.4*		*140.0*		*98.3*		*293.6*	
69.9	6	68.3	17	0.0	0	vt		vt		0.0	0	0.0	0
27176.3		*23847.8*		*19568.431*		*18165.662*		*17867.6*		*12393.799*		*13841.417*	

2012					
30/08/2012		06/09/2012**		13/09/2012	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2115.2	19	1969.5	17	1707.7	16
1732.0	8	1773.5	15	1725.4	14
1476.9	21	1402.0	23	1726.4	7
466.8	4	461.6	6	540.6	3
1564.5	16	1856.0	17	1688.6	23
2102.2	11	1388.7	26	1429.6	15
506.5	2	563.9	8	503.4	9
9964.0		*9415.1*		*9321.7*	
316.7	13	392.3	8	345.4	12
32.3	11	32.8	9	29.6	4
266.9	13	324.6	6	335.7	8
615.8		*749.8*		*710.7*	
34.6	16	24.2	14	20.8	23
27.5	18	49.9	10	27.6	22
24.0	11	34.9	15	49.7	26
221.4	10	155.1	3	176.0	22
106.2	19	162.7	19	138.9	14
24.1	14	0.0	0	0.0	0
52.6	5	45.2	25	38.3	8
0.0	0	0.0	0	0.0	0
122.6	21	226.3	12	165.5	28
44.5	7	37.3	11	30.0	7

224.6	16	198.9	8	144.3	26
39.5	7	0.0	0	0.0	0
204.3	17	85.8	18	59.7	11
184.3	20	175.7	21	144.3	19
26.7	10	26.2	15	26.2	16
188.1	25	181.3	17	200.9	16
19.0	22	30.0	14	22.8	20
22.4	9	19.1	19	15.5	10
184.8	20	246.8	14	163.8	20
32.7	22	46.0	9	29.0	15
28.6	12	33.0	8	27.0	9
30.0	9	41.4	9	35.3	11
0.0	0	0.0	0	0.0	0
217.3	7	204.8	15	217.7	13
17.0	6	33.5	12	28.5	23
39.0	10	45.2	11	35.7	20
39.0	17	47.6	25	34.9	12
2154.8		*2151.0*		*1832.4*	
171.3	10	160.3	23	96.1	11
196.3	17	135.5	17	102.0	12
169.5	11	88.0	7	77.4	7
96.1	2	79.3	8	61.0	8
215.0	11	103.0	14	100.4	6
254.9	9	147.2	20	116.7	20
94.4	3	87.9	2	68.3	5
356.0	14	175.0	23	133.2	20
10.3	9	12.5	17	10.6	7
94.8	3	39.8	7	46.9	9
19.6	11	19.8	13	16.3	17
52.5	5	26.7	21	20.5	19
22.6	10	18.7	20	14.4	17
1753.4		*1093.9*		*863.7*	

56.7	12	42.6	5	48.8	10
14.3	21	8.1	12	0.0	0
42.6	14	45.7	6	52.5	6
47.4	9	45.5	11	47.5	9
27.7	3	35.1	21	43.1	10
34.9	13	35.7	9	35.6	7
48.9	13	43.5	13	47.9	12
0.0	0	0.0	0	0.0	0
49.0	10	71.3	10	98.2	3
26.5	14	30.8	10	34.0	9
22.7	18	24.4	14	27.5	7
370.8		*382.9*		*435.0*	
44.6	12	51.5	8	51.3	2
14903.303		*13844.165*		*13214.873*	

Table S10

Volatile components determined for *Vitis vinifera* L. cv. Sauvignon Blanc variety obtained from 3 parcels at Bairrada Appellation, from 2010, during maturation and for 2011 and 2012 harvests, only maturity was considered.

		SB-SM1													
		2010										**2011**		**2012**	
		28/07/2010		04/08/2010		11/08/2010		18/08/2010 **		25/08/2010		12/08/2011**		30/08/2012**	
^{1}Dtr(s),^{2}Dtr(	Compound	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C6 compounds															
194, 0.640	Hexanal	3762.6	4	2128.3	4	4402.4	3	4865.3	3	3830.8	7	3276.1	7	2922.9	6
206, 0.630	3-Hexenal	1553.2	8	2229.3	2	1849.9	14	1674.4	17	1179.8	13	774.2	13	2282.5	15
230, 0.651	2-Hexenal	5021.7	7	5877.6	9	6421.4	2	5653.4	10	7921.7	7	5471.5	11	4659.7	24
242, 0.630	3-Hexen-1-ol	2084.1	7	1818.4	17	1818.4	17	291.0	14	686.2	13	360.1	16	1389.3	17
248, 1.076	2-Hexen-1-ol	2927.1	4	3476.5	9	2482.4	7	3813.0	8	2688.1	7	3215.4	10	978.1	16
266, 0.903	1-Hexanol	0.0	0	2617.7	10	1483.8	12	2956.0	13	2756.0	10	2257.8	8	1318.9	21
296, 0.930	2,4-Hexadienal	1130.1	11	3163.7	11	2814.4	9	2531.4	5	1345.3	15	664.0	17	1857.4	16
	Sub-total	*16478.8*		*21311.5*		*21272.9*		*21784.5*		*20407.9*		*16019.2*		*15408.7*	
Aromatic alcohols															
420, 3.014	Benzyl Alcohol	64.1	5	237.8	8	404.2	11	412.7	8	551.2	11	vt		802.7	11
446, 1.426	α,α-Dimethyl Benzyl	12.8	16	20.4	16	27.9	16	22.9	5	24.6	5	0.0	0	13.1	15
470, 1.960	2-Phenylethanol	122.5	20	299.8	12	427.3	3	508.5	5	400.3	8	122.4	16	802.3	8
	Sub-total	*199.4*		*558.0*		*859.4*		*944.1*		*976.2*		*122.4*		*1618.2*	
Monoterpenic compounds															
314, 0.440	α-Pinene	67.9	11	21.6	3	12.5	15	18.7	13	35.1	10	17.4	11	14.4	31
344, 0.457	β-Pinene *	52.8	13	67.3	15	83.0	7	114.4	8	110.5	7	19.5	9	354.3	8
356, 0.570	β-Myrcene	54.8	10	163.8	12	51.0	16	51.0	16	0.0	0	35.8	17	0.0	0
362, 0.520	3-Carene	127.0	19	284.5	19	284.5	19	24.6	9	0.0	0	19.0	13	0.0	0
368, 0.790	α-Phellandrene	6.5	11	6.5	11	27.3	17	0.0	0	0.0	0	0.0	0	0.0	0
392, 0.405	*m*-Cymene	0.0	0	55.7	10	56.0	9	49.6	12	40.0	13	0.0	0	33.2	5
398, 0.476	Limonene *	111.4	15	111.4	15	70.0	3	1517.5	19	179.3	19	20.8	5	59.9	7
404, 0.476	1,8-Cineole	0.0	0	280.9	10	280.9	10	148.1	14	125.3	13	159.3	11	161.4	14
416, 0.560	β-Ocimene	0.0	0	39.9	17	12.0	11	12.0	11	48.0	5	0.0	0	0.0	0
428, 0.678	Linalool oxide (isom	73.9	13	401.7	11	121.7	20	118.4	7	72.6	9	171.7	16	154.7	8
434, 0.727	Dihydromyrcenol	0.0	0	0.0	0	445.2	6	752.1	10	606.3	11	vt		570.9	13
440, 0.560	α-Terpinolene	35.0	18	64.4	15	34.8	12	28.1	8	15.8	13	13.1	15	0.0	0
440, 0.790	Linalool oxide (isom	34.3	18	34.3	18	34.3	18	34.3	18	18.0	11	0.0	0	0.0	0
446, 0.700	Dihydrolinalool	0.0	0	16.4	11	8.6	2	19.9	13	33.0	14	0.0	0	0.0	0

452, 0.746	Linalool *	0.0	0	590.9	8	695.7	2	550.4	7	498.6	6	228.3	16	227.8	3
464, 0.600	Rose oxide (isomer)	vt		39.5	9	5.8	7	5.8	7	22.9	12	6.0	3	0.0	0
464, 0.844	Fenchol	vt		761.5	18	1614.3	15	138.2	10	259.4	3	293.3	9	168.3	25
470, 0.646	Hotrienol	vt		29.9	18	11.7	11	38.0	15	43.3	9	33.5	10	44.9	7
470, 0.780	Camphenal	0.0	0	0.0	0	0.0	0	15.8	12	0.0	0	0.0	0	0.0	0
482, 0.890	1-Terpineol	vt		55.1	8	42.8	17	35.9	14	0.0	0	0.0	0	0.0	0
488, 0.690	Cosmene	19.1	18	34.0	8	29.5	7	24.0	17	0.0	0	0.0	0	0.0	0
500 , 0.970	β-Terpineol	vt		30.3	4	14.3	9	39.1	4	0.0	0	0.0	0	0.0	0
512, 0.635	Nerol oxide	24.2	16	124.7	15	78.3	9	85.2	12	68.4	5	63.9	17	58.4	7
518, 0.834	Ocimenol	0.0	0	26.6	18	257.7	18	232.0	11	199.3	17	0.0	0	148.4	8
518, 1.200	*m/z* 68, 94, 79, 109 (	23.0	13	23.0	13	23.0	13	175.2	6	163.6	13	67.7	13	0.0	0
524, 0.860	Borneol	0.0	0	36.0	10	61.7	14	31.9	8	0.0	0	0.0	0	0.0	0
530, 0.884	Mentha-1,5-dien-8-ol	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	76.2	9
530, 0.984	Menthol *	vt		210.0	11	195.0	14	204.6	11	218.8	8	25.3	21	131.7	19
536, 0.715	Terpinen-4-ol	47.8	2	127.3	16	75.4	10	65.1	18	38.6	8	20.2	13	45.5	7
536, 1.269	Cymen-8-ol	0.0	0	35.9	16	24.8	10	48.7	12	24.3	11	vt		44.0	9
542, 0.835	α-Terpineol *	120.7	18	571.8	18	286.1	4	723.5	2	594.8	2	115.9	20	418.0	10
554, 0.900	Safranal	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	12.5	14	0.0	0
560, 0.850	Verbenone	vt		280.5	16	34.5	14	52.6	12	57.4	17	0.0	0	55.1	12
566, 0.703	Menth-1-en-9-al	vt		102.3	13	107.1	13	68.5	13	40.1	14	43.5	15	47.9	7
572, 1.340	2-Hydroxycineole	29.2	17	64.2	8	52.8	15	61.2	7	24.2	17	0.0	0	0.0	0
584, 0.873	Geraniol (isomer 1) *	vt		229.7	12	242.4	11	225.8	6	244.0	10	179.9	9	160.5	12
584, 0.943	β-Citronellol *	0.0	0	71.1	17	63.8	5	46.1	6	34.1	12	vt		12.9	17
590, 0.737	Geraniol (isomer 1) *	43.4	9	785.0	17	980.1	8	1187.6	12	1705.1	8	603.2	9	1449.8	16
596, 0.976	Citral (isomer 1)	vt		101.6	15	115.2	12	135.1	17	98.4	2	28.8	22	111.3	21
602, 0.815	Carvone *	vt		50.5	19	20.1	11	34.3	15	43.2	10	38.2	18	43.1	13
626, 0.775	Citral (isomer 1)	33.5	11	126.7	9	169.7	11	222.0	11	118.4	10	75.7	12	332.9	8
	Sub-total	*904.6*		*6056.2*		*6723.6*		*7335.2*		*5781.1*		*2292.5*		*4925.3*	
C$_{13}$ Norisoprenoids															
566, 0.532	*m/z* 159, 91, 131	151.6	5	275.2	3	165.0	10	165.2	12	42.3	6	0.0	0	128.4	11
620, 0.595	Vitispirane	259.6	19	229.6	19	185.4	15	205.6	10	0.0	0	26.0	17	152.0	11
632, 0.517	Theaspirane (isomer	410.6	19	324.4	11	309.3	9	319.1	11	55.7	6	71.0	9	264.3	11
644, 0.528	Theaspirane (isomer	vt		183.6	12	107.4	15	97.8	9	24.9	20	19.6	13	100.2	13
668, 0.790	TDN	0.0	0	18.4	11	12.7	9	0.0	0	0.0	0	92.3	18	0.0	0
674, 0.681	β-Damascenone (isor	vt		89.4	4	38.7	15	75.9	4	0.0	0	0.0	0	73.9	14
700, 0.702	β-Damascenone (isor	271.2	16	824.2	13	666.0	5	662.0	2	445.7	6	134.6	18	405.4	13
736, 0.648	Geranylacetone *	253.5	8	367.2	11	460.4	4	400.0	4	176.5	7	77.5	11	683.5	13
742, 0.850	5,6-Epoxy-β-ionone	0.0	0	20.8	13	9.2	11	12.1	16	0.0	0	0.0	0	16.6	4
760, 0.868	3,4-Dehydro-β-iononי	0.0	0	39.7	17	17.4	6	15.6	19	0.0	0	0.0	0	49.4	26

778, 0.635	α-Iso-methyl ionone	vt		114.0	16	73.4	11	33.3	15	14.2	20	0.0	0	24.5	10
784, 0.717	β-Ionone *	43.3	11	137.9	16	68.2	15	63.3	2	0.0	0	20.6	4	20.7	11
900, 0.894	Methyl dihydrojasmo	0.0	0	252.2	3	196.3	16	147.5	20	369.3	69	33.5	8	156.1	15
	Sub-total	*1389.8*		*2876.6*		*2309.3*		*2197.5*		*1128.6*		*475.0*		*2075.0*	
Sesquiterpenic compounds															
656, 0.583	Longipinene epoxide	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
680, 0.469	α-Copaene	vt		27.8	15	21.2	15	19.0	8	27.6	3	10.3	17	84.3	13
712, 0.508	Longifolene	0.0	0	7.4	9	12.5	11	21.9	19	13.0	14	23.1	12	71.4	22
724, 0.541	α-Humulene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
756, 0.630	Aromadendrene	41.4	18	22.8	11	32.1	6	32.1	6	0.0	0	17.4	13	83.8	13
762, 0.450	α-Muurolene	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
790, 0.660	α-Farnesene	vt		95.1	9	126.0	15	72.1	3	58.2	5	38.0	16	81.6	13
796, 0.525	γ-Cadinene	7.5	13	20.8	8	29.8	7	22.5	17	44.6	10	24.2	9	39.5	17
808, 0.630	Calamenene	54.9	16	13.5	14	12.3	18	12.3	18	0.0	0	0.0	0	0.0	0
826, 0.629	α-Calacorene	12.6	19	18.5	9	15.8	14	15.8	14	0.0	0	10.4	16	26.6	13
832, 0.880	Nerolidol	0.0	0	0.0	0	0.0	0	164.4	2	99.1	3	72.4	12	80.1	11
862, 0.726	Caryophyllene oxide	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
886, 0.690	β-Eudesmol	0.0	0	0.0	0	156.2	16	104.6	25	124.2	4	51.5	9	62.4	13
912, 0.654	*m/z* 119, 91, 191, 10	vt		160.2	14	112.0	8	84.9	9	87.1	3	59.7	13	112.5	15
	Sub-total	*116.5*		*366.2*		*517.8*		*549.6*		*453.9*		*306.9*		*642.1*	
	Total	*19089.1*		*31168.5*		*31683.0*		*32810.8*		*28747.6*		*19216.0*		*24669.3*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

vt - vestigial

GC×GC peak area x 10^4 (arbitrary units)													
SB-SM2													
2010										**2011**		**2012**	
28/07/2010		04/08/2010		11/08/2010		18/08/2010**		25/08/2010		12/08/2011**		30/08/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2075.3	5	2883.5	2	4644.9	2	3556.8	15	4890.3	14	3330.5	12	1265.6	8
1809.6	14	1910.5	16	1772.0	13	2016.2	9	1331.8	8	661.6	12	2001.1	12
6977.2	13	5665.4	9	6774.0	9	6867.2	9	7079.7	16	4572.1	14	2264.9	25
1858.8	11	905.6	6	905.6	6	318.2	18	450.1	12	345.2	20	1466.1	30
2764.5	17	4392.2	16	3127.1	10	2742.0	5	3032.5	8	2608.2	2	1082.8	22
0.0	0	2085.0	14	2485.0	3	2806.8	18	2471.6	6	2193.0	1	1366.8	15
1658.5	1	2705.2	10	2900.9	2	2568.8	10	1658.5	10	672.9	0	2389.1	12
17143.8		*20547.4*		*22609.5*		*20876.0*		*20914.5*		*14383.5*		*11836.3*	
173.6	10	386.5	15	461.9	2	555.4	1	628.0	6	vt		768.3	15
16.9	18	34.5	13	20.9	12	43.1	6	23.3	14	0.0	0	20.1	16
59.8	4	231.0	16	583.4	1	478.6	3	462.1	6	143.4	12	924.7	5
250.3		*652.0*		*1066.2*		*1077.0*		*1113.4*		*143.4*		*1713.0*	
62.4	7	17.5	13	39.7	6	29.8	8	39.6	4	10.8	9	18.6	3
35.7	16	202.0	4	79.9	7	119.3	13	90.7	17	27.9	16	453.8	6
121.8	14	288.2	6	57.0	6	63.2	5	0.0	0	38.7	20	0.0	0
129.1	7	390.8	14	71.1	12	46.1	9	45.4	6	19.0	6	0.0	0
8.1	13	8.1	13	18.8	20	21.8	14	0.0	0	0.0	0	0.0	0
vt		100.9	18	58.1	14	56.5	7	39.4	12	vt		44.9	12
200.8	20	74.8	16	277.7	15	173.2	2	194.4	12	13.4	21	62.6	7
vt		512.3	9	385.6	19	186.3	6	127.6	13	198.0	15	210.0	11
0.0	0	75.7	13	35.9	10	44.1	7	54.5	9	0.0	0	0.0	0
74.6	14	69.7	11	68.8	8	96.6	5	61.7	12	174.6	16	150.4	6
vt		vt		735.2	5	745.1	4	587.0	1	vt		542.9	4
41.8	2	95.1	19	34.6	15	55.1	13	22.5	20	35.0	16	0.0	0
35.9	14	35.9	14	35.9	14	35.9	14	25.9	15	vt		vt	
0.0	0	16.7	4	23.2	14	20.0	13	21.7	13	vt		vt	

vt		743.0	6	678.6	2	616.3	11	569.2	7	553.4	17	229.0	11
0.0	0	11.2	15	11.2	15	11.2	15	7.3	4	6.2	4	0.0	0
vt		555.5	17	725.4	5	1136.3	8	394.8	15	617.8	3	262.5	4
0.0	0	29.9	18	46.0	6	54.0	5	32.9	17	21.5	4	52.2	2
0.0	0	0.0	0	17.3	18	20.0	5	0.0	0	0.0	0	0.0	0
vt		64.7	1	27.5	19	34.7	12	vt		vt		vt	
36.0	15	36.7	14	18.0	5	24.5	7	0.0	0	0.0	0	0.0	0
0.0	0	29.2	6	15.3	17	17.9	16	0.0	0	0.0	0	0.0	0
14.7	14	82.8	16	70.3	12	82.0	10	52.4	9	98.0	10	47.8	24
vy		67.3	19	262.1	8	392.0	7	312.0	10	0.0	0	175.3	24
0.0	0	0.0	0	186.2	15	330.7	2	285.4	3	59.4	4	vt	
0.0	0	15.4	6	25.1	12	65.6	6	vt		0.0	0	vt	
0.0	0	0.0	0	0.0	0	vt		vt		vt		72.5	7
vt		222.2	8	250.2	8	204.5	10	214.3	4	21.4	14	155.2	15
45.2	18	148.5	13	79.2	9	102.2	7	80.4	1	12.4	13	53.7	10
vt		72.9	8	57.0	10	82.9	5	31.3	15	vt		41.7	5
122.2	13	433.7	8	939.2	2	941.5	1	697.7	11	117.9	20	658.4	11
0.0	0	27.7	3	40.7	4	29.1	18	0.0	0	18.9	12	0.0	0
vt		112.6	3	50.1	7	100.3	12	104.1	9	vt		57.0	9
vt		55.8	19	45.4	7	85.2	2	48.4	10	60.0	12	58.9	15
38.5	8	124.8	19	53.8	2	63.1	2	25.2	8	0.0	0	vt	
vt		309.0	10	383.9	1	312.6	7	284.0	8	138.0	14	177.6	5
vt		91.7	13	62.6	15	53.8	6	24.6	3	vt		17.6	13
28.0	9	731.6	12	989.2	12	1102.1	3	1358.6	5	592.4	5	1950.9	7
vt		101.2	15	142.9	8	138.5	11	112.0	8	35.8	7	207.9	26
vt		41.0	5	29.3	7	27.2	12	43.5	8	30.8	16	47.6	7
35.0	6	121.9	9	197.8	17	198.8	7	187.0	18	68.6	7	404.9	20
1029.9		*6117.9*		*7325.7*		*7920.1*		*6175.6*		*2969.6*		*6154.0*	
136.5	5	261.0	13	185.5	19	129.4	16	36.0	18	0.0	0	197.0	25
211.2	16	193.0	6	380.2	11	246.8	9	0.0	0	26.0	21	196.6	28
247.1	11	214.8	17	205.3	14	179.6	8	142.9	11	78.1	16	269.8	12
0.0	0	169.0	7	81.8	15	50.9	11	40.7	17	17.3	16	110.1	10
0.0	0	16.2	10	0.0	0	0.0	0	0.0	0	68.8	8	0.0	0
0.0	0	101.7	10	39.1	17	41.7	4	0.0	0	0.0	0	93.8	19
542.9	17	1102.2	5	728.9	2	606.0	13	441.5	14	154.9	21	492.5	17
253.1	12	498.5	16	280.9	11	607.9	8	454.4	12	164.4	14	919.5	5
0.0	0	24.1	9	17.4	16	24.2	10	12.0	11	0.0	0	21.6	12
0.0	0	32.1	14	25.5	12	25.8	15	10.1	8	0.0	0	81.2	11

0.0	0	64.7	5	78.4	9	13.6	7	9.8	6	0.0	0	23.3	9
62.2	17	208.2	13	141.3	6	239.7	16	73.6	4	39.5	15	40.6	9
0.0	0	340.6	11	260.8	8	146.7	8	226.2	47	21.9	16	176.1	24
1453.0		*3226.2*		*2424.9*		*2312.3*		*1447.3*		*571.0*		*2622.1*	
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	26.5	21	0.0	0
0.0	0	0.0	0	25.5	8	22.2	9	25.2	7	12.7	9	85.5	17
0.0	0	14.2	16	21.9	13	30.1	12	28.4	10	34.8	16	87.1	8
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
40.1	11	61.7	2	33.9	8	33.9	8	0.0	0	27.4	15	64.7	4
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
vt		96.0	3	123.6	7	88.3	16	42.2	5	55.3	7	87.9	17
8.0	6	32.9	8	32.9	8	32.9	8	89.8	12	0.0	0	45.1	4
58.3	12	15.8	12	9.3	9	6.3	17	0.0	0	0.0	0	0.0	0
15.8	20	27.3	7	14.1	4	15.1	17	0.0	0	21.5	14	40.5	13
0.0	0	0.0	0	131.2	13	172.4	11	100.7	14	83.4	16	92.5	11
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
vt		100.5	6	123.9	9	232.7	10	118.6	18	58.2	14	68.7	13
vt		199.7	7	116.4	17	86.8	5	63.6	7	64.4	16	112.5	8
122.2		*548.1*		*632.8*		*720.8*		*468.6*		*384.3*		*684.5*	
19999.1		*31091.6*		*34059.0*		*32906.2*		*30119.4*		*18451.7*		*23009.9*	

SB-SM3											
2010								**2011**		**2012**	
28/07/2010		04/08/2010		11/08/2010**		18/08/2010		12/08/2011**		30/08/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2917.3	17	2916.2	5	4516.7	3	4260.7	1	3469.5	10	1344.2	20
1391.7	13	1679.6	12	1743.7	9	1839.4	8	490.4	18	2829.7	22
4118.5	14	6637.9	4	6370.3	5	6303.7	7	4898.3	5	2275.4	13
2728.7	17	995.5	5	995.5	5	192.6	10	497.4	13	2261.8	7
3487.5	8	4688.4	13	3449.9	4	2721.1	5	2994.7	4	1134.0	18
0.0	0	2323.9	6	2615.3	11	2734.9	10	2003.0	12	1212.9	21
1345.9	13	2729.4	9	2813.9	5	2655.7	3	583.0	15	2418.0	2
15989.6		*21970.9*		*22505.3*		*20708.0*		*14936.2*		*13475.9*	
133.8	6	346.2	12	424.3	5	514.6	7	0.0	0	960.4	10
16.6	7	21.7	9	21.8	6	27.6	13	0.0	0	21.8	9
116.1	14	276.4	5	540.9	5	457.6	5	170.7	11	1120.2	7
266.5		*644.3*		*987.0*		*999.7*		*170.7*		*2102.4*	
59.8	16	35.2	5	26.9	12	19.6	7	10.8	8	17.9	11
52.3	3	101.8	2	132.5	18	152.2	6	22.4	18	489.1	12
81.7	5	149.1	6	149.1	6	65.8	7	38.8	4	0.0	0
182.5	6	375.8	7	55.0	10	29.1	16	22.0	19	0.0	0
15.1	19	33.7	7	21.8	14	0.0	0	0.0	0	0.0	0
104.1	4	80.6	7	63.3	12	47.8	5	0.0	0	45.7	19
126.0	17	454.2	8	15.0	7	282.4	14	15.3	19	60.5	2
vt		85.4	6	186.3	6	vt		251.3	7	261.2	9
0.0	0	0.0	0	21.7	14	40.4	19	0.0	0	0.0	0
47.3	5	277.8	14	76.8	13	123.6	19	177.2	6	193.9	18
0.0	0	0.0	0	727.1	3	647.9	3	0.0	0	577.4	8
44.6	14	75.5	18	51.6	6	37.1	19	11.5	15	0.0	0
20.6	3	20.6	3	20.6	3	43.3	17	0.0	0	0.0	0
0.0	0	7.1	8	18.8	6	12.6	19	0.0	0	0.0	0

vr		608.0	8	638.2	8	641.9	3	378.9	18	315.9	24
0.0	0	0.0	0	7.2	7	7.2	7	6.6	16	0.0	0
vt		662.8	12	746.1	14	1726.8	18	754.0	15	332.1	8
vt		49.1	12	39.1	16	27.2	11	18.8	12	62.2	10
0.0	0	vt		16.6	4	0.0	0	0.0	0	0.0	0
vt		57.9	4	30.3	6	62.5	2	0.0	0	0.0	0
28.1	15	30.7	6	28.0	10	25.3	7	30.8	6	0.0	0
0.0	0	31.6	16	27.8	11	4.5	8	0.0	0	0.0	0
27.0	19	115.3	13	85.2	6	55.0	6	113.0	15	53.5	10
0.0	0	136.6	9	341.4	10	256.4	20	0.0	0	206.1	20
14.8	16	186.2	15	124.0	13	314.6	9	117.7	16	0.0	0
0.0	0	12.0	6	26.2	18	0.0	0	0.0	0	0.0	0
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	85.6	6
vt		112.9	7	233.3	6	144.7	15	20.9	16	159.2	9
37.4	17	121.3	7	104.9	18	84.9	9	14.6	14	60.1	10
vt		68.4	10	45.9	4	33.0	5	0.0	0	55.7	4
105.1	12	477.3	5	832.9	3	814.7	9	111.0	30	685.8	7
vt		40.7	4	29.1	18	25.0	15	20.6	12	0.0	0
vt		59.7	12	66.9	8	49.6	5	vt		64.9	7
vt		93.5	3	69.7	7	107.8	3	58.6	31	63.4	5
25.4	8	58.9	18	65.2	16	59.6	6	0.0	0	0.0	0
vt		244.7	15	333.0	11	401.5	16	160.3	19	174.8	17
vt		74.5	17	50.5	17	44.1	4	vt		17.7	27
115.7	14	736.8	11	1120.9	12	1265.1	17	612.9	6	2092.4	6
80.6	1	80.6	1	136.8	11	128.3	5	35.5	9	218.0	23
vt		48.3	15	27.6	17	31.5	6	39.1	15	50.7	13
45.0	7	127.4	8	220.0	11	159.2	13	65.6	4	435.3	14
1213.0		*5932.0*		*7013.2*		*7972.0*		*3108.4*		*6779.1*	

130.3	5	323.8	8	198.8	17	139.1	17	0.0	0	250.7	5
165.7	1	317.8	16	329.9	5	63.7	3	30.6	16	268.3	10
519.0	4	383.5	7	264.0	11	306.5	6	50.1	13	283.2	10
vt		198.8	4	109.8	15	101.8	11	15.6	24	184.1	3
0.0	0	18.3	12	0.0	0	0.0	0	52.8	14	0.0	0
vt		84.3	12	31.0	15	44.7	13	0.0	0	219.1	27
262.0	15	756.4	3	810.0	8	758.1	14	178.4	13	594.0	12
373.3	16	444.9	13	349.0	19	594.3	15	179.1	15	884.4	11
0.0	0	22.7	3	19.4	9	30.5	4	0.0	0	20.6	13
0.0	0	46.2	6	16.3	12	23.8	2	0.0	0	104.0	12

vt		131.9	3	18.8	14	25.3	5	0.0	0	37.1	17
68.8	12	170.6	12	59.1	15	95.0	7	33.7	22	49.3	6
0.0	0	324.1	5	150.3	29	159.7	5	36.0	13	305.0	14
1519.2		*3223.2*		*2356.5*		*2342.7*		*576.5*		*3199.9*	
0.0	0	0.0	0	0.0	0	0.0	0	25.0	9	0.0	0
vt		30.5	7	23.3	5	24.5	15	13.9	13	88.2	7
31.4	18	62.1	12	82.5	13	80.3	13	76.3	18	116.5	25
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
36.9	11	85.0	3	29.1	17	32.1	6	20.1	13	53.8	19
0.0	0	0.0	0	vt		vt		0.0	0	vt	
vt		93.4	3	134.0	13	71.5	8	66.3	16	92.2	5
8.8	13	44.3	4	28.3	10	28.0	12	0.0	0	38.2	7
62.6	8	12.0	3	16.2	10	14.9	28	0.0	0	0.0	0
12.8	12	16.1	3	14.2	20	12.8	17	17.4	13	29.7	6
vt		vt		127.5	9	117.0	19	88.3	16	96.2	13
0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
vt		94.9	4	105.0	9	147.7	18	64.2	13	89.4	13
vt		160.9	4	107.6	8	133.0	10	70.5	24	129.4	11
152.5		*599.1*		*667.6*		*661.9*		*442.0*		*733.7*	
19140.8		*32369.6*		*33529.7*		*32684.3*		*19233.8*		*26291.0*	

Table S11

Volatile components determined for *Vitis vinifera* L. cv. Baga variety obtained from 3 parcels, at Bairrada Appellation, from 2010, during maturation, and for 2011 and 2012 harvests, only maturity was considered.

		BA-VA1													
		2010												2011	
		20/08/2010		27/08/2010		03/09/2010		10/09/2010		17/09/2010**		24/09/2010		03/10/2011**	
[1]Dtr(s),[2]Dtr(	Compound	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C_6 compounds															
194, 0.640	Hexanal	1121.7	16	2180.7	15	4770.4	6	3867.6	13	3573.6	10	3960	8	2765.9	19
206, 0.630	3-Hexenal	273.2	18	667.9	10	690.1	6	458.9	11	335.2	8	671.3	5	467.3	18
230, 0.651	2-Hexenal	2218.4	11	4569.9	14	5039.6	12	3213.4	14	3466.1	15	5367.2	7	2313.1	9
242, 0.630	3-Hexen-1-ol	872.4	4	954.3	4	966.2	10	449	10	504.4	16	866.7	5	459.4	14
248, 1.076	2-Hexen-1-ol	798.3	14	3041.5	8	2371	11	2324.7	9	2924.6	9	1719.5	14	2965	17
266, 0.903	1-Hexanol	994.1	12	2542.9	13	2116.4	7	2727.2	13	2547.7	19	2276.7	11	2984.2	9
296, 0.930	2,4-Hexadienal	233.5	5	773.4	11	844.5	17	316	8	327.7	20	639.9	8	256.9	19
	Sub-total	*6511.6*		*14730.6*		*16798.2*		*13356.8*		*13679.3*		*15501.3*		*12211.8*	
Aromatic alcohols															
420, 3.014	Benzyl Alcohol	170	14	250.8	8	417.3	5	709	9	604.1	4	595.7	3	507.9	8
446, 1.426	α,α-Dimethyl Benz	0	0	0	0	0	0	0	0	vt		36.6	6	0	0
470, 1.960	2-Phenylethanol	95.6	4	257	20	282.8	10	327.5	15	432.5	16	425.8	8	378.1	1
	Sub-total	*265.6*		*507.8*		*700.1*		*1036.5*		*1036.6*		*1058.1*		*886*	
C_9 Norisoprenoid															
506, 0.761	Norinone	16.8	21	44.9	5	47.4	12	52.1	4	52.0	7	49.2	11	59.7	13
Monoterpenic compounds															
314, 0.440	α-Pinene	26.9	12	16.9	6	15.5	10	15.5	10	19.5	6	35.1	5	7	2
344, 0.457	β-Pinene	32.1	15	31.3	19	38.5	10	20.4	14	20.3	19	18.3	6	0	0
392, 0.405	*m* -Cymene	19.9	9	39.7	8	48	8	31.3	5	32.5	14	17.3	19	18.4	8
398, 0.476	Limonene	0	0	31.7	18	35.9	20	23.5	10	93.2	8	43.8	8	15.8	14
404, 0.476	1,8-Cineole	0	0	0	0	0	0	0	0	vt		61.0	14	85.1	15
416, 0.560	β-Ocimene	312.7	11	208.2	11	157.8	20	210.1	3	192.7	2	226.8	6	0	0
428, 0.678	Linalool oxide (iso	vt		84.7	13	96.3	5	107.8	4	145.1	9	40.2	18	vt	
434, 0.727	Dihydromyrcenol	24.0	19	102.7	10	72.4	12	64.2	5	24.9	16	27.9	13	33.6	7
440, 0.560	α-Terpinolene	0	0	426.9	5	400.5	6	433.5	15	417.8	10	459.6	12	0	0
446, 0.700	Dihydrolinalool	19.8	9	18.8	9	18.7	3	14.6	7	0	0	0	0	0	0
452, 0.746	Linalool	0	0	19.1	11	27.1	11	35	2	21.5	6	58.6	16	63.5	10

464, 0.600	Rose oxide (isomer	151.6	8	171.7	9	156.8	9	160.8	2	159	5	116.2	5	0	0
464, 0.844	Fenchol	114.8	7	83.2	13	59.3	14	52.6	4	59.3	7	57.1	5	69.8	5
470, 0.646	Hotrienol	0	0	13.1	9	21.9	20	23.8	8	23.8	8	14.4	10	26.8	16
470, 0.780	Camphenal	vt		54.8	7	61.4	7	42.2	4	46.8	7	59.7	9	19.5	20
482, 0.890	1-Terpineol	vt		27.4	12	58.1	4	28	20	22.6	3	34.5	13	0	0
512, 0.635	Nerol oxide	0	0	0	0	0	0	vt		16.3	6	34.8	5	0	0
524, 0.860	Borneol	vt		133.6	3	223.9	19	203.3	14	169	5	143.8	14	156.7	19
530, 0.984	Menthol*	0	0	0	0	0	0	0	0	vt		23.8	19	38.9	9
536, 0.715	Terpinen-4-ol	vt		152.9	8	217.6	15	175.4	12	111	12	153.3	7	29.6	19
536, 1.269	Cymen-8-ol	78.4	18	27.1	18	29.9	16	25	4	25	4	14	10	14.4	11
542, 0.835	α-Terpineol*	0	0	20.1	13	31.4	3	26.1	16	20.9	7	24.5	12	258.4	18
548, 0.850	Dihydrocarvone	vt		280.3	4	251.3	13	236.7	11	178.5	5	220	16	0	0
560, 0.850	Verbenone	0	0	0	0	0	0	0	0	vt		vt		38.9	5
566, 0.703	Menth-1-en-9-al	vt		53.9	11	60	13	51.9	15	48.2	4	45.6	17	0	0
584, 0.873	Geraniol (isomer 1	0	0	0	0	0	0	vt		53.5	12	110.4	5	227.3	13
584, 0.943	β-Citronellol*	vt		83.5	13	161.6	11	162.1	4	192.4	18	139.8	18	164.3	21
590, 0.737	Geraniol (isomer 2	0	0	0	0	0	0	0	0	0	0	0	0	372.1	14
596, 0.976	Citral (isomer 1)	23.7	13	145.6	17	164.1	4	171.9	7	151.2	6	114.1	12	26.7	17
602, 0.815	Carvone*	68.3	18	284.5	10	254.9	3	269	5	289.7	13	246.3	15	0	0
626, 0.775	Citral (isomer 2)	vt		36.2	16	46.9	14	42.1	10	27.3	15	26.9	9	0	0
	Sub-total	*872.2*		*2547.9*		*2709.8*		*2626.8*		*2562*		*2567.8*		*1666.8*	
C$_{13}$ Norisoprenoids															
566, 0.532	*m/z* 159, 91, 131	0	0	14.4	17	15.4	10	11.9	8	10.9	10	10.9	10	0	0
620, 0.595	Vitispirane	292.2	6	58.7	2	27.9	7	27.2	10	42.2	4	34.6	12	0	0
632, 0.517	Theaspirane (isome	127.6	14	180.9	19	120.6	20	82.1	3	76.8	1	100.8	9	11.9	9
644, 0.528	Theaspirane (isome	vt		134.5	19	81.6	19	68.7	5	42.9	11	55.2	10	8.0	2
674, 0.681	β-Damascenone (i	vt		49.8	6	45.1	13	34.5	12	17.2	8	26.6	12	0	0
680, 0.840	*m/z* 142, 157, 115	59.5	4	70.2	5	122.9	13	188.4	6	173.9	2	144.2	19	0	0
700, 0.702	β-Damascenone (i	74.4	6	538.3	9	571.7	10	425.9	7	327.5	5	343.1	6	186.6	24
736, 0.648	Geranylacetone *	270.5	18	390.4	8	428.1	3	352.4	8	308.2	9	245.2	18	192.8	7
742, 0.850	5,6-Epoxy-β-ionon	0	0	0	0	13.2	23	23.2	13	13.2	23	19.8	13	69.4	15
778, 0.635	α-Iso-methyl iono	0	0	vt		31.0	16	13.5	14	14	10	21.8	8	14	8
784, 0.717	β-Ionone *	vt		113.3	13	154.6	9	87.3	6	32.7	5	28.1	11	16.8	14
900, 0.894	Methyl dihydrojasr	0	0	27.9	12	27.1	9	20.5	10	19.3	18	88.1	47	97.8	13
	Sub-total	*824.2*		*1578.4*		*1639.2*		*1335.6*		*1078.8*		*1118.4*		*597.3*	
Sesquiterpenic compounds															
650, 0.583	δ-Elemene	0	0	0	0	17.7	1	22	6	23.8	8	25.4	6	45.1	6
686, 0.510	β-Bourbonene	0	0	vt		56.1	13	59.4	3	56.1	13	55.8	6	14.1	17
718, 0.481	β-Caryophyllene	0	0	0	0	0	0	vt		10.3	18	11.3	0	46.4	10

756, 0.630	Aromadendrene	vt		59.1	12	64.3	5	60.7	6	62.2	1	62.8	6	vt	
796, 0.525	γ-Cadinene	0	0	0	0	0	0	0	0	vt		vt		0	0
826, 0.629	α-Calacorene	41.7	4	41.7	4	33.4	6	51.5	5	59.1	5	70.6	2	0	0
832, 0.880	Nerolidol	vt		60.7	7	124.1	13	75.2	14	79.2	9	84.1	8	0	0
886, 0.690	β-Eudesmol	vt		190.1	13	223.2	5	199	7	173.8	13	199.7	1	12.1	15
	Sub-total	*41.7*		*351.6*		*518.8*		*467.8*		*464.5*		*509.7*		*117.7*	
Diterpenic compound															
1116, 0.929	Phytol	0	0	vt		45.1	7	58.8	2	57.0	10	60.2	2	82.4	9
	Total	8532.1		19761.2		22458.6		18934.4		18930.2		20864.7		15621.7	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

vt - vestigial

GC×GC peak area x 10^4 (arbitrary units)															
		BA-VA2													
2012		**2010**													
05/08/2012**		20/08/2010		27/08/2010		03/09/2010		10/09/2010		17/09/2010		24/09/2010		01/10/2010**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2634.7	14	1885.1	10	2920.6	10	4377	8	4302.8	8	4791.8	9	3741.4	10	4770.2	3
408.2	5	259.1	18	511.5	12	817.1	2	746	9	503	5	507.3	14	674.1	6
2764.6	14	1637.9	12	4925.4	2	4528.9	9	4124.4	7	5030.3	4	6234.9	20	3529.6	14
453.8	12	767.4	3	727.1	8	948.4	12	869.3	6	802.5	5	556.2	9	750	5
1746.1	10	794.9	18	3336.7	12	3600.9	14	3253.2	10	3498.1	17	2853.2	14	2658	13
1860.6	16	1017.6	7	2383.2	8	2182	14	516.7	12	2461.8	3	1653.5	5	3955.4	15
235.5	6	235.9	13	585.8	4	674.3	16	657.3	19	477.2	4	540.8	3	481.2	6
10103.5		*6597.9*		*15390.3*		*17128.6*		*14469.7*		*17564.7*		*16087.3*		*16818.5*	
548.4	11	168.4	2	209.1	4	671.6	12	707.8	12	621.4	6	640.2	3	600	6
vt		0	0	0	0	0	0	vt		vt		33.0	5	31.8	13
419.7	23	110.9	12	298.1	5	297	7	324	16	518.8	10	430.9	3	500.5	9
968.1		*279.3*		*507.2*		*968.6*		*1031.8*		*1140.2*		*1104.1*		*1132.3*	
50.7	3	12.0	12	58.8	7	54.5	5	60.1	3	55.8	6	58	6	50.8	10
22.5	9	32.9	19	10.7	14	19.3	5	18.9	12	11.6	13	28.8	17	7.6	7
41.3	14	22.9	4	19.9	8	27.1	11	23.9	13	24	7	16.7	7	18.4	16
12.7	27	18.2	6	38.6	5	37.1	7	37.5	8	37.5	12	17.5	10	40.2	17
10.6	15	0	0	26.2	4	38.8	6	35.4	10	90.3	10	49.6	3	vt	
133.2	19	0	0	0	0	0	0	0	0	0	0	0	0	vt	
0	0	376.2	15	141.4	16	167.8	20	148.9	29	86.8	3	254	13	231.7	7
48.8	6	vt		76.3	4	86.2	7	129	18	18.2	4	0	0	0	0
0	0	21.7	10	99	17	83.9	12	77.8	4	29.3	14	40.5	3	29.9	2
0	0	vt		463.5	6	469.3	11	447.1	5	631.4	4	430.3	4	430.1	7
0	0	0	0	17.2	9	17.2	9	17.2	9	0	0	0	0	0	0
180.2	29	0	0	25.7	12	40.3	6	38	10	25.1	8	63.7	3	33	13

0	0	79.0	10	144.1	14	161.9	13	162.9	16	109.7	6	127.7	9	101.6	1
56.7	6	78.3	3	40.5	3	39.6	17	48.7	4	95.6	12	92.3	6	89	3
78.3	22	0	0	13.4	9	13.4	22	23.4	12	15.6	16	14.3	11	13.9	10
0	0	vt		60.9	10	43.6	18	41.9	3	40.1	4	57.7	2	52.5	5
0	0	vt		47.0	15	28.4	3	24.8	17	34.5	6	38.4	15	38.5	16
0	0	0	0	0	0	0	0	vt		23.7	12	17.4	5	18	15
83.7	25	vt		209.9	11	197	37	201.1	7	226.5	13	167.4	15	134.1	11
78.5	11	0	0	0	0	0	0	vt		vt		36.8	15	13.1	19
67.8	13	vt		139.8	19	175.4	19	174.1	20	120.5	16	178.7	11	167.6	15
16.6	27	32.8	10	24.1	17	20.3	10	20.3	10	20.3	10	19.4	14	87.6	14
257.7	13	0	0	25.2	7	25.7	10	33.5	14	31.4	2	25.4	9	25.8	13
0	0	vt		302.6	3	281.2	12	247.5	9	177	10	208.7	12	239.1	7
35.1	18	0	0	0	0	0	0	0	0	0	0	vt		vt	
0	0	vt		54.9	14	50	18	40.9	12	44.4	5	40.6	4	43	6
195.5	17	0	0	0	0	0	0	0	0	0	0	vt		64.6	17
137.3	28	vt		135.6	20	153.1	22	172.2	3	288.9	9	138.7	12	132.7	11
231.4	6	0	0	0	0	0	0	0	0	0	0	0	0	vt	
33.4	8	34.2	11	127.4	6	162.5	18	154.9	5	175	17	124	26	236.2	20
0	0	136.6	14	288.5	23	351.5	7	317.9	11	401.3	11	328.6	9	302	6
27.7	12	vt		56.3	6	48.5	8	36.6	15	25.9	9	37.5	14	50.9	6
1749		*832.8*		*2588.7*		*2739.1*		*2674.4*		*2784.6*		*2554.7*		*2601.1*	
22.4	25	0	0	14.9	8	25.1	13	18.8	14	29.5	9	18.2	7	20.9	8
51.5	9	84	19	64.4	16	75.4	3	79	8	58.1	6	16.2	16	0	0
16.9	29	279.7	19	194.7	12	210.3	7	86.7	3	87	6	277.4	12	160.9	5
9.1	17	vt		161.8	14	173.2	15	89.1	4	67.1	11	13.1	4	23.3	7
29.1	31	vt		38.4	9	30.4	5	20	15	30.7	13	21.7	11	31.5	13
0	0	60.5	12	93.3	5	95.3	8	102.3	17	98.6	8	86	11	94.3	27
711.7	8	89.3	8	445.7	2	514.6	2	525.3	16	440.4	1	424.9	6	418.7	3
291.5	9	263.6	11	434.2	8	411	4	297.4	11	233.6	15	279.4	18	234.9	9
vt		0	0	0	0	6.3	4	13.9	17	13.9	17	11.5	8	0	0
27.5	11	0	0	21.7	18	21.1	22	21.1	22	22.3	5	17.1	18	19.9	15
33.5	24	vt		132.3	15	163.6	1	103.8	8	32.2	15	23.5	7	37.2	9
105.8	19	vt		19.2	14	19.9	21	18.3	19	32.5	18	21.5	3	16.2	7
1299		*777.1*		*1620.6*		*1746.2*		*1375.7*		*1145.9*		*1210.5*		*1057.8*	
77.7	12	0	0	0	0	21.6	4	26.6	3	30.5	5	30	6	20.8	5
135.2	16	vt		vt		62.2	3	63.8	4	65.8	3	68.5	5	15.4	20
46.5	5	0	0	0	0	0	0	0	0	0	0	vt		vt	

41.2	3	vt		36.9	10	62.0	2	58	16	58.5	4	159.1	11	59.8	2
26.9	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25.2	15	40.9	2	40.9	2	50.4	1	58.1	6	62.9	5	65.7	3	vt	
vt		vt		116.7	18	117.2	8	111.2	16	137.5	12	82	4	134.8	8
15.3	10	vt		209.7	3	225.5	14	221.3	19	244.6	4	124.2	15	231.4	16
368.0		*40.9*		*404.2*		*538.9*		*539.0*		*599.8*		*529.5*		*462.2*	
80.9	10	0	0	vt		54.1	5	60.6	7	63.6	8	73.4	9	65.7	6
14619.2		8540		20569.8		23230		20211.3		23354.6		21617.5		22188.4	

														BA-SM1	
		2011		**2012**		**2010**									
08/10/2010		03/10/2011**		21/09/2012**		20/08/2010		27/08/2010		03/09/2010		10/09/2010		17/09/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3699.8	15	2395.1	18	1236.1	15	1401.5	8	3345.9	8	3799.8	10	4254.8	7	4618.4	8
529.4	7	460.2	16	574.6	8	154.9	17	867	4	714.5	9	746	2	570	8
4003.5	13	3642.5	6	2281.1	9	1798.1	19	5180.5	2	5763.2	8	3875.4	11	4452.5	1
478.7	1	418.1	19	316.5	19	682.1	1	1027	6	1002.4	20	495.5	13	771.6	4
1889.3	7	3285.6	9	1093.7	21	399.9	18	3444.7	13	2921.9	12	3145.7	16	2369.4	16
2989.4	8	3442.8	10	1063.9	10	1020.7	7	2424.5	19	1742	5	1333.4	20	4062.3	6
404.1	18	269.8	13	235.4	6	57.3	8	725.3	1	1114.5	20	688.2	13	367.1	17
13994.2		*13914.1*		*6801.3*		*5514.5*		*17014.9*		*17058.3*		*14539*		*17211.3*	
569.2	10	658.5	8	620.3	10	170.5	15	193.2	4	473.5	8	556.5	5	556.8	5
35.1	21	0	0	vt		0	0	0	0	0	0	0	0	0	0
482.6	6	252	13	645.6	7	90.1	2	283.1	15	209.7	7	348.4	11	454.6	5
1086.9		*910.5*		*1265.9*		*260.6*		*476.3*		*683.2*		*904.9*		*1011.4*	
48.7	11	41.2	5	86.2	13	9.8	1	48.9	7	48.9	4	53.1	3	48.7	9
30.4	19	8.7	6	23.4	14	23.7	8	16.3	11	11.2	20	11.2	20	9.1	9
43.7	11	0	0	42.7	14	27.8	17	41.9	11	22.4	16	18.6	18	36.9	11
17	19	12.8	13	13.4	10	17.9	3	48.6	10	42.7	15	33.2	19	44	17
0	0	30.2	12	17.8	26	0	0	31.8	17	33.2	2	27.7	14	44.4	8
vt		113.5	10	155.5	19	0	0	0	0	0	0	0	0	0	0
272.8	11	vt		vt		206.2	17	95.5	7	173.9	14	212.3	5	103.1	3
0	0	0	0	54.4	21	0	0	51.6	5	56.7	15	106.9	4	112.1	11
25.8	13	47.6	7	vt		23.2	18	78.9	10	62.3	12	71.6	8	27	19
530.4	9	vt		vt		vt		437.6	6	216.4	1	136.5	19	549.3	19
0	0	0	0	0	0	12	13	11.6	10	11.6	10	15.5	29	0	0
36.9	45	104.2	8	461.4	14	0	0	24.9	11	27.2	5	29.4	15	40.5	19

143.5	66	0	0	vt		82.2	9	113.7	19	126.4	13	123	12	81.2	10
0	0	41.4	9	57.3	5	109.8	16	43.1	11	43.9	15	43.9	15	43.9	15
0	0	32.8	15	88.4	15	vt		18.1	6	11.9	11	15.5	12	20.5	14
54.5	41	19.1	12	vt		vt		61.2	5	38.4	10	39.6	19	60.3	1
32.5	18	0	0	vt		vt		45.0	3	28.5	15	31.9	4	51.2	15
7.8	6	0	0	0	0	0	0	0	0	0	0	0	0	11.8	12
192.6	19	171.9	17	106.6	7	vt		125.5	20	137.6	28	154.1	14	157	12
18.4	6	55.7	10	72.9	7	0	0	0	0	0	0	0	0	0	0
116.3	15	26.4	10	82.3	24	vt		137.6	23	134.6	21	173.3	4	120.4	5
25.3	18	26	19	33.9	9	34	10	27.2	9	22.4	14	22.4	7	24.6	10
32	12	308.8	11	284.3	8	0	0	25.6	16	24.5	4	27	13	26.1	3
219.8	4	vt		vt		vt		256.0	13	257.8	19	252.1	13	89.1	3
vt		34.5	17	33.3	11	0	0	0	0	0	0	0	0	0	0
58.5	15	vt		vt		0	0	54.1	9	40.8	13	40.4	2	29.4	3
116.4	12	203.1	11	266.5	3	0	0	0	0	0	0	0	0	0	0
108.8	19	159	20	167.6	25	vt		156.7	16	172.3	2	133.9	12	162.9	20
vt		308.9	14	231	10	0	0	0	0	0	0	0	0	0	0
51.1	16	17	21	34.3	10	35.6	13	195.4	17	176.8	13	145.2	15	127.5	14
164.1	18	vt		vt		67.4	8	257.6	16	183.9	8	206.6	13	259.9	13
46.3	5	vt		37.2	16	vt		41.5	17	31.7	17	25.6	8	46.6	5
2344.9		*1721.6*		*2264.2*		*639.8*		*2397*		*2089.1*		*2097.4*		*2278.8*	
30.9	10	0	0	25.5	3	0	0	27.1	10	26.2	15	13.6	6	26.3	6
0	0	0	0	57.5	9	130.6	19	32.1	13	41.6	19	43.6	7	35.9	5
58.3	12	16	8	19.4	22	347.5	16	134.8	20	186.8	4	65.1	3	57.1	3
29.6	9	9	4	11.2	6	vt		102.6	12	82	9	42.9	10	28.5	19
30.2	5	9.6	11	32.7	13	0	0	36.3	12	41.9	6	22.3	12	25.6	8
63.2	18	0	0	0	0	58.8	15	75.4	8	66.8	7	192.2	18	185.8	8
527.1	10	263.1	27	850.7	14	47.6	13	581.7	12	568.4	15	440.9	9	387.4	8
240.3	12	245.5	23	359.1	9	276.2	6	349.1	8	411.8	7	218.6	3	178.6	14
0	0	76.5	5	0	0	0	0	6.3	4	9.6	18	6.2	15	5.7	8
18.1	15	10	1	42	5	0	0	0	0	0	0	17.4	12	26.3	5
30.1	17	12.1	13	53.1	19	vt		107.4	14	98.9	12	87.4	3	43.2	14
21.6	18	61.9	20	280.6	18	0	0	14.2	16	15.8	18	12.9	26	18.4	20
1049.4		*703.7*		*1731.8*		*860.7*		*1467*		*1549.8*		*1163.1*		*1018.8*	
0	0	47.2	9	87.1	10	0	0	0	0	22.7	15	24.8	15	24.9	8
0	0	25.2	31	136	22	0	0	vt		52.7	15	82.4	9	55.3	5
vt		42.1	7	61.5	8	0	0	0	0	0	0	0	0	0	0

184.1	9	9.1	16	61.8	6	vt		68.2	10	64.9	6	64.9	6	61.3	14
0	0	0	0	19	13	0	0	0	0	0	0	0	0	0	0
0	0	7.5	3	21.6	4	61.9	13	18.8	6	17.2	20	14.2	12	14.8	18
136.4	10	0	0	0	0	vt		88.8	13	89.3	3	111.6	18	110.1	6
186.3	17	13.2	20	14.9	13	vt		179.6	13	184.7	8	202.8	10	198.5	7
506.8		*144.3*		*401.9*		*61.9*		*355.4*		*431.5*		*500.7*		*464.9*	
62.4	13	94.9	14	105.5	16	0	0	vt		50.7	8	61.3	12	73.6	11
19093.3		17530.3		12656.8		7347.3		21759.5		21911.5		19319.5		22107.5	

						2011		**2012**	
24/09/2010		01/10/2010**		08/10/2010		03/10/2011**		21/09/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4121.4	10	4843.7	11	3597.4	4	2939.4	15	1413.9	26
488.9	9	522.9	15	513.9	9	602.6	10	552.9	21
4590	17	3467.6	15	4851	10	4297.6	10	1878.8	9
744.8	15	518.4	12	330	15	429.4	12	436.7	18
3192.6	16	2240.7	19	1958	16	1735.6	8	2556.4	5
2124.3	17	1747.4	11	1641.7	23	3129.2	20	15195.8	2
516.6	10	430	19	524.4	20	383.2	8	414	29
15778.6		*13770.7*		*13416.4*		*13517*		*22448.5*	
575	5	606.4	4	584.6	4	451.9	8	436.8	19
29.7	3	38.4	2	29.9	9	vt		vt	
379.1	5	414.4	9	420.8	4	225.3	8	373.6	15
983.8		*1059.2*		*1035.3*		*677.2*		*810.4*	
47.7	13	46.8	5	44.4	14	49.1	9	45.4	9
30.5	12	0	0	0	0	7.6	6	7.7	6
21.5	15	19.7	10	19.8	15	0	0	37.7	18
19	7	37.2	11	19.6	8	14.3	22	12.5	22
50.8	2	0	0	0	0	17.3	22	9.5	11
vt		vt		vt		113.4	15	102.3	12
195.9	3	195.8	6	218.7	13	vt		vt	
0	0	vt		vt		0	0	49.8	18
20.4	13	0	0	0	0	43.3	11	0	0
416.3	3	413.1	4	404.9	5	0	0	0	0
0	0	0	0	0	0	0	0	0	0
53	5	36	18	41.7	11	101	8	183.2	9

109.6	15	109	18	97.8	15	vt		vt	
59	13	94	2	0	0	54.6	23	38.6	15
11.7	16	19.4	10	0	0	42.8	18	73.6	16
45.9	15	56.8	6	55.4	7	39.4	19	vt	
31.9	3	29.3	17	39.0	14	vt		vt	
14.5	7	0	0	0	0	0	0	0	0
165.7	7	113.5	18	99.0	7	162.8	16	78.7	8
15.8	14	0	0	0	0	37.2	16	59.4	4
145.9	5	130.9	9	126.2	67	25.7	13	45.6	22
26.2	12	23.9	11	34.2	1	33	11	31.6	17
29.5	5	25.9	15	32.2	5	189.6	3	208.3	35
218	4	226.9	19	193.9	7	0	0	vt	
0	0	vt		vt		59	14	30.8	12
32.2	9	51.3	17	39.5	2	0	0	0	0
0	0	32.1	8	74.4	16	98.8	11	195.8	26
137.6	13	131.4	19	116.2	6	77.4	17	46.2	26
0	0	0	0	0	0	164.3	11	138.5	23
83.6	16	110.8	13	86.2	12	20.5	14	24.1	17
290.2	9	241.8	19	219.4	6	0	0	vt	
25.8	7	26.2	10	63.3	9	vt		30.6	7
2250.5		*2125*		*1981.4*		*1302*		*1404.5*	
16.2	9	17.3	17	0	0	0	0	20.5	25
30.9	15	0	0	0	0	0	0	29.0	25
49.2	15	45.5	7	30.3	4	19.6	20	14.3	29
23.4	9	12.5	7	10.8	12	19	20	8.1	5
16.3	14	23.2	11	17.9	9	8.2	8	25.2	15
162	8	148.2	7	117.4	4	0	0	vt	
238.1	19	186.4	17	193.1	14	174.9	14	576	15
352.6	8	279.8	7	299.4	18	142.7	6	313.6	28
0	0	0	0	0	0	63.9	8	vt	
14.6	4	19.8	20	22.5	11	0	0	24.3	19
29.8	8	52	17	31.7	17	23.5	14	18.3	5
77	8	25.9	19	26.6	18	54.4	10	156	24
1010.1		*810.6*		*749.7*		*506.2*		*1185.3*	
23.3	6	0	0	0	0	65.1	2	51.2	11
19.6	6	26.7	17	0	0	15.5	8	49.4	12
0	0	0	0	0	0	58.1	8	36.9	15

62.1	1	49.3	19	42.3	8	7.8	5	31.8	9
0	0	vt		vt		0	0	13.1	25
14.8	17	14.7	5	0	0	6.1	5	17.9	18
122.3	9	114.3	6	133.8	11	0	0	0	0
188.1	17	214.7	6	216	5	13.3	12	12.6	19
430.2		*419.7*		*392.1*		*165.9*		*212.9*	
62.6	3	68.6	8	59.9	7	79.5	8	62.6	5
20563.5		18300.6		17679.2		16296.9		26169.6	

Table S12

Volatile components determined for *Vitis vinifera* L. cv. Castelão variety obtained from 3 parcels and 3 years of harvests, during maturation, at Bairrada Appellation.

		2010											
		20/08/2010		27/08/2010		03/09/2010		10/09/2010		17/09/2010**		24/09/2010	
^{1}Dtr(s),^{2}Dtr	**Compound**	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C_6 compounds													
194, 0.640	Hexanal	1791	9	3773.4	21	3715.3	10	3701.1	4	3968.6	10	3607.7	10
206, 0.630	3-Hexenal	1154.9	2	625.5	12	684.5	6	784	18	746.5	18	538.2	12
230, 0.651	2-Hexenal	1510.8	26	5894.6	3	5641.8	10	5068.9	4	3817.6	11	5819.4	13
242, 0.630	3-Hexen-1-ol	846.6	10	717.6	6	850.6	8	774	7	831.4	15	475.4	6
248, 1.076	2-Hexen-1-ol	960.2	6	2897	20	2085.1	13	2524.6	4	3067.9	10	2466.1	9
266, 0.903	1-Hexanol	811.9	6	857.3	4	1913.6	26	2812.8	4	2009.8	14	3120.8	20
296, 0.930	2,4-Hexadienal	72.3	13	912.6	5	778.5	8	978.8	17	871.5	4	707.9	22
	Sub-total	*7147.7*		*15678*		*15669.4*		*16644.2*		*15313.3*		*16735.5*	
Aromatic alcohols													
420, 3.014	Benzyl Alcohol	114.4	6	244.5	15	430.8	7	377.2	11	397.9	20	578.1	10
446, 1.426	α,α-Dimethyl Benzyl alc	vt		31.2	11	40.9	8	26.9	18	30.3	14	32	18
470, 1.960	2-Phenylethanol	80.9	3	106.4	9	112	11	230.4	15	310.3	9	45.2	5
	Sub-total	*195.3*		*382.1*		*583.7*		*634.5*		*738.5*		*655.3*	
C_9 Norisoprenoid													
506, 0.761	Norinone	37.4	8	39.6	3	35.5	7	39.9	8	39.8	7	37.2	9
Monoterpenic compounds													
314, 0.440	α-Pinene	34.6	8	35.6	19	24.3	18	22.3	9	13.9	11	15.5	20
338, 0.480	Dehydroxylinalooloxide	22.1	21	50.7	13	85.4	2	vt		vt		0	0
344, 0.457	β-Pinene *	31.4	13	38.2	12	31	8	33.2	9	69.1	4	30.2	12
392, 0.405	*m* -Cymene	0	0	35.1	1	30.5	18	37.1	15	24.8	16	29.8	11
398, 0.476	Limonene *	9.7	9	49.4	9	90.8	5	97.3	2	159	2	175.5	3
404, 0.476	1,8-Cineole	262.8	6	233.4	7	188.7	8	109.2	7	118.4	9	124.5	7
428, 0.678	Linalool oxide (isomer 1)	52.6	5	109.3	12	42.8	16	48.8	19	71.8	16	52.6	22
434, 0.727	Dihydromyrcenol	vt		71.5	2	77.6	6	49.5	7	56.1	16	64.2	8
440, 0.560	α-Terpinolene	27.7	7	18.8	12	20.9	6	22	12	22.1	17	12.4	13
440, 0.790	Linalool oxide (isomer 2)	61.6	2	46.1	6	47.8	9	33.6	8	56.6	2	vt	

446, 0.700	Dihydrolinalool	0	0	26.6	4	37.9	7	63	7	55.3	16	33.5	2
452, 0.746	Linalool *	78.7	10	225.5	17	305.8	19	249.2	1	477.8	11	434.6	17
464, 0.600	Rose oxide (isomer)	0	0	0	0	12.4	13	19.2	15	37	15	15.2	15
464, 0.844	Fenchol	0	0	34.4	15	47	33	92.6	12	68.5	4	38.1	10
470, 0.646	Hotrienol	989.7	8	1061.8	16	788.0	11	766.4	7	609.1	8	546.1	11
470, 0.780	Camphenal	vt		48.2	5	43.1	5	69.8	2	51.2	15	46.1	13
494, 1.050	Pinocarveol	0	0	0	0	0	0	41.1	10	42.8	6	vt	
506, 1.190	Pinocarvone	0	0	0	0	0	0	26.5	16	35.8	5	vt	
512, 0.635	Nerol oxide	157.3	22	154.0	15	87.0	2	71.3	10	137.6	20	56.4	15
518, 0.834	Ocimenol	0	0	181.1	7	186.5	13	258.6	13	266.7	19	252.5	11
518, 1.200	*m/z* 68, 94, 79, 109 (alco	48.2	20	58.5	5	60.5	1	87.6	4	128	17	68.0	4
524, 0.860	Borneol	0	0	0	0	0	0	0	0	0	0	0	0
530, 0.884	Mentha-1,5-dien-8-ol	0	0	0	0	vt		32.4	16	26.9	7	vt	
530, 0.984	Menthol *	12.4	13	306.7	13	400.9	9	413.6	4	293.9	6	304.1	8
536, 0.715	Terpinen-4-ol	32.1	17	52.4	13	46.9	3	31.2	15	39.9	13	25.9	14
536, 1.269	Cymen-8-ol	0	0	30.2	9	42.2	5	52.4	4	53.5	6	54.3	8
542, 0.835	α-Terpineol *	392.4	14	608.1	6	656.0	9	506.6	5	329.1	5	502.6	16
560, 0.850	Verbenone	vt		54.9	4	137.0	8	118.8	18	104.0	7	109.8	17
566, 0.703	Menth-1-en-9-al	198.3	14	171.5	16	197.4	7	160.7	16	194.4	7	193.6	11
572, 1.340	2-Hydroxycineole	0	0	0	0	0	0	0	0	0	0	0	0
578, 0.700	*m/z* 93, 121, 119, 136 (al	23.1	20	23	11	17.6	8	18	12	24.7	9	17	14
584, 0.873	Geraniol (isomer 1) *	21.4	18	95.6	10	167.0	10	308.6	13	425.6	7	339.1	15
584, 0.943	β-Citronellol *	vt		102.1	16	105.8	18	131.8	14	436.0	3	382.9	2
590, 0.737	Geraniol (isomer 2) *	vt		232.6	5	362.5	15	568.7	19	786.6	4	613.0	10
596, 0.976	Citral (isomer 1)	0	0	0	0	0	0	204.9	2	72.2	3	86.2	9
602, 0.815	Carvone *	0	0	39.3	5	59.7	14	37.6	16	38.1	17	40.6	15
626, 0.775	Citral (isomer 2)	vt		51.3	12	44.5	8	50.8	2	123.8	5	96.7	10
	Sub-total	*2456.1*		*4245.9*		*4445.5*		*4834.4*		*5450.3*		*4761.0*	
C$_{13}$ Norisoprenoids													
566, 0.532	*m/z* 159, 91, 131	8.3	14	26.6	20	19.2	8	0	0	0	0	0	0
620, 0.595	Vitispirane	276.3	15	194	23	88	7	34.9	16	19.7	7	22.6	14
632, 0.517	Theaspirane (isomer 1)	18.9	16	0	0	0	0	0	0	0	0	0	0
644, 0.528	Theaspirane (isomer 2)	20.2	12	vt		0	0	0	0	0	0	0	0
674, 0.681	β-Damascenone (isomer)	0	0	25.8	7	33.3	7	34.7	8	38.3	15	21.5	2
680, 0.840	*m/z* 142, 157, 115 (keton	vt		93.8	5	102	18	178	19	104	12	106.6	5
700, 0.702	β-Damascenone (isomer)	62.5	14	508.2	10	512.8	10	349.6	10	424.7	11	372.3	8
736, 0.648	Geranylacetone *	416.5	10	447.5	8	475.1	3	531.1	13	404.4	11	387.1	4
742, 0.850	5,6-Epoxy-β-ionone	0	0	0	0	0	0	11.6	11	21.0	23	0	0
760, 0.868	3,4-Dehydro-β-ionone	0	0	0	0	0	0	0	0	vt		0	0

778, 0.635	α-Iso-methyl ionone	16.2	7	22	8	23	10	44.5	16	50.2	6	16.4	12
784, 0.717	β-Ionone *	22	17	21.6	18	27.1	8	54.5	3	89.5	8	35.5	10
900, 0.894	Methyl dihydrojasmonate	vt		68.0	10	87.4	13	35.7	4	69.0	6	24.1	13
	Sub-total	*840.9*		*1407.5*		*1367.9*		*1274.6*		*1220.8*		*986.1*	
Sesquiterpenic compounds													
650, 0.583	δ-Elemene	0	0	0	0	0	0	37.2	20	35.4	7	28.8	4
680, 0.469	α-Copaene	20.3	27	67.8	7	71	6	121.8	11	143.4	9	207.2	18
724, 0.541	α-Humulene	0	0	0	0	0	0	17.7	21	8	2	44.3	10
756, 0.630	Aromadendrene	93.2	1	144.7	9	34.3	5	155.3	5	34.2	5	142.3	11
762, 0.450	α-Muurolene	vt		77.2	5	50.6	2	74.1	5	vt		0	0
790, 0.660	α-Farnesene	0	0	0	0	46.8	6	vt		0	0	0	0
808, 0.630	Calamenene	21.2	9	16.3	10	12.1	17	16.4	7	16.8	10	20.9	7
826, 0.629	α-Calacorene	36.5	12	15.1	11	24.1	9	15.7	6	17	15	19.4	10
832, 0.880	Nerolidol	vt		189.3	31	135.5	17	145.3	16	196.7	15	179.8	6
862, 0.726	Caryophyllene oxide	vt		96.3	4	74.6	4	26.3	16	27.4	12	0	0
886, 0.690	β-Eudesmol	vt		264.8	16	195.3	6	272.2	17	304.4	14	232.9	5
912, 0.654	*m/z* 119, 91, 191, 109 (al	57.7	4	361	7	465.6	1	133.9	19	201.5	18	92.6	15
	Sub-total	*228.9*		*1232.5*		*1109.9*		*1015.9*		*984.8*		*968.2*	
Diterpenic compound													
1116, 0.929	Phytol	0	0	0	0	84.2	1	105.7	6	145.8	11	143	4
	Total	*10906.3*		*22985.6*		*23296.1*		*24549.2*		*23893.3*		*24286.3*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

vt - vestigial

CA-SM1													
2011													
08/08/2011		16/08/2011		22/08/2011		29/08/2011		05/09/2011		12/09/2011		19/09/2011**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3065.9	8	2215.7	8	3006.1	8	2828.3	9	3430.9	13	3878.8	22	2743	5
780.7	14	1018.7	17	933.9	5	774.4	4	659.8	7	940.2	16	675.2	14
4071	11	5510	18	3851.3	11	5360.7	15	6207.3	2	8496	10	6706.1	7
470.7	13	2222.9	22	748.5	5	268.1	7	427.1	19	315.7	15	303.1	21
2420.1	25	2486.7	3	2147.4	7	2269.4	11	1778.6	16	1837.8	7	1576.8	6
1940	13	1655	15	2478.1	10	2429.4	17	2314.9	5	2431.4	7	3559.6	13
511.7	9	676.4	21	362.5	13	353.2	16	558.4	13	733.2	23	613.1	4
13260.1		*15785.4*		*13527.8*		*14283.5*		*15377*		*18633.1*		*16176.9*	
152.8	6	201.4	27	185.9	22	174.9	26	159.8	32	188.9	9	125.2	27
0	0	0	0	0	0	0	0	vt		vt		0	0
65.6	10	46.9	13	72.5	14	130.1	7	136.0	7	192.9	23	251.9	8
218.4		*248.3*		*258.4*		*305.0*		*295.8*		*381.8*		*377.1*	
75.3	15	50.8	18	13.8	34	26.6	16	12.8	20	7.2	12	8.6	2
0	0	0	0	0	0	0	0	0	0	0	0	0	0
40.3	4	22.8	11	20.7	8	18.7	11	0	0	0	0	0	0
62	21	49.4	4	38.9	5	13.6	20	36.8	12	31.3	9	238.9	9
32.7	18	18.7	22	15.3	24	17.3	25	17.6	5	21.1	22	15.6	17
57.6	23	54.8	8	35.9	11	21.2	14	60.5	2	152.7	23	153.6	10
42.6	9	46.5	14	134.5	13	121.4	10	79.9	9	121.5	11	92.4	8
160.1	13	248.5	17	172.1	2	147.1	16	24.7	13	11.7	20	60.2	19
43.2	21	52.8	3	28.6	10	38.1	8	67	8	80.3	2	89	3
16.5	23	8.3	9	23.7	10	10.6	6	10.7	11	13	15	0	0
185.8	12	234.5	6	210.3	10	223	21	22.6	16	26.2	23	17.6	16

0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	vt		vt		202.1	23	228.4	20	192	11
30.9	14	16.4	15	19.8	9	21	13	12.9	16	10.2	11	9.5	10
31.4	19	23	10	0	0	0	0	0	0	0	0	0	0
223.5	13	745.9	17	591.8	4	714.5	5	618.5	22	245	12	216.8	20
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
102.1	9	100.1	23	81.4	4	35.9	10	60.2	14	51.7	11	56.7	12
0	0	vt		49.0	6	40.9	6	54.3	19	68.9	14	35.4	5
0	0	32.9	19	35.3	23	38.9	4	43.5	22	42.8	9	40	7
0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.2	14	34.7	7	42.7	13	167.4	23	87.5	12	100.3	17	75	4
34.3	16	27.5	9	25.8	8	18.7	8	31.8	6	22.3	18	20.5	23
0	0	0	0	0	0	0	0	0	0	0	0	0	0
112.9	10	183.7	18	212.7	8	183.3	21	127.8	22	107.3	14	91.1	9
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
59.8	14	62.1	14	59.1	4	43.5	7	39.6	18	30	19	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
149.8	14	267.7	16	288.1	11	234.3	17	186.8	10	169.4	14	109.2	11
84.6	3	182.4	5	202.5	9	170	12	143.6	24	142.2	8	111.3	5
0	0	0	0	0	0	73.5	15	480.7	5	641.3	8	749.6	9
0	0	0	0	0	0	0	0	26	6	26.7	3	35.3	12
30.7	6	30.5	4	43.6	8	62	5	48.9	16	60.7	12	64	6
0	0	0	0	vt		45.9	8	34.2	10	33.8	11	35.1	7
1530		*2443.2*		*2331.8*		*2460.8*		*2518.2*		*2438.8*		*2508.8*	

17.1	4	15.3	6	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
102.3	13	158.3	14	59.3	12	37.8	9	40.6	17	40.4	8	32.3	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	vt		vt		104.0	9	146	15	171	14
0	0	0	0	0	0	0	0	0	0	0	0	0	0
195.3	8	255.2	7	302.9	13	253.0	7	146.5	17	160.2	3	168.5	15
125.1	12	256.9	10	170.6	13	152.1	3	129.5	19	115.8	16	74.2	7

0	0	0	0	0	0	0	0	0	0	0	0	0	0
32.1	14	34.8	1	31.5	9	44.1	6	52	10	36.1	16	44.9	13
17.7	8	30.1	10	42.3	8	40.5	8	55	10	41	9	40.4	11
489.6		*750.6*		*606.6*		*527.5*		*527.6*		*539.5*		*531.3*	
19.8	11	30.1	10	10.4	10	13.1	7	14.4	25	11.7	14	11.6	6
0	0	vt		34.1	9	33.8	15	66.3	10	53.4	5	44.3	8
0	0	0	0	0	0	0	0	0	0	0	0	0	0
33.9	3	46	9	55.2	4	78.7	1	49.4	11	45.9	7	32.6	9
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	96.2	1	114.1	12	123.7	7	102.2	1	100.3	8
16.6	3	17.4	12	10.5	7	7.5	10	6.9	9	7	7	0	0
13.7	37	18.3	9	17.2	12	14	7	13.3	34	7.5	5	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	vt		vt		48.8	10	53.9	4	50.7	4
0	0	87.8	3	124.3	14	70.9	11	75.7	8	75.3	13	66.7	4
84		*199.6*		*347.9*		*332.1*		*398.5*		*356.9*		*306.2*	
0	0	0	0	0	0	56.5	4	62.7	4	58.7	9	62.6	4
15657.4		*19477.9*		*17086.3*		*17992*		*19192.6*		*22416*		*19971.5*	

2012										2					
24/08/2012		31/08/2012		07/09/2012		14/09/2012		21/09/2012**		20/08/2010		27/08/2010		03/09/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
1869.5	12	3060.1	4	2608.4	23	3062.7	7	3282.5	14	1307.3	2	3986	20	4491.6	7
322.8	29	589.2	21	308.7	12	709.7	9	821.2	7	1216.1	2	646.1	19	659	8
2255.6	12	1690	19	2205.5	5	3367	8	3865.2	3	1438.3	13	4118.9	13	4846.4	8
67.1	25	172.9	30	226.8	15	516	10	589.5	6	535.7	7	795.8	3	872	6
683.3	13	964.9	18	5314	15	3706	9	3615.9	3	851.4	15	5049.2	21	4285.9	5
595.3	10	1316	22	1026.7	19	2145.3	10	2257.2	19	879.7	4	593	3	363.4	8
59.4	20	305.5	3	928.7	7	1224.9	17	1720.9	24	80.9	8	785.5	3	797.4	4
5853		*8098.6*		*12618.8*		*14731.6*		*16152.4*		*6309.4*		*15974.5*		*16315.7*	
194.2	9	341.4	16	354.9	10	476	8	442.7	13	147.6	7	240.0	19	395.3	13
vt		38.4	18	53.8	18	49.1	11	49.3	20	vt		36.0	15	37.9	12
125	17	166.5	29	307.8	3	389.2	5	371.7	8	77	3	110.2	14	218	19
319.2		*546.3*		*716.5*		*914.3*		*863.7*		*224.6*		*386.2*		*651.2*	
32.6	8	36.2	10	33.4	8	32	3	28.3	17	33.1	12	37.9	11	37.7	5
12.3	9	9.4	3	9	4	8.5	4	8.2	4	16.1	10	22.3	6	14	16
50.5	8	42.2	13	34.1	13	0	0	0	0	15.1	14	32.9	3	46	13
19.4	19	310.3	10	59	4	62.3	10	62.3	10	30.1	6	33.8	6	38.8	11
24	21	62.5	14	59.6	5	66.2	8	80.5	6	0	0	36.2	9	39.7	3
50	13	104.7	16	110.4	13	110.6	15	99.3	3	10	8	102.8	8	341.9	8
149.1	28	277.8	13	554.3	7	696.1	8	725	6	212	18	43.2	10	98.9	8
25.9	10	55.8	16	67.9	15	67.9	15	67.9	15	45.3	7	64.2	13	36.9	9
0	0	61.2	8	64.1	1	65.9	6	65.8	5	vt		60.7	4	74.2	13
0	0	0	0	0	0	0	0	0	0	11.4	13	18.3	10	17.1	10
0	0	0	0	0	0	vt		vt		68.5	7	65.1	4	39.6	11

0	0	0	0	0	0	0	0	0	0	0	0	18.1	5	35.9	14
210.5	21	280.7	13	504.7	13	737.9	18	681	17	77.7	10	230.4	20	195.3	23
0	0	0	0	0	0	0	0	0	0	0	0	vt		13.1	19
9.9	10	34.9	12	39.7	18	45	7	48	13	0	0	36.2	8	22.5	19
237.6	21	396.2	15	348	29	482.2	3	488.7	5	438.6	12	940.2	13	552.6	9
0	0	0	0	0	0	0	0	0	0	0	0	37.3	19	37	15
vt		51.1	9	46.9	19	48.2	8	46.9	19	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
162.9	9	233.5	22	193.8	9	89.1	2	73.8	4	48.3	12	91	13	44.3	7
vt		62.1	4	57.2	7	53.5	6	36.4	8	vt		178.4	6	145.5	8
0	0	0	0	0	0	0	0	0	0	26.4	6	54.6	2	63.8	6
0	0	42.7	9	53.8	22	58.9	7	57.1	24	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60.6	8	59.4	3	74.1	4	65.9	4	66	6	vt		137.3	11	245.7	14
87.2	12	89.7	13	83.3	19	78.5	7	76.3	9	35.6	5	66.9	16	28.4	12
vt		47.2	20	48	8	50.3	5	51.6	5	vt		39.9	22	26.6	20
180.8	15	439.1	14	367.4	20	427.6	13	593.1	7	13.5	16	321.2	8	460.0	8
vt		62.3	1	36.7	5	38.9	12	42.8	5	vt		72.5	12	51.2	16
67.4	16	65.5	1	59.4	7	57.6	8	55.1	9	153.6	5	150.5	15	142.6	13
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	16.5	4	12.7	16
188.1	7	198.1	14	70.8	6	62.8	9	44.2	9	vt		109.2	14	192.3	26
vt		163.4	14	90.2	2	81.4	11	63.7	4	vt		215.0	11	149.6	11
164.6	16	306.4	6	368.3	18	390.8	16	387	2	20.4	15	492.4	12	596.2	2
20.7	7	126.1	20	67.3	24	63.9	8	62	4	0	0	0	0	vt	
27.7	22	26.1	26	29.7	18	19.6	14	20.3	20	vt		77.0	8	69.9	13
58.4	8	53.7	10	65.5	30	62.2	9	55.4	12	vt		48.2	10	51.8	9
1807.6		*3662.1*		*3563.2*		*3991.8*		*4058.4*		*1222.6*		*3812.3*		*3884.1*	
8.1	4	23.4	18	21.3	15	23	10	0	0	6.2	8	19.4	13	14.3	9
83	4	115.9	19	98.6	4	112.6	20	89	9	184.8	12	145.4	6	47.2	17
30.3	7	62.1	6	57	7	60	8	47.8	6	12.5	23	0	0	0	0
15.5	19	28.6	15	30.9	10	18.4	13	17.4	30	16.9	17	vt		0	0
vt		34.7	14	38.8	3	28.8	7	31.8	16	vt		24.3	15	30.2	7
0	0	0	0	0	0	0	0	0	0	vt		77.2	8	85.1	7
65.1	11	214.7	25	316.9	19	332.3	8	250.2	11	59	10	444.4	16	466.2	8
319.7	11	378.3	10	233.9	20	94.7	10	82.1	4	215.1	20	384.7	20	407.9	16
vt		41.4	9	36	5	34.2	19	21.5	13	0	0	0	0	vt	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

22	27	32.1	14	36.9	15	39.7	10	32.7	21	23.1	4	16.6	5	17.7	10
31.3	19	46	10	51.4	12	64.1	6	52.6	5	16.7	21	21.7	4	44.3	13
127.5	16	110.5	10	119.4	6	118.5	11	112.5	17	vt		126.5	15	85.9	7
702.5		*1087.7*		*1041.1*		*926.3*		*737.6*		*534.3*		*1260.2*		*1198.8*	
0	0	12.2	10	16.6	17	25.8	15	30.8	9	0	0	0	0	21.1	12
31.8	5	45.5	8	27.8	16	35.4	8	39.4	4	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40.2	23	77.5	13	68.5	5	74.1	5	68.3	4	18.7	19	153.3	20	66.6	6
0	0	0	0	0	0	vt		0	0	vt		92.8	12	125.6	12
56.1	22	70.4	9	119.2	10	99	10	93.7	7	0	0	0	0	39.2	13
12.2	17	12.1	18	10.7	23	8	4	6.6	5	21.5	9	18.8	7	6.7	2
19.6	11	10.4	18	12.4	18	11.3	16	8.4	8	27.2	14	27.2	18	34.2	5
0	0	0	0	0	0	0	0	0	0	vt		130.8	14	110.5	9
0	0	0	0	0	0	0	0	0	0	100.2	8	101.1	5	88.9	2
0	0	18.4	31	20.3	10	17.9	11	18.2	12	vt		194.8	9	172.1	21
65.2	27	221	28	124.2	16	139.7	23	126.6	23	73.0	12	335	10	199.5	12
225.1		*467.5*		*399.7*		*411.2*		*392*		*240.6*		*1053.8*		*864.4*	
0	0	55.8	13	80.1	5	159.2	8	151.5	11	0	0	vt		58.9	3
8940		*13954.2*		*18452.8*		*21166.4*		*22383.9*		*8564.6*		*22524.9*		*23010.8*	

GC×GC peak area x 10^4 (arbitrary units)

												CA-SM2			
010														**2011**	
10/09/2010		17/09/2010**		24/09/2010		08/08/2011		16/08/2011		22/08/2011		29/08/2011		05/09/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3909.5	12	4146.4	7	3937.4	7	2627.3	22	2119.9	21	2497.9	21	2620.3	13	2873.9	17
792.3	8	825.3	1	500.1	14	688.3	13	651.7	20	1051.6	7	548.2	6	666.7	19
4029.7	10	4148.8	15	4491.3	9	5655.3	8	4362	9	5274.7	23	4760.9	17	6632.8	23
651.1	13	860.4	14	528.6	6	477.6	23	344.4	11	296.4	25	233	7	493.3	13
3789.8	6	3241.4	11	2997.4	13	2405.1	8	1915.4	15	1480.4	2	2059.7	15	1960	6
762.8	4	3139.9	1	2991.6	6	2134.5	17	2249	20	2272.6	9	1640.1	14	2266.1	11
922	17	1117	2	769.7	7	572.8	20	456.1	12	379.9	13	230.7	4	559.5	4
14857.2		*17479.2*		*16216.1*		*14560.9*		*12098.5*		*13253.5*		*12092.9*		*15452.3*	
392.5	12	388.1	10	572.5	1	176.6	22	125.0	22	158.2	26	186.0	29	165.8	20
22.5	11	30.8	4	22.6	6	0	0	0	0	0	0	0	0	0	0
206.1	11	287.8	13	47.4	7	62.8	7	96.0	23	84.8	16	90.1	11	175.0	27
621.1		*706.7*		*642.5*		*239.4*		*221.0*		*243.0*		*276.1*		*340.8*	
39.5	3	38.1	11	35.8	14	78.3	6	13.2	22	10.8	9	11	14	17.5	22
14.2	16	15.2	11	11.5	6	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	53.6	23	26.9	16	17.8	8	17.4	12	0	0
43.5	5	64	11	42	5	89.6	8	42.2	12	34.1	9	10.5	22	53.4	24
29.6	18	28.1	13	19.5	11	35.4	13	19.9	22	18.3	4	15.9	4	16.7	24
116.3	9	152	3	184.8	19	59	7	44.5	12	33.8	11	20.9	9	18.4	23
132.8	4	55.2	6	250.6	12	36.5	11	64.9	16	97.6	4	90.4	3	233.8	19
40.2	11	171.9	8	50.8	15	134.4	23	223	3	188.4	18	116.3	12	22.7	15
37.7	9	43.1	14	63.1	7	38.9	16	38.9	4	28.3	19	27.5	8	70.4	9
17.9	9	18.9	18	13.5	21	26.6	13	21	22	19.3	12	10.8	13	10.1	6
29.3	12	27.4	5	vt		140.4	10	222	14	201.5	20	158.8	16	15.8	21

44.1	10	63.3	4	29.4	10	0	0	0	0	0	0	0	0	0	0
317.6	16	326.2	15	278.7	13	0	0	0	0	0	0	0	0	327	22
32.8	13	55.7	4	19.1	18	17.8	11	39.8	24	25.7	9	12.5	19	13.6	10
87.8	10	55.2	5	47.2	11	31.1	10	22.2	9	0	0	0	0	0	0
291.1	12	386	7	211.8	12	173.8	21	728.6	13	483.7	4	473.1	9	478	21
67.7	3	41.9	11	34	7	0	0	0	0	0	0	0	0	0	0
35.5	5	40.6	11	vt		0	0	0	0	0	0	0	0	0	0
14.1	9	16.7	9	0	0	0	0	0	0	0	0	0	0	0	0
36.3	15	75.4	7	55.4	3	0	0	0	0	0	0	0	0	0	0
281.4	9	165.2	4	226.7	6	95.9	12	88.7	15	62.6	9	30.8	6	41.7	14
81.3	12	46.5	3	82.1	7	0	0	0	0	45.9	3	35	12	37.1	12
0	0	vt		0	0	0	0	27.2	8	29.7	6	25.1	14	31.7	9
29.4	14	25.2	6	0	0	0	0	0	0	0	0	0	0	0	0
207.3	14	293.6	12	299.4	6	27.0	20	31.3	8	35.7	13	139.9	2	51.5	19
29.1	19	42.4	0	20.9	10	23.0	23	26.7	6	20.8	8	16	12	15.4	10
59.4	9	49.9	19	27.1	6	0	0	0	0	0	0	0	0	0	0
316.9	9	440.1	22	538.7	11	104.7	8	174	15	204.4	5	174.5	14	93.2	13
135.4	11	97.2	7	44.7	9	0	0	0	0	0	0	0	0	0	0
184.0	3	172.5	3	0	0	0	0	0	0	0	0	0	0	0	0
vt		vt		0	0	44.6	7	44.8	6	40.1	6	35	11	32.7	4
12.7	14	22.3	20	15.3	21	0	0	0	0	0	0	0	0	0	0
489.5	7	542.7	4	151.4	20	128.8	18	220.4	5	240.9	11	204.1	14	153.1	12
253.2	20	267.8	11	71.2	12	73.4	6	160.8	7	196.5	5	169.3	4	117.2	20
632.9	5	671.8	6	625.7	8	0	0	0	0	vt		62.8	6	470.4	16
67.9	11	72.1	8	55.2	7	0	0	0	0	0	0	0	0	23.3	10
31.7	6	39.7	11	36.5	8	30.4	2	29	10	36.6	13	41.4	23	43.2	19
283.3	5	126.7	17	101.5	2	0	0	0	0	0	0	51.1	10	33.1	6
4483.9		*4712.5*		*3607.8*		*1364.9*		*2296.8*		*2061.7*		*1939.1*		*2403.5*	
0	0	0	0	0	0	25.3	8	22.8	29	0	0	0	0	0	0
25.2	9	62.6	8	23.3	10	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	73.4	10	133.3	20	32.4	17	36.2	13	34.1	10
34.5	6	33.8	14	17.9	23	0	0	0	0	0	0	0	0	0	0
99.1	15	101.3	7	63.1	7	0	0	0	0	0	0	0	0	0	0
331.6	17	379.1	5	251.2	6	0	0	0	0	0	0	vt		90.0	5
553	8	363.5	12	358.5	14	0	0	0	0	0	0	0	0	0	0
12.5	7	19.5	7	0	0	187.5	8	303.3	11	254.2	29	192.9	4	124.9	15
0	0	vt		0	0	139.2	20	177.8	6	162	13	135.7	15	120.5	16

23.8	19	45.3	4	15.2	22	0	0	0	0	0	0	0	0	0	0
51.5	13	89	6	30.6	18	29.5	12	22.4	1	19.1	14	18.5	12	40.6	13
67.6	10	59	10	30.7	12	16.3	25	20.7	4	23	10	21.8	9	28.1	13
1198.8		*1153.1*		*790.5*		*471.2*		*680.3*		*490.7*		*405.1*		*438.2*	
49.3	1	83.7	6	23.8	16	74.9	131	25.2	8	10.1	22	9.5	19	14.1	11
53.8	9	33.8	9	123	16	0	0	vt		33.5	10	32.7	21	54.4	20
14.7	5	16.4	8	45	8	0	0	0	0	0	0	0	0	0	0
58.4	9	55.6	10	75	5	vt		43.1	11	34	2	34.9	14	44.4	7
119.1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	vt		94.2	3	101.8	4	118.2	11
15.8	11	17.3	12	13.3	8	17.6	7	16.9	21	10.1	3	7	3	6.3	8
13.3	16	13.3	12	11.4	11	15.4	20	21.9	21	11.8	32	9.8	4	9.2	1
172	19	173.1	20	165.8	20	0	0	0	0	0	0	0	0	0	0
42.7	14	55.2	16	0	0	0	0	0	0	0	0	0	0	0	0
201.7	17	193.7	4	231	17	0	0	0	0	0	0	vt		48.2	8
153.1	14	111.3	12	142.7	22	vt		44.8	10	96.7	1	94.3	4	52.4	7
893.9		*753.4*		*831*		*107.9*		*151.9*		*290.4*		*290*		*347.2*	
98.6	9	125.2	10	132.5	20	0	0	0	0	0	0	44.8	10	40.6	13
22193		*24968.2*		*22256.2*		*16822.6*		*15461.7*		*16350.1*		*15059*		*19040.1*	

						2012									
12/09/2011		19/09/2011		26/09/2011**		24/08/2012		31/08/2012		07/09/2012		14/09/2012		21/09/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3758.7	13	2990.6	10	4250.1	13	1358.8	28	2254.5	30	3083.6	23	3452.2	14	3087	14
1579.1	19	724.3	14	1002.4	4	527.7	29	383.8	18	324.1	31	468.1	8	851.8	28
3257	13	8254	25	6353.4	21	1765.9	15	1853.3	14	2368	9	3727.4	15	5146.8	4
356.8	14	884.5	12	564.3	2	503.9	22	204.8	13	302	9	458.5	12	585.7	9
1881.5	9	1722.1	10	2729	1	1469.7	16	1590.6	20	7141.8	12	4892.6	19	4839.6	27
2211.9	6	2251	8	2198.9	8	2379.4	20	1849	18	816.5	10	2201.7	20	2915.2	11
695.6	11	555.5	15	727	5	61.4	27	331.4	14	714.7	12	1274.8	17	1280.1	13
13740.6		*17382*		*17825.1*		*8066.8*		*8467.4*		*14750.7*		*16475.3*		*18706.2*	
147.9	12	104.9	16	109.1	20	140	33	348.5	9	321.2	26	363.3	8	391.1	5
0	0	vt		vt		vt		29.5	20	52.8	9	21	16	26.7	3
196.2	14	222.5	19	198.1	4	112.9	16	134.5	16	310.6	5	331.6	14	348.8	4
344.1		*327.4*		*307.2*		*252.9*		*512.5*		*684.6*		*715.9*		*766.6*	
8.7	16	9.1	8	9.3	5	26	13	32.1	13	25.3	15	27.1	18	23.2	12
vt		vt		0	0	10.4	13	8.9	13	8.1	7	6.9	8	5.9	10
0	0	0	0	0	0	46.5	9	35.1	6	31.2	10	0	0	0	0
277.4	61	170.7	20	16.7	6	17.5	22	260.8	25	35.8	14	51.4	24	57.5	5
15.5	14	9	20	17.3	16	15.2	27	35.7	14	52.6	7	61.4	5	65.7	12
21.3	14	55.1	7	21	13	43.2	4	41.7	2	50.6	21	47.2	13	64.2	20
179.2	14	67.4	11	93.3	10	84	9	130	19	378.7	15	459	7	618.9	12
25.4	26	90.6	6	140.7	9	16.9	15	51.5	17	46	14	39.9	14	51.3	26
77	16	71.2	2	80.1	2	0	0	24	7	16.8	15	52.7	3	44.6	17
85.5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22.1	4	19.5	8	29.4	12	0	0	0	0	0	0	vt		vt	

0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
250.1	11	182.2	22	229.6	11	216.3	11	197.2	6	319.1	13	392.7	12	387.5	7
8.2	6	7.1	4	8.3	7	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	11.1	21	38.1	26	28.6	16	35.8	15	38.1	14
267.5	12	358.1	20	411.4	8	137.5	27	250.8	17	250.1	14	375.3	11	406.6	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	vt		26	11	24.1	28	22.2	7	22.1	17
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	130.9	21	154.3	25	49.1	10	39.3	23	42.5	23
30.4	20	40.4	4	37	8	vt		55.5	11	46.1	26	40.4	7	24	3
29.4	4	35	11	23.8	21	0	0	0	0	0	0	0	0	0	0
38.8	14	34.3	12	38.8	12	vt		30.7	9	42	16	38.9	8	41.5	10
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60.1	15	42.7	11	50.7	16	56.1	9	56.5	7	68.9	4	57.9	6	58.5	12
14.2	16	16.6	20	17	25	63.2	7	69	11	70.3	14	58.1	15	58.2	12
vt		vt		0	0	0	0	22.3	18	37.4	12	27.7	14	29.2	11
104.9	6	85.6	6	93.8	10	121.3	10	318.5	14	329.2	15	387.3	21	454.3	10
0	0	vt		0	0	vt		51.5	4	38.7	10	34.7	12	35.3	10
0	0	vt		0	0	62.9	7	61.1	5	34.8	11	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131.1	20	96.1	10	88.9	3	175.1	12	178.6	26	52.5	9	34.3	22	13.8	32
121.3	23	61.4	11	78.8	6	vt		124.8	15	88.7	1	80.1	9	54.2	2
482	5	648.5	4	633.3	10	118.5	11	92.5	24	324.3	10	349	10	241.1	21
24.1	6	26.6	23	25.1	13	45.9	8	95.8	21	56.6	7	49.9	10	52.2	3
45.9	8	51.6	10	31.4	3	27.2	25	22.1	18	19.9	20	10.8	25	0	0
34.8	8	35.8	4	0	0	53.0	5	44.2	19	57.6	21	54.6	20	27.6	4
2346.2		*2205.5*		*2166.4*		*1452.7*		*2477.2*		*2557.8*		*2807.5*		*2894.8*	
0	0	0	0	0	0	7.3	5	12.6	25	20.5	2	21.3	16	0	0
0	0	0	0	0	0	42.9	14	97.4	10	92.2	10	102	22	83.5	7
0	0	0	0	0	0	41	6	68.9	11	51.9	14	41.9	16	31.4	15
39.4	15	28.7	2	22.9	14	18.7	7	53.8	9	44	12	27.9	17	15.5	32
0	0	0	0	0	0	0	0	25.9	15	30.5	7	25.9	17	29.8	12
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150.9	12	159.5	21	151	24	63.1	8	187.6	21	284.3	13	305.9	14	133.6	27
0	0	vt		vt		375.6	13	324.2	2	219.3	27	88.9	21	73.5	11
143	13	136.3	9	157.6	27	0	0	29.7	18	24.8	14	28.1	20	11.2	6
93.6	1	67	1	46.7	11	0	0	0	0	0	0	0	0	0	0

vt		0	0	0	0	24	20	31.2	8	24	18	35.7	3	28.5	16
28.4	12	25.6	11	24.4	14	30.6	15	50	15	42.8	5	58.5	7	46.6	7
32.9	3	27.4	6	25.9	10	104.6	23	98.6	13	108.3	4	119.3	18	91.9	30
488.2		*444.5*		*428.5*		*707.8*		*979.9*		*942.6*		*855.4*		*545.5*	
11.8	24	8.3	10	10.5	5	0	0	0	0	9.4	7	13.4	29	29.8	8
32.8	20	35.2	8	38	9	18.4	24	28.6	12	17.3	18	27	15	33.2	7
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36.5	11	20.1	13	22.9	14	21.9	19	61.5	10	63.9	10	49.8	42	42.5	7
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91.8	4	76.3	5	71.6	3	38.8	23	47.1	7	58.8	10	58.2	20	52.5	12
5.9	3	0	0	0	0	10	10	9.2	17	6	6	7.1	8	5.1	6
8.5	2	0	0	0	0	9.9	4	14.4	29	13.5	19	18.5	24	8.6	4
0	0	vt		0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48.7	5	47.7	9	39.6	11	vt		14.8	22	18.2	9	15.9	13	15.3	14
41.8	15	43.6	7	51	6	66.5	12	214.3	20	102	8	107.4	16	130.6	15
277.8		*231.2*		*233.6*		*165.5*		*389.9*		*289.1*		*297.3*		*317.6*	
42.7	2	50.2	12	55.0	9	0	0	54.2	21	55.2	13	126.5	4	136	5
17248.3		*20649.9*		*21025.1*		*10671.7*		*12913.2*		*19305.3*		*21305*		*23389.9*	

2010															
20/08/2010		27/08/2010		03/09/2010		10/09/2010		17/09/2010**		08/08/2011		16/08/2011		22/08/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
1084.9	20	3301.8	6	4517	10	3907.7	15	4221.4	11	3071.9	16	3521.2	23	3652	15
1194.2	1	1654.1	21	1312.3	18	756.4	4	702.5	15	833.6	17	627.5	22	802.6	14
1223.1	12	3386.1	11	3411.6	14	3848.9	15	4417.4	4	4030.5	19	5036.7	16	5235.3	9
544.7	17	612.2	15	533.6	16	540.4	6	587.2	16	1099.9	15	784.9	23	348.5	18
1008.1	7	4902.9	7	3410.7	10	3440.5	8	3430.5	12	3354.7	8	2916.3	12	1299.6	15
855.7	7	554.4	6	1403.6	16	1865.3	3	1719.8	12	3153.6	8	2220.3	7	2297	8
48.4	15	1711.9	21	1845.5	13	868.6	11	840.2	10	584.2	15	420.2	22	310.3	4
5959.1		*16123.4*		*16434.3*		*15227.8*		*15919*		*16128.4*		*15527.1*		*13945.3*	
166.1	5	262.1	14	424.3	5	370.4	19	434	3	116.9	25	96.0	16	108.9	6
0	0	20.5	7	31.6	3	24.1	12	34.5	5	0	0	0	0	0	0
86.4	1	92.6	9	97.1	10	178.8	30	238.5	15	57.1	21	84.3	13	75	11
252.5		*375.2*		*553.0*		*573.3*		*707.0*		*174.0*		*180.3*		*183.9*	
31.8	15	35.8	20	32.3	27	34.2	17	33.9	18	13.1	19	26.7	12	10.5	20
49.1	18	19.8	16	25.4	7	17.3	3	13.7	13	0	0	0	0	0	0
17.4	13	26.8	4	31	3	0	0	0	0	21.6	9	37	10	15.4	14
14	21	46.4	9	27.4	15	18.6	13	34.3	15	22.2	19	48.1	15	19.3	14
0	0	35.8	12	26.4	9	25.8	11	26	18	27.5	18	17.5	23	16.9	22
9.7	6	510.7	13	143.3	19	144	9	222	7	36.9	10	41.3	8	28.7	6
196.6	12	232.6	8	102.7	7	170.2	18	93.5	2	28.6	18	39.8	5	75.8	15
48.4	12	52.3	23	34.8	13	51.4	19	50.7	3	106.7	9	177.4	3	61.9	8
0	0	54.9	2	73.4	9	42.8	7	50	9	0	0	24.0	23	51.5	4
11.6	20	15.4	12	14.7	7	18.5	13	23.6	8	11.6	13	9	9	16.6	25
15.6	18	24	9	25.5	18	32.7	7	26.7	12	112.9	17	160.7	19	303.8	6

0	0	13.7	8	31.7	17	39.5	15	58	19	0	0	0	0	0	0
36.8	9	187.9	14	215.7	9	195.3	15	279.9	9	0	0	0	0	0	0
0	0	0	0	14	19	15.1	11	18.2	15	16.8	21	15.2	17	13.2	3
vt		27.2	2	29.7	7	81.1	18	77.2	19	25.8	14	20.4	10	0	0
434.7	10	520.9	13	328.1	12	353.7	3	182.5	12	150.4	16	424.5	9	178.5	23
0	0	34.1	13	34.1	9	44.5	7	38	2	0	0	0	0	0	0
0	0	0	0	51.9	14	44.8	17	40.9	5	0	0	0	0	0	0
0	0	0	0	67.8	4	36.3	7	17.5	16	0	0	0	0	0	0
38.3	19	61.5	15	59.9	13	38.2	18	46.8	13	0	0	0	0	0	0
vt		176.1	19	203.6	6	247	16	255.1	19	65.6	8	53.5	8	28.5	14
29.4	8	33.9	3	47.6	7	60.9	2	36.3	9	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	vt		21.9	17	28.1	9
0	0	0	0	0	0	27	10	31.1	3	0	0	0	0	0	0
vt		224.9	26	206.5	16	308.4	8	386.6	8	26.5	17	31.8	16	27.9	1
32	4	36.4	11	34.9	15	28.1	14	32.5	2	19.9	16	21.6	9	18.9	10
0	0	35.7	7	33.9	5	52	5	65.2	5	0	0	0	0	0	0
vt		206.9	4	200.4	6	230.7	19	261.8	16	80.9	7	96.7	6	97.4	7
vt		45.9	21	46.7	2	122.6	9	119	10	0	0	0	0	0	0
190.7	9	108.2	11	149.1	14	35.3	10	52.7	4	0	0	0	0	0	0
0	0	0	0	0	0	vt		0	0	38.9	9	39.6	8	37.8	5
13.6	8	16.1	10	16.3	14	11.1	6	25.5	6	0	0	0	0	0	0
vt		137.5	12	122.5	19	106.9	19	255.3	11	114	17	230	19	235	9
vt		125.6	17	256.5	8	75	19	159.3	17	62.2	5	94.6	7	80.2	8
vt		248.2	18	468.0	12	315.3	9	664.5	15	0	0	0	0	0	0
0	0	0	0	vt		49.3	5	54.5	2	0	0	0	0	0	0
vt		32.6	19	74.5	3	35.3	8	45.7	10	19.8	13	26	6	26.5	22
vt		58.5	16	53.4	4	53.7	3	55.3	3	vt		vt		vt	
1137.9		*3350.5*		*3251.4*		*3128.4*		*3799.9*		*988.8*		*1630.6*		*1361.9*	

6.5	17	15.1	7	21.7	20	0	0	0	0	22.3	6	8.6	20	0	0
102.2	21	41.2	16	101.3	11	53.2	11	25.1	12	0	0	0	0	0	0
28	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23.2	17	0	0	0	0	0	0	0	0	51.1	7	43.9	18	21.9	21
0	0	20.3	3	27.3	16	37	13	21	11	0	0	0	0	0	0
vt		165.4	19	132.7	19	109.5	11	94.6	7	0	0	0	0	0	0
100.5	7	432.6	8	422.9	16	224.3	21	244.7	21	0	0	0	0	0	0
274.3	5	415.4	10	331.4	8	501.2	8	401.1	9	0	0	0	0	0	0
0	0	0	0	0	0	10.4	5	13.6	20	142.8	26	218	8	128.1	6
0	0	0	0	0	0	0	0	0	0	121.9	2	169.2	6	111.6	21

16.4	6	19.1	8	29	13	29.5	4	37.9	12	0	0	0	0	0	0
30.6	4	26.2	13	13.4	5	70.9	2	48.7	10	22	20	22.6	12	19	13
0	0	39	12	70	9	66.5	4	48.2	19	11.1	3	18.1	3	22.1	8
581.7		*1174.3*		*1149.7*		*1102.5*		*934.9*		*371.2*		*480.4*		*302.7*	
0	0	0	0	0	0	30.8	3	32	6	11.7	8	10.2	6	6.2	7
0	0	0	0	27.5	9	34	8	29.8	4	0	0	0	0	28.6	2
0	0	0	0	0	0	8.1	1	16.6	9	0	0	0	0	0	0
39.8	7	63.7	11	53.2	4	41.4	12	75	9	vt		30.0	21	31.2	5
vt		65.6	2	51.5	6	67.6	2	0	0	0	0	0	0	0	0
0	0	vt		48.5	13	0	0	0	0	0	0	0	0	0	0
14.4	7	8.2	2	8.8	9	10.3	4	17.2	13	14.8	8	11	13	8.5	3
23.7	8	25.3	6	11.5	8	11.6	9	21.9	4	11.6	9	15.2	16	10.4	7
vt		109.6	21	196.6	2	132.9	21	110.6	7	0	0	0	0	0	0
vt		106.5	7	60.2	13	57.7	8	78.4	9	0	0	0	0	0	0
vt		157.1	14	179.3	20	239.6	23	242.3	9	0	0	0	0	0	0
63.6	6	252.5	15	107	19	141.9	18	121.8	17	vt		52.4	3	64.3	14
141.5		*788.5*		*744.1*		*775.9*		*745.6*		*38.1*		*118.8*		*149.2*	
0	0	0	0	53.1	4	92.3	7	108.9	13	0	0	0	0	0	0
8104.5		*21847.7*		*22217.9*		*20934.4*		*22249.2*		*17713.6*		*17963.9*		*15953.5*	

CA-SM3															
2011															
29/08/2011		05/09/2011		12/09/2011		19/09/2011		26/09/2011**		03/10/2011		24/08/2012		31/08/2012	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2336.2	23	3296.7	10	4440.3	6	4256.6	5	3499.8	13	3610.7	9	1622.7	23	2768.4	26
519.4	14	2556.4	8	896	20	603	11	780.1	10	734.6	14	243.9	31	362.2	30
3999.4	23	6247.1	17	4824.1	20	8017	13	8290.8	4	8750.2	2	1822.4	17	2620.7	29
181.3	26	298.2	17	760.7	16	732.2	15	682.5	10	592.8	15	164.2	15	43.2	22
2227.9	17	1876.8	21	1753.9	17	1922.3	13	2323.8	15	2710.3	12	1282.8	22	1241.3	3
2158.2	19	2060.3	10	2383.2	4	3722	4	2306.1	8	2766.9	4	1561.1	8	1876.1	6
207.9	4	447.5	11	497	17	537.8	8	536.4	6	445.5	1	72.6	23	434.4	10
11630.3		*16783*		*15555.2*		*19790.9*		*18419.5*		*19611*		*6769.7*		*9346.3*	
142.3	27	118.3	22	141.3	23	148	18	160.0	20	189.7	6	113.2	16	148	3
0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	22
90.9	23	117.9	11	119.6	12	109.4	10	118	16	99.4	18	71.4	7	90.1	6
233.2		*236.2*		*260.9*		*257.4*		*278.0*		*289.1*		*184.6*		*264.1*	
12.3	16	9.5	21	7.9	2	8.1	1	26.9	13	14.7	21	19.3	16	23.6	18
0	0	0	0	0	0	0	0	0	0	0	0	6.2	4	4.6	10
16.5	18	0	0	0	0	0	0	0	0	0	0	19.8	28	10.2	4
11.9	14	36.5	8	187.9	9	194.5	7	19.6	25	9.7	25	16.9	12	239.2	14
8.6	9	35.2	8	40.9	2	6.1	11	17.1	3	13	18	17.3	23	11.2	15
27	19	44.6	21	51.9	7	35	5	16.5	4	24.6	15	29.5	28	25.8	22
67.9	18	108.6	4	57.4	4	49.2	6	207.8	20	217.4	17	59.2	5	87.5	5
69.7	24	20	6	13.4	24	17.3	23	21.7	21	168.6	18	19.5	14	32.3	12
60.3	5	36.4	22	54.9	8	57.2	9	95.1	13	42.5	17	0	0	14.0	21
17.2	15	32.2	4	19.7	6	0	0	0	0	0	0	0	0	0	0
265.4	8	24.5	16	16.5	2	17.7	20	17	15	13.9	24	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53.2	5	259.7	21	338.4	20	181.1	6	270.9	15	123.8	16	135.1	15	180.9	22
11.1	12	12.8	18	8.6	6	8.9	4	9.7	6	82	6	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	8.5	9	19.1	2
181.4	16	143.9	16	199.9	16	172.1	23	149.2	20	58.6	5	121.9	28	215.9	12
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.9	10
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	101.5	3	100.2	26
21.9	16	27.3	13	24.8	17	25.1	12	21.8	14	0	0	vt		36.3	10
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	10	24.3	17	30	7	28.4	7	44	9	0	0	vt		32.3	15
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39.3	12	42.4	15	40.8	10	41.4	21	31.2	3	41.1	16	46	16	37.2	7
13.1	18	14	7	15.3	14	12.7	12	12.1	12	13.9	7	43.9	8	66.9	12
0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.5	16
72.4	11	69.7	8	69.1	22	76.6	4	34.8	10	32.2	10	135	8	300.9	21
0	0	0	0	0	0	0	0	0	0	0	0	vt		45.8	13
0	0	0	0	0	0	0	0	0	0	0	0	33.7	17	22.2	16
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
228.1	14	166.5	6	124.2	13	94	4	89.8	11	83.8	4	153.5	12	149.5	26
61	11	65	19	58.4	6	65.1	5	64.4	4	53.4	5	vt		83.1	3
41.7	8	399.5	13	150.9	18	61.5	4	570.2	10	564.9	9	85.4	8	67.9	9
0	0	23.7	7	22	7	27	7	23.8	10	24	7	34.3	4	94.2	9
48.6	18	39.2	23	39.9	8	41.9	14	27.6	6	24.2	6	24.9	24	19.6	17
33.7	8	34.4	11	44.6	11	42.6	13	0	0	0	0	41.8	8	39.3	15
1373		*1660.4*		*1609.5*		*1255.4*		*1744.3*		*1591.6*		*1133.9*		*1973.5*	

0	0	0	0	0	0	0	0	0	0	0	0	6.4	3	15.3	6
0	0	0	0	0	0	0	0	0	0	0	0	30.1	8	83.8	11
0	0	0	0	0	0	0	0	0	0	0	0	20.3	27	12.8	25
17.2	21	20.5	12	17.7	17	18.1	14	0	0	0	0	11.6	17	7.8	10
0	0	0	0	0	0	0	0	0	0	0	0	0	0	20.8	11
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vt		59.5	10	65.9	9	114	15	97.5	3	91.4	6	53.3	17	217	18
0	0	0	0	0	0	0	0	0	0	0	0	110.8	16	144.9	23
97	22	116.8	14	138	13	113.6	8	130.6	19	158.6	19	0	0	0	0
111.4	8	108.2	11	69	8	63.8	6	61.5	5	74.6	4	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0	20.3	7	18.3	12
15	3	25.3	18	23.6	23	24.6	10	23.9	9	13.8	15	18.5	18	28.1	7
16.3	7	21.8	18	30.1	13	26.1	15	19.5	18	12.4	16	85.5	6	82.1	6
256.9		*352.1*		*344.3*		*360.2*		*333*		*350.8*		*356.8*		*630.9*	
17.9	10	16.2	16	10.4	5	7	9	8.5	5	7.1	9	0	0	0	0
30.1	9	29.8	11	20.3	14	18.6	11	19.8	8	18.2	12	15.2	16	13.4	21
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31.6	11	27.1	16	23.2	13	0	0	0	0	0	0	16.7	17	40.9	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86.0	10	75.1	6	62.7	3	42.3	4	40.7	15	30.4	7	25.2	37	36.5	9
6	7	5.8	10	4.1	7	0	0	0	0	0	0	10.2	25	9.3	8
7.9	16	8.5	3	0	0	0	0	0	0	0	0	9.6	12	9.1	7
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	21.2	16	27.1	10	20	10	25.7	19	22.2	5	vt		20.8	26
40.8	8	33.1	10	43.5	6	30.7	7	37.3	14	36.8	16	36.9	10	115.2	18
220.3		*216.8*		*191.3*		*118.6*		*132*		*114.7*		*113.8*		*245.2*	
40.5	9	38	16	30.6	11	30.2	16	33.1	11	26.4	14	0	0	52.1	2
13766.5		*19296*		*17999.7*		*21820.8*		*20966.8*		*21998.3*		*8578.1*		*12535.7*	

2012					
07/09/2012		14/09/2012		21/09/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2062.4	6	3857.7	8	3031.1	26
330.8	14	884.2	28	864.3	21
1872.3	75	3544.6	22	3991	15
191.3	18	659.2	24	881.4	8
5383.2	11	4346.2	11	4469	24
1398.5	25	2537	5	3110.2	17
1041.3	26	1447.3	7	1299.7	16
12279.8		*17276.2*		*17646.7*	
215.2	14	322.5	9	308.3	3
32.4	7	20.4	3	23.9	22
225.4	14	314.6	9	289.3	10
473.0		*657.5*		*621.5*	
18.9	21	16.1	8	17.2	24
3.8	9	4.2	8	2.7	18
10.9	25	0	0	0	0
35	3	44.8	13	53.6	5
12.4	21	11.6	27	11	23
39.4	10	38	27	28.2	28
213	15	934.4	128	206.6	14
26.9	19	34.8	14	39.4	17
29.1	16	48	9	33.9	20
0	0	0	0	0	0
0	0	0	0	0	0

0	0	0	0	0	0
315.6	17	335.8	23	374.4	15
0	0	vt		vt	
27.3	10	31.2	9	34	5
258.1	10	290.7	8	293.3	25
0	0	0	0	0	0
16.2	14	17.3	14	15.4	18
0	0	0	0	0	0
31.4	14	21.8	24	30.4	23
34.9	22	25.7	18	12.2	6
0	0	0	0	0	0
35.4	13	34.6	8	36.5	4
0	0	0	0	0	0
47.3	26	36.1	21	33	12
53.9	5	60.3	28	33.3	21
18.3	13	25.9	14	18.5	22
333.7	19	277.2	11	351.6	5
27.1	13	27.2	15	22.6	24
29.9	13	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
41.5	11	24	24	13.4	18
78.6	17	78.4	23	31.8	31
206.2	19	145.3	26	126.7	20
49.3	13	46.2	6	38.6	17
11.9	9	0	0	0	0
28.5	12	24.4	13	27.2	22
2015.6		*2617.9*		*1868.3*	
16.9	28	15.3	20	0	0
89	7	52.5	14	32.1	10
16.5	24	17.2	31	21.4	21
9.7	4	9.4	14	14	11
19.5	12	10.2	14	10.5	26
0	0	0	0	0	0
212.5	7	192.9	19	146	31
68	17	64.2	9	49	18
0	0	20.0	20	13.6	17
0	0	0	0	0	0

21.5	10	19.4	18	18.2	25
23.1	11	28.3	8	32	6
97.7	13	66.1	15	58.4	16
574.4		*495.5*		*395.2*	
6.8	13	8.6	7	9.5	6
13.1	28	17.2	12	17.9	15
0	0	0	0	0	0
43.3	25	38.3	6	34.1	10
0	0	0	0	0	0
44.8	9	68.9	6	49.4	11
4.8	15	4.4	9	3	12
8.2	6	7.1	9	6.3	5
0	0	0	0	0	0
0	0	0	0	0	0
14.5	14	13.9	14	13.4	10
97.7	19	89.5	5	79.1	9
233.2		*247.9*		*212.7*	
46.4	21	63	26	63	7
15641.3		*21374.1*		*20824.6*	

Table S13

Volatile components determined for *Vitis vinifera* L. cv. Touriga Nacional variety obtained from 3 parcels and 3 years of harvests, during maturation, at Bairrada Appellation.

		2010											
		24/08/2010		31/08/2010		07/09/2010		14/09/2010		21/09/2010		28/09/2010	
[1]Dtr(s),[2]Dtr	**Compound**	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C_6 compounds													
194, 0.640	Hexanal	1501.7	8	3015.8	2	4524.9	21	3430.1	19	4203.9	16	4409.4	15
206, 0.630	3-Hexenal	357.5	1	1662.9	7	1172	20	980	12	612.2	16	631.4	8
230, 0.651	2-Hexenal	2061.2	7	3316.1	9	6881.8	6	6089.1	13	6860.5	11	4446.2	2
242, 0.630	3-Hexen-1-ol	310	18	1125.3	16	771	13	388	21	122	9	195.2	4
248, 1.076	2-Hexen-1-ol	643.5	5	4076.4	12	2854.6	5	1887.7	4	2126.9	10	1163	19
266, 0.903	1-Hexanol	542.4	7	1725.7	17	2260.7	18	3426.2	22	3287.8	2	8408.4	5
296, 0.930	2,4-Hexadienal	120.7	8	1223.9	7	942.3	17	714.5	11	319.6	15	437.5	11
	Sub-total	5537.0		16146.1		19407.3		16915.6		17532.9		19691.1	
Aromatic alcohols													
420, 3.014	Benzyl Alcohol	33.4	5	133.6	19	162.4	9	294.7	7	326.7	16	260	7
446, 1.426	α,α-Dimethyl Benzyl alcohol											vt	
470, 1.960	2-Phenylethanol	14.8	11	148.5	7	232.8	15	175.8	16	245.3	14	391	6
	Sub-total	48.2		282.1		395.2		470.5		572		651	
C_9 Norisoprenoid													
506, 0.761	Norinone	21.6	3	27.3	23	37.2	9	40.5	12	37.9	14	38.5	8
Monoterpenic compounds													
314, 0.440	α-Pinene	31.5	23	37	13	53.6	3	109.6	18	118.2	8	7.2	3
338, 0.480	Dehydroxylinaloolo	0	0	29.2	10	35.2	4	0	0	0	0	0	0
344, 0.457	β-Pinene *	12.6	11	26.2	4	31.7	21	23	5	13.6	7	13.1	15
362, 0.520	3-Carene	64.1	7	46.1	12	22.5	19	40.6	3	0	0	0	0
392, 0.405	*m*-Cymene	58.7	5	70.8	6	44.6	10	23.7	20	14.6	19	12.2	14
398, 0.476	Limonene *	vt		94.7	5	31.2	23	29.5	8	31.4	10	73.5	8
404, 0.476	1,8-Cineole	80.2	10	70.4	1	51	3	52	3	55.4	20	60.7	3
416, 0.560	β-Ocimene	33.2	2	202.8	6	163.3	23	132.5	21	124.3	3	185.9	3
428, 0.678	Linalool oxide (isom	88.1	1	51.7	18	63.7	14	21.1	10	12.1	10	16.9	9
434, 0.727	Dihydromyrcenol	vt		296.5	4	314.8	18	231.6	10	265.6	19	226.9	9
440, 0.560	α-Terpinolene	52.9	4	72.8	22	44.2	2	19.6	15	31.5	9	27.7	16

440, 0.790	Linalool oxide (isom	118.1	5	32.7	17	25.8	23	13.4	20	12.4	5	18.7	6
446, 0.700	Dihydrolinalool	vt		28.3	13	15.3	18	17.4	8	14.4	4	10.7	6
452, 0.746	Linalool *	460.0	9	573.7	14	628.5	13	792	21	799.1	11	843.7	8
464, 0.600	Rose oxide (isomer)	17.8	14	45.9	6	8	6	8.8	1	0	0	0	0
470, 0.646	Hotrienol	479.1	9	479.9	10	635.7	8	403	4	424.9	21	430	19
500 , 0.970	β-Terpineol	68.3	22	29.1	18	30.7	10	0	0	0	0	0	0
512, 0.635	Nerol oxide	21.3	22	66.5	16	90.8	19	38.8	19	30.6	12	36.7	8
518, 1.200	*m/z* 68, 94, 79, 109	47.0	17	81.8	5	103.6	7	85.4	5	86.2	2	81.9	2
524, 0.860	Borneol	44.0	5	71.2	8	104.9	18	51.4	12	92.4	20	75.5	8
530, 0.984	Menthol *	vt		127.4	20	119.9	17	167.1	4	118.9	15	99.6	7
536, 0.715	Terpinen-4-ol	36.1	15	52.4	13	62.3	21	34.7	9	18.2	16	16.6	19
536, 1.269	Cymen-8-ol	0	0	0	0	46.7	6	22.2	13	22.9	10	27	9
542, 0.835	α-Terpineol *	160	8	417.5	18	393.9	5	364.6	8	238.8	16	278.5	18
560, 0.850	Verbenone	25.4	9	45.8	1	40.3	7	30.6	14	30.5	5	24.3	9
566, 0.703	Menth-1-en-9-al	0	0	11.5	21	18.1	6	15.4	29	97.9	6	21.8	16
584, 0.873	Geraniol (isomer 1)	vt		68.8	5	106.9	9	158.3	14	83.6	4	174.3	4
584, 0.943	β-Citronellol *	vt		77.0	11	156.9	13	184.6	35	60.3	14	41.2	4
590, 0.737	Geraniol (isomer 2)	284.5	13	631.4	14	777.9	14	805.2	13	800.2	15	789.7	10
596, 0.976	Citral (isomer 1)	0	0	21.2	16	31.3	9	41.9	5	50.5	3	51.5	3
602, 0.815	Carvone *	vt		47.2	19	48	17	28.5	17	26.4	12	52.1	11
626, 0.775	Citral (isomer 2)	vt		57.5	18	58.9	12	47	22	36.6	9	70.5	14
	Sub-total	2182.9		3965		4360.2		3993.5		3711.5		3768.4	
C_{13} Norisoprenoids													
566, 0.532	*m/z* 159, 91, 131	10	5	70.7	11	14.8	13	13.1	20	0	0	0	0
620, 0.595	Vitispirane	78.7	16	93.7	11	79.9	2	0	0	0	0	0	0
644, 0.528	Theaspirane (isomer	0	0	0	0	0	0	0	0	0	0	0	0
674, 0.681	β-Damascenone (iso	102.2	19	154.5	29	288.2	7	170.0	23	167.6	13	204.2	6
700, 0.702	β-Damascenone (iso	147.4	7	516.1	9	516.1	17	291.7	17	291.2	10	278.0	16
736, 0.648	Geranylacetone *	312	4	492.3	5	488.6	14	508.1	8	294.7	11	272.6	8
742, 0.850	5,6-Epoxy-β-ionone	vt		22.8	20	8.9	9	8.4	4	6.5	3	6.6	18
760, 0.868	3,4-Dehydro-β-iono	0	0	0	0	0	0	0	0	0	0	0	0
778, 0.635	α-Iso-methyl ionone	16.9	13	19.4	4	14.8	7	0	0	0	0	0	0
784, 0.717	β-Ionone *	0	0	74	6	92.1	4	18.5	11	18.5	7	27.5	8
900, 0.894	Methyl dihydrojasm	0	0	43.5	11	61.9	15	66.6	14	64.5	1	63.8	16
	Sub-total	667.2		1487		1565.3		1076.4		843		852.7	
Sesquiterpenic compounds													
650, 0.583	δ-Elemene	0	0	0	0	0	0	vt		11.7	13	10.9	10
680, 0.469	α-Copaene	0	0	20.2	12	28.5	8	26.7	11	29.4	16	26.0	19
686, 0.510	β-Bourbonene	0	0	17.3	18	23.8	14	24.5	13	28.0	7	31.3	10

756, 0.630	Aromadendrene	17.7	19	16.4	6	17.8	10	23.9	18	20.8	13	38.5	16
790, 0.660	α-Farnesene	0	0	0	0	8.4	6	10.7	8	13.5	17	19.5	10
808, 0.630	Calamenene	0	0	vt		15.4	18	18.4	13	21.3	9	16.3	21
826, 0.629	α-Calacorene	0	0	0	0	8.4	19	10.4	11	13.9	1	19.3	11
832, 0.880	Nerolidol	vt		98.8	7	89.9	9	81.2	14	82.4	13	103.9	9
850, 0.751	Globulol	22.2	19	22.0	6	30.2	8	90.0	7	76.3	4	54.7	3
862, 0.726	Caryophyllene oxide	0	0	0	0	18.7	11	21.3	31	17.0	18	19.4	15
886, 0.690	β-Eudesmol	83.2	0	125.2	13	156.1	22	91.4	9	78.8	7	74.8	13
912, 0.654	*m/z* 119, 91, 191, 1	0	0	109	17	74.2	16	70.8	1	63.8	8	62.1	10
	Sub-total	123.1		408.9		471.4		469.3		456.9		476.7	
Diterpenic compound													
1116, 0.929	**Phytol**	0	0	0	0	0	0	42.7	7	36.7	3	37.8	7
	Total	*8580.0*		*22316.4*		*26236.6*		*23008.5*		*23190.9*		*25516.2*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

*** Grapes not available

vt - vestigial

											TN-SM1				
														2011	
05/10/2010		12/10/2010**		19/10/2010		11/08/2011		18/08/2011		25/08/2011		01/09/2011		08/09/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4667.8	14	2801.9	6	2598.7	18	2373.1	15	3325.3	10	4447.8	17	2638.2	16	4203.8	16
403.5	21	636.5	7	681.7	8	906.4	17	907.7	21	738.8	23	475.9	14	714.4	9
5366.8	15	7669.7	11	6622.8	6	4430.2	7	5034.6	15	6007	10	6113.4	10	9934.2	16
131.1	9	436.3	16	586.0	20	455.9	25	395.7	15	599.8	14	386.2	16	245.2	19
938.9	18	1530.5	9	2160.9	25	1600.5	5	2513.7	18	2101	14	1035.3	15	1608.9	4
5851.6	12	1916.4	18	2023.8	42	1561.4	21	2181.6	19	2407.3	2	2187.2	10	2569.3	14
296.2	13	219.4	8	489.4	7	528.6	20	749.4	12	527.4	16	262.8	16	770.1	7
17655.9		15210.7		15163.3		11856.1		15108		16829.1		13099		20045.9	
376.2	20	435.2	10	506.2	13	210.2	10	188.4	18	162	8	234.6	7	300.9	5
vt		vt		vt											
352.0	16	397.8	27	415.2	19	191.5	4	233.5	11	302.7	2	254.4	6	242	14
728.2		833		921.4		401.7		421.9		464.7		489		542.9	
34	7	34.1	16	37.5	9	21.3	22	27.3	19	23.9	18	20.7	20	20.7	22
5.4	5	4.6	4	0	0	vt		21.4	17	11	12	10.7	7	25.3	17
0	0	0	0	0	0	23.5	11	20.6	11	11.5	6	0	0	0	0
12.9	4	13.6	4	10.3	8	35.2	16	40.4	8	35.5	7	22.8	16	64.4	20
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.2	20	7.6	15	5.5	12	48.5	19	105.9	23	107.2	7	9.6	4	23.8	20
60.4	4	44.1	10	36.1	8	62.2	20	101.7	7	68.5	23	80.6	6	118.1	5
87.2	3	61.5	16	42.1	10	38.8	20	45.8	4	35.8	16	40.7	3	48.1	4
165.9	12	178	5	125	12	0	0	0	0	0	0	0	0	0	0
17.9	10	23.3	5	20.8	5	150.4	21	131.7	10	75	15	24	13	39.1	5
118.9	9	99.6	8	173.1	7	45.1	18	58.4	12	40.6	5	48.6	7	71.4	13
19	5	8.9	11	11.9	8	80.4	20	250.5	23	48.7	15	34.3	7	171.5	16

10.6	6	12.2	13	17.5	6	0	0	0	0	0	0	0	0	0	0
6.8	3	7.8	4	0	0	0	0	0	0	0	0	0	0	0	0
947.8	19	1081.5	29	947.9	17	143.2	9	516.2	18	1105	4	928	16	192.9	6
0	0	0	0	0	0	32.2	3	36.9	6	29.5	7	29	8	0	0
416.7	8	463.3	29	424.0	12	168.6	20	526.3	22	561.3	24	514.9	17	601.6	11
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.9	9	0	0	0	0	82.0	13	69.4	18	45.4	8	47.5	7	45.9	15
84.3	4	79.5	3	62	6	81.3	8	121.3	16	24.8	12	22.2	7	34.4	11
53.1	10	44.4	15	58.8	18	259.6	19	530.5	17	401.2	15	340.3	9	46.4	14
72	7	62.2	13	57.4	11	39.1	22	31.6	11	25.4	8	21.8	7	82.7	11
18.6	17	16.5	15	14.7	19	0	0	0	0	0	0	14	20	23.5	14
21.7	5	17.3	7	19.1	12	20.0	15	36.5	13	19.9	12	20.4	9	22.9	20
240.9	15	250.8	17	256.6	22	23.7	16	35.2	12	49.7	19	220.9	13	261.7	16
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77.9	4	60.4	2	18.8	6	0	0	0	0	0	0	0	0	0	0
171.1	34	172.3	4	145.5	10	348.9	5	518.2	13	460.8	15	0	0	0	0
34.9	17	35.4	11	0	0	124.7	19	252.1	16	149.2	16	146	19	91.6	15
775.8	19	877.8	12	815.7	8	246.2	7	603.5	10	645.9	9	745.2	11	889.2	4
40.2	13	41.4	4	0	0	vt		50.0	1	23	17	32.3	7	55.7	8
20	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36.8	16	37	14	60.6	6	33.9	15	62	6	67.6	16	17.1	15	193.3	3
3550.9		3701		3323.4		2087.5		4166.1		4042.5		3370.9		3103.5	

0	0	0	0	0	0	22.4	13	37.5	16	16.4	6	0	0	0	0
0	0	0	0	0	0	58.5	16	27.5	18	16.8	21	0	0	0	0
0	0	0	0	0	0	118.4	13	188.1	38	105.2	8	0	0	0	0
126.1	7	150.3	35	117.2	8	210.5	18	210.3	6	184.7	22	21.4	10	9.3	14
242.9	10	253.6	13	218.7	9	0	0	0	0	0	0	29.4	12	141.1	16
327.9	4	277.9	18	182	34	170.3	11	271.9	12	225.2	11	161.1	11	133.5	2
0	0	0	0	0	0	28.2	22	49.6	22	112.4	19	168.3	17	154.4	19
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15.2	18	10.3	4	14.1	42	21.9	14	20.2	19	13.7	19	18.2	4	21.4	9
65.5	11	64.0	6	60.5	4	vt		24.6	15	22.4	7	19.8	12	101.3	8
777.6		756.1		592.5		630.2		829.7		696.8		418.2		561	

18.4	7	23.7	4	19.1	13	23.2	11	43.7	22	30.2	7	26.7	11	14.5	5
25.8	21	29.1	19	27.6	28	10.5	7	19.3	10	25.1	14	27.2	18	33.5	18
27.6	14	26.3	11	20.9	11	7.4	12	10.6	17	12.9	7	10.4	9	16.5	21

39.7	26	42.0	13	45.4	13	16.3	15	24.8	9	27.0	12	26.5	11	27.4	16
25.8	13	28.6	17	37.9	5	0	0	0	0	14.3	17	14.9	8	15.3	3
14.2	16	12.8	5	13.6	21	0	0	0	0	7.3	8	7.4	18	6.9	16
16.8	12	13.4	13	10.3	2	0	0	0	0	0	0	12.7	10	18.4	28
90.1	10	77.0	5	84.8	13	vt		110.7	8	118.7	21	108.7	14	89.6	19
45.7	3	43.2	3	42	9	vt		15.9	11	22.8	10	31.0	7	27.8	10
22.4	9	18.3	19	21.4	12	0	0	0	0	10.4	13	12.5	17	11.8	14
82.1	21	79.7	7	77.4	16	vt		89.3	14	84.1	19	67.7	8	70.1	10
46.7	13	51.7	3	46.2	6	48.5	11	50.3	18	30.8	3	31.9	4	42.5	5
455.3		445.8		446.6		105.9		364.6		383.6		377.6		374.3	
37.8	7	40.1	1	39	3	0	0	0	0	0	0	0	0	0	0
23239.7		*21020.8*		*20523.7*		*15102.7*		*20917.6*		*22440.6*		*17775.4*		*24648.3*	

								2012							
15/09/2011		22/09/2011		29/09/2011		06/10/2011**		31/08/2012		07/09/2012		14/09/2012		21/09/2012	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3687.2	11	2994.9	20	3780.7	3	2739.8	4	2603.4	8	2983.3	4	2698.4	23	2411.6	16
602.5	4	677	6	792.8	16	792.7	9	1286.1	14	1164.1	9	2281.2	26	1517.5	7
8820.5	10	7065.2	15	6473.4	7	6767.5	21	1468.2	26	1729.6	13	3760.7	25	3749.7	27
237.7	17	471.3	5	539.1	5	488.0	12	189.2	3	272.6	13	409.4	22	464.2	18
1461.5	16	1531.9	2	1300.7	8	1873.1	12	2674.5	13	1625.3	26	2500.2	4	1818.7	10
2340.8	9	3529.2	14	3290.9	8	3705.4	9	2381.4	7	2628.2	9	2785.8	17	2112.3	19
695.2	21	461.7	21	924	14	564.6	5	554	17	688.5	6	548	17	458.3	6
17845.4		16731.2		17101.6		16931.1		11156.8		11091.6		14983.7		12532.3	
303.8	5	347.8	6	295.9	8	313.5	12	38.8	17	109.1	15	158.9	21	240	9
		vt		vt		vt						vt		vt	
221.5	14	202.4	23	257.6	20	194.9	10	26.1	16	87.9	3	131	18	186.3	22
525.3		550.2		553.5		508.4		64.9		197		289.9		426.3	
19.3	12	22.1	12	20.4	9	23.5	11	21.6	10	24.4	8	27	16	30.3	12
13.5	12	13	8	10.1	10	0	0	25	12	30.8	10	33.9	11	47	10
0	0	0	0	0	0	0	0	54.1	7	56.3	15	25.9	16	33	11
25.9	23	19.2	3	35.7	8	16.5	21	17.5	15	30.6	7	26.4	14	34.3	9
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	10	11.2	14	30.2	21	8.9	20	21.8	2	41.3	5	38.9	6	38.8	9
173.5	12	164.8	11	137.3	17	167.1	23	144.5	15	119.8	13	126.7	20	93.3	12
23.3	20	24.2	6	98.7	2	98	12	45.1	8	47	9	52.1	9	28.3	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70.5	5	69.2	4	59.6	6	39.5	15	141.1	24	100.4	2	82.8	4	60.6	8
60.6	2	52.2	2	61.3	6	29.4	5	200.5	17	215.6	11	186	10	193.7	17
86.6	21	45.2	2	36.9	6	20.3	6	0	0	0	0	0	0	0	0

9.9	8	10.5	11	11.9	15	6.4	7	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
247.7	17	770.5	7	851.6	1	1020.6	4	166.0	22	709.0	9	818.7	7	749.9	8
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
435.2	13	489.5	16	491.6	21	443.9	13	190.1	13	759.1	14	831.3	16	703.7	12
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41.7	20	31	9	25.2	11	18.3	14	18.5	18	23.4	8	62.7	10	65	14
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36.6	14	39.8	7	36.3	9	0	0	39.9	6	48.6	6	56.8	11	50.7	11
43.1	7	69.8	5	69.5	10	28.8	20	51.7	15	48.5	9	68.3	13	60.8	18
55.1	22	50.5	2	48	7	25.7	17	53.3	18	58.4	6	48.4	10	52.4	20
26.2	14	27.1	17	35.5	13	16.9	14	0	0	vt		14.1	4	31.6	19
331.7	11	253.6	11	253.5	10	401.9	19	95.8	12	155.7	19	163.9	22	242.4	4
0	0	0	0	0	0	0	0	40.2	22	49.7	3	54.1	9	30.1	8
0	0	0	0	0	0	0	0	0	0	15.3	25	20.1	8	40.7	7
0	0	0	0	0	0	0	0	vt		45.7	8	23	21	21.6	17
75.8	2	105.8	18	119.9	12	62.7	9	vt		30.9	12	130.3	17	141.4	9
860.8	6	838.4	4	760.1	2	734.2	13	237.8	3	666.2	27	615.2	4	719.6	7
56.8	3	51.2	13	32.9	19	29.3	22	vt		48.0	11	43.3	16	45	28
0	0	0	0	0	0	0	0	vt		75.8	13	72.7	8	42.2	6
111.5	19	139.3	10	133.4	13	39.9	21	vt		71.3	11	55.9	25	42.4	21
2807		3276		3339.2		3208.3		1542.9		3447.4		3651.5		3568.5	

0	0	0	0	0	0	0	0	20.6	9	37.5	9	21	15	19	7
0	0	0	0	0	0	0	0	145.1	22	163.7	11	81.7	7	66.6	10
0	0	0	0	0	0	0	0	124.3	13	191.8	8	124.1	12	107.7	14
8.1	5	0	0	0	0	0	0	89.4	5	86	8	70.6	7	80.1	2
170.2	5	117	17	113.8	12	81.4	21	191.2	2	323.3	12	283.1	11	244.2	13
120.4	17	176.8	7	175.9	7	104.2	26	126.7	13	229.9	8	190.5	23	136.6	13
93.9	6	102.6	8	113.4	23	69.2	22	0	0	0	0	vt		10.3	11
0	0	0	0	0	0	0	0	67.1	21	67.8	8	21.1	9	17.7	11
0	0	0	0	0	0	0	0	28.6	8	33.7	18	28.7	11	26.2	10
21.2	6	20	10	14	11	0	0	20.8	10	28	6	24.4	19	25.6	11
41.9	5	46.6	7	56	4	54.4	3	0	0	0	0	27.6	22	31.6	6
455.7		463		473.1		309.2		813.8		1161.7		872.8		765.6	

14.7	14	12.6	11	16.5	5	17.9	12	0	0	0	0	3.4	6	4.2	12
32.6	16	30.2	4	27.9	9	26.8	20	21.3	6	48.6	9	57.1	18	59.6	7
16.3	13	20.8	15	19.1	10	19.3	6	13.2	4	20.0	23	17.8	14	24.7	18

25.8	12	28.1	20	36.9	20	37.2	18	10.1	13	21.2	23	25.4	23	27.2	23
20.8	7	24.3	6	31.8	12	39.5	10	30.1	18	33.1	16	30.9	17	35.8	23
8.7	5	8.3	13	8.3	14	8.2	16	4.7	12	5.3	10	6.2	14	12.2	7
15.3	18	20.8	10	17.3	19	28.3	12	14.1	8	27.6	12	24.1	12	29.5	21
73.9	12	64.2	27	67.9	15	63.1	17	53.5	17	62.4	11	53.5	19	30.3	18
39.1	25	37.1	12	30.1	23	29.5	20	38.7	10	47.9	6	54.3	8	61.1	17
13.2	16	14.2	13	15.8	13	15.7	9	0	0	12.4	13	13.5	9	16.3	12
64.3	13	71.5	7	68.7	15	63.9	12	42.6	12	125.5	9	125.9	16	114.1	20
42.7	18	40.2	7	39.7	5	31.1	11	21.6	26	30.2	23	45.1	7	48.3	15
367.4		372.3		380		380.5		249.9		434.2		457.2		463.3	
42.4	5	45.3	5	39	5	40.8	5	0	0	0	0	35.6	11	44.5	26
22062.5		*21460.1*		*21906.8*		*21401.8*		*13849.9*		*16356.3*		*20317.7*		*17830.8*	

				2010											
28/09/2012		05/10/2012**		24/08/2010		31/08/2010		07/09/2010		14/09/2010		21/09/2010		28/09/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2405.1	22	2556.1	10	1339.9	13	3312.7	10	3057	15	3867.4	13	4694.1	15	3575.8	5
680.5	19	655.2	13	294.7	17	1307.9	4	1008.9	4	693.3	12	572.1	14	395.9	13
4704.2	16	3875.2	24	1965.8	19	4525.5	5	6283.7	11	4126.6	4	5224.0	5	5321.4	6
620.1	8	529.0	32	534.6	1	727.4	3	926.2	12	727.4	12	550.6	9	580.1	5
1811.7	24	2323.2	6	1880.2	11	4673.8	4	1964.4	16	1391.7	13	1501.3	4	1180.8	11
2100.9	19	2296.6	19	928	7	2506	4	3097.9	6	3298.8	6	2979.6	7	6718.8	4
347.3	5	546.2	23	84.3	14	1059.8	5	862.6	10	485.3	5	444.3	11	313	11
12669.8		12781.5		7027.5		18113.1		17200.7		14590.5		15966		18085.8	
290	23	285.9	7	32.8	6	147.9	10	297.6	10	298.9	4	398.4	20	448.7	15
vt		vt						vt		vt		vt		vt	
216.7	26	184.6	13	14.6	9	165.6	5	370.6	17	299	14	281.7	14	348.3	16
506.7		470.5		47.4		313.5		668.2		597.9		680.1		797	
36.8	16	31.3	8	33.1	4	39.8	2	44.9	10	42.4	17	43.9	8	41.6	11
6.2	6	0	0	39.4	4	44.5	13	66.3	5	9.5	4	15.6	18	6.6	5
50.1	10	28.8	8	33.8	5	37.3	6	25.7	6	0	0	0	0	0	0
24.9	26	24.3	19	39.9	16	25.5	9	28.2	12	43.5	13	19.3	9	33.3	8
0	0	0	0	17.8	14	33.6	16	43.5	5	0	0	0	0	0	0
28.9	5	27.3	22	75.7	11	51.4	8	17.2	20	23.3	8	17	6	12.7	20
80.6	9	79.4	9	0	0	81	7	22.7	20	54.8	14	78.1	3	173.7	10
31.4	8	26.3	19	54.4	19	97.8	21	60.9	6	56.1	9	63.4	2	54.5	3
0	0	0	0	44.8	5	89.3	20	206.7	7	140.3	24	166.4	12	188.2	1
46.7	9	56.2	16	109.2	2	48	5	68.3	15	23.7	6	24.6	18	27.3	21
121.4	14	173.9	17	0	0	350.7	17	347.3	13	285.6	17	271.2	20	251.9	5
0	0	0	0	78.8	1	52.2	7	21.8	10	43.4	4	24.9	13	33.5	21

0	0	0	0	101.8	2	64.3	2	40	22	27	21	29.7	18	18.6	18
0	0	0	0	4.9	5	28.5	14	17.5	14	24.3	12	10.8	13	16.3	12
801.6	17	898.0	4	481.8	15	608.9	21	516.3	8	694.4	20	790.9	17	780.0	13
0	0	0	0	35.4	14	72.7	4	7.3	3	10.9	16	6.3	29	5.7	9
881.5	8	1012.3	4	345.4	15	439.2	7	688.9	20	454.9	11	579.5	20	517.5	6
0	0	0	0	34.9	12	33.1	4	46	3	0	0	0	0	0	0
73.3	14	66.1	26	22.1	12	46.2	8	93.2	13	50.6	6	41.7	18	40.6	18
0	0	0	0	42.2	2	102.5	16	232.8	18	118.5	14	82	13	98.1	2
65.7	22	62.1	5	28.6	19	81.3	16	119.6	19	50.3	8	90.8	11	98.7	21
74.9	13	68.3	10	0	0	161.3	16	173.1	26	115.4	18	109.4	19	177.9	8
43	7	52.2	7	75.3	6	78.4	15	39	13	41.4	14	29.4	14	29.1	18
21.1	8	23.4	23	0	0	0	0	41.9	5	27.2	6	24.1	9	29.9	6
214.9	19	144	9	350.3	3	354.8	11	485.4	9	838.2	10	502.5	17	657.1	6
44.1	11	27.7	5	16.8	6	35.8	15	41.4	13	28.5	18	34.8	3	19.5	7
32	9	34.4	11	0	0	13.1	7	32.6	14	76.5	2	90.1	1	86.9	2
46.3	23	56	8	vt		62.5	15	153.5	18	235.7	22	221.2	7	237.5	20
107.8	14	170.8	22	vt		61.0	12	147	6	135.5	10	211.5	7	145.2	14
810.4	11	874.5	11	310.4	10	658.5	21	717.1	6	1002.2	6	1091.8	5	902.9	12
40.2	7	51.1	7	vt		36.8	2	55.8	11	63.3	21	67.6	15	58	12
50.3	12	28.6	16	vt		34.4	5	65.5	8	31.2	16	26.3	5	43.7	20
44.5	13	26.6	23	vt		41.7	16	94.7	16	102.2	9	61.2	7	88.1	19
3741.8		4012.3		2343.7		3926.3		4717.2		4808.4		4782.1		4833	
13.3	21	17.8	28	26.9	17	65.6	8	29.3	6	34.9	6	0	0	0	0
63.1	6	54.9	4	138.5	22	88.3	7	31.5	4	0	0	0	0	0	0
95.5	7	62.9	15	0	0	0	0	0	0	0	0	0	0	0	0
68.6	4	63.3	12	220.4	19	339.7	4	299.1	10	165.6	4	190.1	21	193.9	11
234.7	14	100.9	9	175.4	30	505.4	13	564.9	7	285.2	15	273	10	290.8	8
149	29	98	9	308.2	21	562.6	14	623.2	21	723.1	2	518.7	20	529.3	15
7.6	8	0	0	vt		22.3	11	13.3	16	15.2	10	12.3	10	11.4	18
18.2	11	20.5	24	0	0	0	0	0	0	0	0	0	0	0	0
26.9	27	35.1	24	41.8	18	17.6	22	16.4	5	0	0	0	0	0	0
21.4	11	18.9	26	vt		70.5	9	91.4	2	19.8	6	23.2	6	16	7
52.2	7	48.9	19	vt		48.5	13	42.6	3	66.5	2	59.2	13	57.7	13
750.5		521.2		911.2		1720.5		1711.7		1310.3		1076.5		1099.1	
9.8	3	9.5	8	0	0	21.3	9	16.3	13	14.9	16	12.3	15	12.8	7
54.9	9	55.0	14	23	16	32	19	24	15	26.6	13	23.5	13	22.9	10
24.5	7	23	10	13.6	19	20.4	5	22.0	10	25.3	7	24.2	7	27.1	15

37.1	16	35.1	12	26.5	7	35.2	16	44.5	23	43.9	16	39.5	17	43.2	9
42.8	16	42.0	21	15.3	9	28.3	2	38.3	21	29.5	12	37.3	15	39.8	18
14.6	22	15.0	11	7.1	4	23.4	14	31.4	3	28.3	19	28.9	23	26.1	14
16.8	18	30.1	19	7.1	4	18.9	10	23.9	14	20.8	13	18.4	18	19.7	22
37.2	23	27.2	12	85.2	15	119.4	13	166.6	11	110	9	110.1	13	107.2	8
63	7	37.2	13	11.3	10	22.0	22	30.1	7	78.5	2	81.5	1	60.6	5
15.7	11	16.3	15	10.3	7	17.3	6	21.4	13	15.3	15	18.2	6	21.4	19
105.7	19	126.6	23	45.2	12	110.2	19	154.5	8	109.6	3	100.5	14	107.2	4
46.2	17	29.6	21	62.9	10	104.2	15	107.9	20	76.9	18	84.6	13	71.4	8
468.3		446.6		307.5		552.6		680.9		579.6		579		559.4	
43.3	6	35.7	13	0	0	0	0	0	0	65.4	5	65	4	75.6	2
18217.2		*18299.1*		*10670.4*		*24665.8*		*25023.6*		*21994.5*		*23192.6*		*25491.5*	

GC×GC peak area x 10^4 (arbitrary units)															
TN-SM2															
				2011											
05/10/2010		12/10/2010**		11/08/2011		18/08/2011		25/08/2011		01/09/2011		08/09/2011		15/09/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
5118.6	14	4032.7	19	2340.9	14	2883.4	20	3237.7	18	2850.8	9	3073	18	3293.5	16
209	20	333.2	16	489.6	47	698	13	641.3	25	446	13	1034.1	19	660.6	6
6465.7	16	5873.5	21	3415	18	5578	14	5041.8	20	5007.1	7	10705.4	13	9514.7	10
616.4	15	532.9	12	594.4	10	431	18	909.8	12	322.9	20	681.0	14	531.8	12
1596.1	11	2182.0	12	1902.5	14	2272.1	16	2038.8	5	1077.3	13	1965.1	22	1889.9	16
6388.5	2	2250.6	9	780.6	8	3127.2	21	2023.7	8	2288	11	3362.7	4	3175	4
219.9	15	281.3	13	340.4	16	544.3	16	266.6	19	213.9	23	638.7	6	524.8	16
20614.2		15486.2		9863.4		15534		14159.7		12206		21460		19590.3	
418.3	11	506.3	13	223.5	2	248.1	13	312.4	5	282.6	21	310	7	336.9	15
vt		vt												vt	
392.7	22	415.2	19	191.9	16	179.5	19	104	23	248.3	3	234.1	4	229.5	0
811		921.5		415.4		427.6		416.4		530.9		544.1		566.4	
40.2	14	38.1	11	29.6	6	26.7	18	28.1	17	24.1	12	22	24	28.9	7
7.6	1	6.2	6	vt		26.5	7	10.7	16	13.6	5	24.6	21	14.7	5
0	0	0	0	41.5	22	28.8	17	22.5	15	0	0	0	0	0	0
20.1	2	13.3	8	39.3	18	78.2	9	26.6	22	45.5	13	59	10	23.5	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9.7	7	6.5	2	54.4	16	91.8	11	116.7	21	16.3	14	16.4	11	21.7	18
141.9	9	133.7	17	42.6	16	91.8	8	115.1	23	115.3	4	210	2	233.1	14
80.8	7	94.5	5	81.2	19	51.7	3	47.5	7	51.5	6	56.1	4	25.1	8
163.2	12	169.8	13	0	0	0	0	0	0	0	0	0	0	0	0
24.3	13	29.3	7	242.9	13	253.5	5	84.1	16	67.9	14	137	11	84.9	5
205.6	11	177.5	21	51.4	11	34.2	10	60.5	10	76.1	8	100.3	17	66.1	3
31.1	8	24.9	20	85.3	10	90.4	22	158.9	28	39.9	11	15.7	18	155.1	21

13.5	9	13.9	7	0	0	0	0	0	0	0	0	0	0	10.4	12
9.4	7	7.2	13	0	0	0	0	0	0	0	0	0	0	0	0
804.0	16	812.1	12	158.7	16	1181.2	24	1938.2	6	1418.9	27	402.5	13	869.8	4
7.7	1	0	0	53.0	6	50.5	12	25.2	24	25.2	15	7	14	7.5	8
529.2	11	544.1	14	301.1	10	695.6	6	596.3	6	581.5	11	649.2	16	672.7	9
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.7	20	20.1	8	144.1	16	75.9	26	106	16	28.2	14	70.2	16	59.3	4
98.6	8	92.9	4	106.7	9	167	8	20.5	16	39	10	110.2	17	42.3	15
78.3	8	75.8	14	284.5	11	515.7	18	376.3	14	282	9	46	3	39.5	5
139.9	11	103.8	8	58.1	6	36.9	10	19.9	5	36	8	100.3	5	39.7	10
25.2	19	17.5	8	0	0	0	0	0	0	21.8	5	25.3	5	68.2	6
22.3	6	27	4	19.3	23	30.8	16	19	13	23.8	8	15.7	11	21.8	14
667.1	10	665.3	6	22.5	8	56.3	23	53.6	25	290.2	14	170.2	18	363.7	16
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.9	2	88.4	3	0	0	0	0	0	0	0	0	0	0	0	0
203	26	188.9	12	330.5	8	823.3	19	460.1	14	0	0	0	0	0	0
134	13	124.8	18	215.9	8	311.6	21	168.9	33	205.4	12	123.8	23	142.4	19
932.5	5	900.8	4	432.4	14	645.9	9	742.6	10	552.5	13	815.6	5	1167.3	18
57.3	11	57.6	8	0	0	73.5	12	23.5	12	33.9	7	59.1	5	57.2	4
53.5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67.9	12	49.5	13	38.8	6	42.2	5	62.4	13	21.6	7	116.8	15	161.4	8
4647.3		4445.4		2804.2		5453.3		5255.1		3986.1		3331		4347.4	
0	0	0	0	39.8	9	32.4	8	32.2	15	0	0	0	0	0	0
0	0	0	0	86.9	3	29.8	13	35	15	0	0	0	0	0	0
0	0	0	0	129.9	14	206	7	103.8	17	0	0	0	0	0	0
138.6	11	140.6	12	214.1	5	277.7	23	269.6	18	19.8	14	8.4	6	13.9	11
242.3	18	268.7	19	0	0	0	0	0	0	36.5	13	160.5	13	210.4	9
491.8	6	481.1	7	193.4	17	466.3	7	256.5	14	189.9	15	173.2	12	118.5	12
0	0	0	0	67.5	3	64.8	8	116.6	11	217.4	5	124.1	17	91.5	4
0	0	0	0	32.6	6	24.2	24	18.3	14	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22.5	9	19.1	8	28.4	8	21.9	15	11.1	10	18.2	9	25.7	26	23	16
63.1	5	65.6	8	0	0	24.7	14	24.3	5	37.8	7	111.1	19	54.2	7
958.3		975.1		792.6		1147.8		867.4		519.6		603		511.5	
14.3	9	11.2	10	vt		56.3	11	31.9	16	31.5	4	25.6	10	17.3	15
21.6	23	21.0	16	0	0	0	0	27.6	7	38.8	5	35.5	7	22.9	9
25.3	7	21.5	10	15.6	18	18.4	16	21.4	12	28.4	17	31.3	15	54.4	7

45.8	16	53.2	23	12.7	12	26.4	15	17.0	7	19.8	12	24.8	4	26.8	16
45.9	14	47.4	3	10.5	21	13.6	12	21.6	11	17.8	13	26.3	5	46.8	5
27.3	6	26.2	9	0	0	7.5	23	8.9	10	8.4	4	8.0	4	5.2	2
17.4	18	19.0	12	22.8	16	9.6	13	10.2	8	7.2	15	11.2	10	11.7	7
109.3	20	110.2	15	53.5	23	84.9	14	65.3	16	62.8	12	52.8	14	43.7	18
52.8	13	54.9	7	42.4	11	63.5	17	59.2	19	46.3	23	44.7	18	40.3	14
22.7	8	21.9	13	0	0	0	0	20.3	8	25.2	12	17.0	9	21.4	12
114.8	21	122.3	8	84.2	10	103.0	12	89.4	12	82.6	7	75.2	12	69.4	9
77.7	15	71.8	14	66.3	21	79.9	9	85.8	5	76.9	13	74.4	0	71.8	10
574.9		580.6		308		463.1		458.6		445.7		426.8		431.7	
75.6	2	77.7	1	0	0	0	0	0	0	0	0	45.3	5	57.2	8
27721.5		*22524.6*		*14213.2*		*23052.5*		*21185.3*		*17712.4*		*26432.2*		*25533.4*	

						2012									
22/09/2011		29/09/2011		06/10/2011**		31/08/2012		07/09/2012		14/09/2012		21/09/2012		28/09/2012	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
3155.8	8	4477.8	9	2471.6	10	2557.7	17	2718.3	10	1989.5	14	2063.3	9	1384.7	16
850.6	7	828.4	9	759.6	19	1587	20	1310.9	17	1969.3	7	1204.7	23	563.7	17
10568.6	20	9599.9	14	8710.7	23	2013.1	20	1653.7	15	2560.4	14	3927.1	16	4771.8	15
653.5	4	986.9	10	824.0	13	212.6	24	204.3	17	369.8	24	517.9	20	644	28
2225.2	12	2098.7	12	2822.2	20	1741.9	11	1647.1	9	1840.7	9	835.7	6	936.6	19
3219.7	3	3611.2	4	3552.2	7	2107.1	17	2066.7	5	2832.8	18	2072.6	10	1821.2	17
784.6	9	884	15	582.4	13	857.6	13	656.4	13	582.5	5	746.7	10	310.1	14
21458		22486.9		19722.7		11077		10257.4		12145		11368		10432.1	
399	10	314.5	13	391.5	8	41	6	164	17	212.7	14	327.7	7	340.3	8
vt		vt		vt						vt		vt		vt	
222.5	19	258.1	15	286.7	22	28.2	15	101.9	5	166.9	20	294.9	6	306.3	23
621.5		572.6		678.2		69.2		265.9		379.6		622.6		646.6	
26.2	8	25.9	19	18.6	19	30.8	4	28.6	5	31.1	5	34	12	41	6
14.8	10	14	14	0	0	39.9	10	35	9	42	5	51	6	36.8	13
0	0	0	0	36.6	24	61	9	65.6	15	63.8	16	61	9	59.9	6
30.5	19	39.7	18	38.9	6	35.7	9	36.9	10	33.4	19	40.2	16	25.8	4
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13.7	6	33.9	8	64.1	16	34	15	48.5	8	47.5	8	52.3	14	48.4	4
183.8	12	184.9	3	57.5	17	178.1	18	139.3	12	133.3	24	115.2	16	106.9	24
24.1	5	172.5	17	40.7	9	57	6	53.8	8	50.1	7	48.8	8	38.6	12
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79.4	6	75.5	6	105.4	12	138.1	20	114.4	12	100.2	5	73.7	17	65.5	7
85.2	17	66.9	0	50.2	4	504.2	6	204.1	10	195.7	9	209.6	19	153.3	13
60.9	9	57.5	7	87.5	11	0	0	0	0	0	0	0	0	0	0

10.8	10	13.6	11	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1395.5	17	979.4	6	1117.7	24	419.8	11	1409.2	16	1100.6	10	1155.4	18	956.8	16
7.7	3	5.3	6	40.7	14	0	0	0	0	0	0	0	0	0	0
528.1	21	628.2	13	553.7	21	177.5	24	1099.5	11	1026.5	20	1096.6	14	1121.2	9
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52.7	10	51.6	11	68.7	5	21.6	14	27.7	26	73.2	12	77.8	14	75.8	10
76	54	36.5	8	86.8	16	0	0	0	0	0	0	0	0	0	0
45.9	8	40.4	11	227.9	12	51.7	4	58.5	7	55.8	9	58.7	5	67.8	6
54.3	7	83.7	19	51.3	13	121.3	19	74.1	5	99.4	19	98.7	24	99	12
65.9	4	65.5	11	0	0	74.1	6	62.6	8	50.3	12	68.8	3	61	4
19.1	11	23.6	15	26	20	0	0	0	0	19.8	17	20.7	15	21.1	11
261.7	10	416.9	16	26.2	5	128.6	21	253.6	8	269.1	14	290.9	17	251.3	24
0	0	0	0	0	0	57.9	21	59.6	8	77.1	9	47.4	8	47.4	5
0	0	0	0	0	0	0	0	27.8	17	26.2	16	51	9	61	6
0	0	0	0	260.9	14	vt		51.6	7	38.7	13	40.9	14	56.1	8
116.5	13	205.7	14	114.7	14	vt		119.1	19	150.1	13	151.4	17	174	17
1017.8	12	1074.1	10	1095.2	9	388.3	14	782.1	13	849.2	12	986.7	17	1025.4	13
52.9	10	40.8	20	0	0	vt		46.1	16	44.3	9	51.7	6	65.3	14
0	0	0	0	0	0	vt		80.4	25	81.4	8	59.7	8	49.4	10
151.5	5	218.8	7	58.1	26	vt		111.8	20	58.5	6	45.6	9	40.3	7
4348.8		4529		4208.8		2488.8		4961.3		4686.2		4953.8		4708.1	
0	0	0	0	26.5	26	22.8	6	51.5	10	21.4	22	21.6	22	22.7	17
0	0	0	0	0	0	153.5	18	194.7	13	116.7	16	85.2	3	73.8	13
0	0	0	0	114.6	17	140.8	15	206.7	6	170.4	15	150.8	22	102.2	6
0	0	0	0	195.9	12	126.2	14	112.2	13	86.1	5	76.8	5	70.3	8
142.3	5	172.8	14	0	0	255.6	10	343.7	14	344.2	14	328.5	19	246	4
165.9	17	183.7	9	150.7	20	166.9	25	259.4	7	236.5	26	221.4	8	239	12
103.6	12	128.4	15	32.1	10	0	0	0	0	0	0	11.2	12	8.1	5
0	0	0	0	0	0	76.5	30	79.1	7	38.5	6	27	21	23.1	12
0	0	0	0	0	0	42.7	21	37.3	9	48	12	43.6	14	42.9	5
25.5	15	18.3	16	17.9	15	21.4	22	35	9	26.1	19	25.4	15	30.1	26
51.7	7	59.8	7	0	0	0	0	0	0	37.7	11	38.8	1	56.1	22
489		563		537.7		1006.4		1319.6		1125.6		1030.3		914.3	
13.1	13	11.3	12	10.2	12	0	0	0	0	0	0	0	0	14.1	8
24.4	10	25.3	13	30.5	9	32.1	12	52.8	23	66.3	8	75.7	7	63.5	7
53.8	9	50.1	4	43.7	23	17.9	18	20.2	21	23.1	9	26.5	12	28.8	6

28.8	6	28.7	7	32.5	19	11.2	21	24.9	11	35.7	17	28.6	11	31.6	11
54.6	10	48.4	9	43.5	13	10.8	17	15.9	29	17.2	7	20.0	12	33.6	15
5.1	6	9.0	3	8.5	7	6.7	27	6	14	6.2	5	7.1	21	8.9	18
10.5	6	10.1	7	7.4	10	14.8	13	31.5	6	33.1	22	38.2	22	45.2	19
44.7	14	42.9	21	32.1	9	32.4	11	54.8	15	47.2	16	38.5	12	36.2	13
40.6	30	39.5	13	42.4	21	51.2	8	103.4	8	114.8	12	99.5	17	80.4	4
18.3	23	17.6	19	17.4	12	0	0	0	0	13.5	21	15.3	14	18.4	13
61.3	15	73.3	9	69.6	15	23.9	11	123.2	2	138.1	13	122	20	133.0	23
69.9	2	66.6	4	63.4	12	38.3	16	35.6	8	49.5	12	51.6	13	40.5	4
425.1		422.8		401.2		239.3		468.3		544.7		523		534.2	
64.3	3	65.3	6	60.7	7	0	0	0	0	42	13	57.5	5	54.2	6
27432.9		*28665.5*		*25627.9*		*14911.5*		*17301.1*		*18954.2*		*18589.2*		*17330.5*	

												2010			
05/10/2012		12/10/2012**		24/08/2010		31/08/2010		07/09/2010		14/09/2010		21/09/2010		28/09/2010	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2296	12	2212.4	14	1776.5	15	3998.7	13	2894	1	4180.3	19	4182.5	17	4127.6	20
486.9	17	410	18	372.7	18	1082.7	6	918.4	2	709.1	9	588.8	14	468.5	5
5277.4	20	5341.1	19	1984.1	12	944.4	8	5759.4	7	4481.7	7	4211.8	18	5526.8	14
446.1	27	474.8	20	468.3	10	433.1	7	726.3	14	170.6	14	151.3	20	186.4	12
1318.9	11	1519.2	7	1119.8	21	2224.9	5	1989.1	14	1357.2	15	1189.9	22	1638.2	7
2272.2	7	1757.3	8	915.1	7	2423.9	8	2762	15	3280.2	11	6584	6	6869.3	7
423.2	12	328.7	12	112.3	4	842.2	6	723.5	12	591.9	7	446.4	14	376.3	17
12520.7		12043.5		6748.8		11949.9		15772.7		14771		17354.7		19193.1	
333.9	1	341.4	11	28.8	16	130.7	22	188.1	7	265.4	21	358.8	11	432.3	12
vt		vt												vt	
302.4	12	296.7	16	19.2	3	170	14	255.1	21	180.5	14	178.3	11	308.6	10
636.3		638.1		48		300.7		443.2		445.9		537.1		740.9	
38.2	13	30.2	20	17.7	10	24.4	13	31	13	36.3	9	34.5	5	31.5	5
29.1	11	15.7	20	43.9	12	43.1	6	51.7	17	72.5	2	85	3	5.3	4
48.6	13	35.1	11	22.1	15	21.5	5	32.3	4	0	0	0	0	0	0
44	7	29.6	6	20.7	15	48	12	32.6	20	17.3	20	11.7	16	24	23
0	0	0	0	9.6	15	25.1	8	12.3	19	0	0	0	0	0	0
36.4	21	31.6	5	64.6	23	33.6	14	33.4	19	18.3	18	12.4	18	21.3	11
85.4	7	82.3	6	0	0	55.9	6	22.5	41	56.6	2	81.3	13	36.5	3
40	5	26	14	37.3	9	45.3	7	50.9	2	75.1	4	50.5	1	59.1	10
0	0	0	0	46.7	19	51.1	4	124.1	5	166.6	5	130.1	10	150.2	12
73.7	22	58.6	12	77.2	22	31.6	12	67.1	15	49.8	11	17.6	6	15.8	18
178.8	22	163.9	23	0	0	357.1	19	220.1	31	225.1	14	243.2	13	187.8	19
0	0	0	0	60.5	13	21.8	15	41.4	5	18.6	16	20.8	18	22.6	16

0	0	0	0	73.2	0	41.1	1	16.8	10	10.5	13	11.4	7	10.4	5
0	0	0	0	0	0	19.5	13	11.6	10	6.4	5	10.5	1	11	15
1557.4	9	1488.1	15	107.2	12	183.8	17	180.3	24	195.6	6	101.4	13	107.9	3
0	0	0	0	24.5	12	6.7	4	7.3	2	7.8	3	0	0	0	0
965.4	2	954.1	18	180.6	19	324.8	6	555.1	9	488.8	17	397.7	15	376.3	16
0	0	0	0	24.7	11	8.2	5	75.7	2	0	0	0	0	0	0
74.9	7	87.7	14	14	4	49.2	15	83.8	14	30	8	42	4	22.9	12
0	0	0	0	18.4	7	40.3	21	108.8	16	53.8	0	56.9	5	53.5	3
87.9	7	95.9	24	19.6	13	108.1	22	88	16	65.7	7	76.5	8	67	8
91.9	18	89.4	24	0	0	119.4	19	130.3	16	104.9	18	105.7	6	97.6	18
48.6	16	60.5	10	46.8	22	37.4	10	62.1	5	27.5	13	17.8	17	17.8	15
28.8	8	31.4	28	0	0	0	0	33.9	11	27.6	12	34.4	10	23.1	7
221.6	10	212.6	21	208.1	25	359.8	18	404.9	4	385.9	14	383.5	23	247.1	21
31.4	3	59.1	12	14.7	5	53.9	8	30.2	9	33	4	30.8	7	19.7	5
49.2	13	45.5	13	0	0	18.7	15	15.5	18	10.5	3	11.9	9	9	6
62.8	6	62.7	1	0	0	51.8	12	116.2	21	104.7	10	74.9	10	70.1	11
212.3	20	219.4	9	0	0	72.6	4	97.1	14	45.8	15	46	11	77.8	11
1263.0	22	1059.1	11	231.1	3	283.4	13	424.4	13	456.8	11	542.0	5	568.0	11
57.8	5	43.2	10	0	0	68.8	3	40.9	15	56.5	18	43.5	4	39	9
53.7	9	41.6	8	0	0	34.4	16	41.1	12	25	15	51.4	7	28.1	8
37.6	22	34.5	16	0	0	42.3	7	51.7	9	53.6	21	56.7	14	98.8	17
5380.3		5027.6		1345.5		2658.3		3264.1		2890.3		2747.6		2467.7	
19.5	12	20.5	12	28.6	13	23.9	5	34.1	9	33	4	0	0	0	0
57.1	12	58.2	5	54.2	11	14.5	2	39.3	11	0	0	0	0	0	0
70.3	13	67.1	4	0	0	0	0	0	0	0	0	0	0	0	0
69.5	8	62.4	14	13.9	17	192.5	30	251.4	21	239.7	32	172.4	23	112.9	7
262.5	8	262.2	14	135.8	27	440.5	5	523.6	19	227.3	12	255.2	12	281.7	13
161.9	25	128	21	242	8	425.5	15	406.5	18	499.5	7	265	13	226.2	28
0	0	0	0	0	0	5.5	16	7.2	10	6.7	28	5.5	8	4.5	7
21.4	27	19.5	23	0	0	0	0	0	0	0	0	0	0	0	0
40.7	4	40.9	8	18.3	1	13	2	16.7	6	0	0	0	0	0	0
22.5	17	21.4	10	0	0	36.4	9	51.6	7	22.8	8	16.4	1	31.2	11
51.6	8	54.7	4	0	0	48.4	18	35.9	6	54.6	15	56.0	16	60.0	10
777		734.9		492.8		1200.2		1366.3		1083.6		770.5		716.5	
13.7	24	13.1	18	0	0	0	0	0	0	12.4	12	13.4	6	8.2	4
59.6	11	59.1	6	0	0	0	0	4.3	7	6.4	10	7.3	11	23.5	13
26.5	13	26.1	24	0	0	0	0	0	0	0	0	0	0	0	0

38.7	6	45.3	26	38.3	10	41.9	18	44.2	13	47.4	21	44.9	19	51.4	11
41.1	10	45.9	16	0	0	0	0	12.7	15	13.5	17	23.6	12	27.2	7
12.8	7	13.3	18	6.1	19	13.5	13	17.4	18	14.6	11	12.0	7	11.5	12
40.2	12	23.4	25	7.3	4	12.8	11	14.6	10	12.5	13	13.4	16	15.2	18
37.0	22	35.2	12	32.5	12	86.8	20	64.3	21	93.4	5	86.7	5	77.5	20
73.5	17	53.9	19	13.7	17	14.6	19	21.9	7	54.1	2	50.1	5	47.8	4
19.4	11	19.3	12	0	0	0	0	19.6	7	21.4	13	17.3	12	16.3	11
142.5	21	133.8	16	0	0	88.9	8	115.2	12	70.5	6	87.7	10	70.8	16
35.4	21	32.6	16	58.9	9	91.1	8	86.3	11	54.1	16	42.9	15	62.2	8
540.4		501		156.8		349.6		400.5		400.3		399.3		411.6	
43.4	9	41.8	14	0	0	0	0	0	0	11.2	11	12.3	12	9.9	8
19936.3		*19017.1*		*8809.6*		*16483.1*		*21277.8*		*19638.6*		*21856*		*23571.2*	

TN-SM3															
						2011									
05/10/2010		12/10/2010**		19/10/2010		11/08/2011		18/08/2011		25/08/2011		01/09/2011		08/09/2011	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4647.7	14	4303.3	8	3327.3	8	2471.6	10	4599.6	6	4299.5	17	3278.3	8	3212.8	17
323	6	383.8	15	707.2	10	306.0	19	605	13	536.4	13	527.3	20	940.1	9
4342.7	4	5076.7	14	4816.5	14	4387.1	23	6088.5	19	6038.2	24	6614.7	17	10888.6	10
322.0	18	414.7	23	407.3	19	524.0	13	394.2	19	312.4	15	336.3	18	415.1	6
1403.9	8	2079.4	16	1746.4	16	1822.2	20	2066.7	20	1631.9	7	650	12	1388.3	15
5918.6	2	3525.3	17	3086.8	4	757.4	7	2055.8	27	2456.6	17	1752	18	2683.3	3
217.9	21	338.7	8	566.3	20	489.0	13	529.8	7	539.6	20	230.5	12	602.6	14
17175.8		16121.9		14657.8		10757.3		16339.6		15814.6		13389.1		20130.8	
387.2	11	375.6	7	366.2	11	191.5	8	200.2	9	201.7	15	202	16	235.1	13
vt		vt		vt										vt	
213.1	8	326.4	5	347.5	16	186.7	22	243.8	14	208.5	16	148.2	27	268.2	9
600.3		702		713.7		378.2		444		410.2		350.2		503.3	
32.3	8	30.4	11	28	14	18.6	19	22.2	10	16.2	20	14.1	12	12.4	14
4.6	5	0	0	0	0	0	0	0	0	7.8	10	9.9	5	11.4	5
0	0	0	0	0	0	36.6	24	15.3	10	16.9	8	0	0	0	0
15.2	18	23.7	14	19.9	18	38.9	6	31.6	15	20.8	14	23.7	14	33.6	14
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7.4	18	17.3	8	18.3	16	64.1	16	26.5	23	13	13	7.0	7	13.8	4
91.4	10	49.5	20	17.7	4	57.5	17	88.4	9	10.5	11	62.6	7	103.7	7
72.3	2	65	13	38.8	14	40.7	9	22	5	24	12	33.8	11	43.9	2
137.8	11	125.4	15	108.9	7	0	0	0	0	0	0	0	0	0	0
15.7	11	22.3	7	19.8	9	105.4	12	80.7	13	41.1	13	25	11	35.4	12
123	25	211.4	20	213.3	10	50.2	4	39.5	5	37.1	10	42.1	8	62.3	10
15.2	14	27.9	19	48.8	9	87.5	11	32.5	18	40.6	98	13.1	15	10.8	20

13.5	19	14.8	17	18.7	6	0	0	0	0	0	0	0	0	vt	
3.7	3	8.1	1	0	0	0	0	0	0	0	0	0	0	0	0
94.9	11	164.6	21	126.8	22	117.7	24	465.7	20	546.2	4	669.7	2	682.6	6
0	0	0	0	0	0	40.7	14	48	13	34.4	6	30.8	4	0	0
354.0	5	306.2	10	380.2	15	153.7	21	277.2	21	381.5	10	306.3	14	335.8	19
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25.2	6	22.2	18	19.2	12	68.7	5	29	15	39.5	14	15.8	12	42.2	15
44.3	5	0	0	0	0	86.8	16	121.5	16	25.1	4	19.3	11	27.3	11
39.9	17	57.6	17	64.4	4	227.9	12	185.6	17	208.3	23	142.5	11	46.5	10
37.5	22	70.1	12	79.3	4	51.3	13	16.8	21	15.4	21	27.2	6	72.4	7
17.8	11	32.2	9	39.5	14	0	0	0	0	0	0	0	0	24.8	16
17.6	4	24.9	14	22.5	16	26	20	17.4	22	13.8	14	16.6	17	19.3	5
169.1	17	188.9	11	122.1	22	26.2	5	34.4	25	12.2	23	186.4	4	228.9	15
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84.3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52.4	9	116.3	135	47.7	16	435.1	18	669.3	20	421.9	14	0	0	0	0
49.3	13	74.3	22	43.7	29	260.9	14	195.3	29	106.3	21	181.1	15	127.4	13
417.8	9	659.3	25	644.3	28	114.7	14	245.3	13	603.7	17	597.2	9	502.3	19
35.2	12	54.9	5	0	0	vt		46.8	10	30.8	11	22.1	15	35.9	13
24.5	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35.4	11	45.1	17	52.7	3	58.1	26	36.2	7	63.1	17	53.7	2	70.2	3
1999		2382		2146.6		2148.7		2725		2714		2485.9		2530.5	

0	0	0	0	0	0	26.5	26	17.9	6	vt		0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	114.6	17	165.1	18	106.6	9	0	0	0	0
118.6	10	122.7	9	142.3	15	195.9	12	236.4	21	101.4	23	0	0	0	0
207.4	13	194.1	11	200.1	14	0	0	0	0	0	0	19.3	16	12.2	20
209.4	19	172.2	15	160.7	14	150.7	20	221.5	7	105.7	15	92.1	14	133	22
0	0	0	0	0	0	32.1	10	50.6	6	101.4	10	122.4	10	128.8	11
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13.2	19	12	1	21	13	vt	0	17.8	8	10.6	9	13.9	7	18.9	19
56.6	12	57.9	15	60.1	12	17.9	15	15.5	20	15.7	12	22.4	5	40	9
605.2		558.9		584.2		537.7		724.8		441.4		270.1		332.9	

9.3	6	8.5	12	8.6	12	11.4	14	15.7	7	20.3	7	20.3	9	20.0	12
35.2	23	39.5	13	37.2	10	0	0	0	0	22.7	8	24.0	5	27.3	9
0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.0	17

46.3	9	41.2	22	38.5	15	15.3	18	21.6	10	37.3	19	34.5	5	33.5	12
28.6	15	25.6	13	26.1	12	0	0	vt		21.2	21	19.3	19	18.3	14
10.4	19	11.2	16	12.5	21	0	0	0	0	6.6	6	5.3	5	5.0	12
13.2	13	10.9	13	11.2	14	0	0	0	0	14.2	19	18.4	13	13.7	5
72.3	9	72.3	11	64.8	9	20.4	13	24.6	11	45.2	15	53.9	21	47.5	17
46.6	17	47.1	9	42.9	10	vt		23.2	13	33.0	22	29.2	11	26.6	19
14.6	13	13.5	17	12.3	13	0	0	0	0	0	0	9.6	15	10.3	13
69.7	5	66.9	7	53.6	16	26.3	24	30.2	17	32.4	26	30.6	21	27.3	16
59.3	7	51.9	4	42.4	28	39.4	12	38.1	13	42.0	5	34.2	6	38.8	3
405.5		388.6		350.1		112.8		153.4		274.9		279.3		280.3	
11.6	8	11.3	19	10.1	5	0	0	0	0	0	0	0	0	0	0
20829.7		*20195.1*		*18490.5*		*13953.3*		*20409*		*19671.3*		*16788.7*		*23790.2*	

				2012											
015/09/2011		22/09/2011***		31/08/2012		07/09/2012		14/09/2012		21/09/2012		28/09/2012		05/10/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
4987.5	9	3447.6	12	3001	23	3041.1	9	2687.7	21	1499.7	11	1455.1	13	2326	10
269.9	14	633.2	18	1641.1	27	1693.4	22	1784.2	16	1197.4	12	522.0	13	751.3	21
6992.1	9	9130.7	20	1958.5	31	2721.2	18	3580.2	30	4863.7	31	3709.5	13	3809.2	29
565.1	13	761.7	19	675.4	25	423.3	7	409.2	26	634.4	23	755.5	12	7324.6	31
1456.8	15	1376.5	11	1572.3	15	1790.4	17	2859.9	17	1685.8	27	1780.0	22	2105.2	27
2492.4	14	3099.0	17	1960.3	8	2468.4	14	3074.6	16	2384.7	16	1858.4	9	1826.3	15
643.3	17	507.8	9	874.9	14	726.4	8	821.8	14	739.2	18	316.3	23	443.2	12
17407.1		18956.5		11683.5		12864.2		15217.6		13004.9		10396.8		18585.8	
311.1	15	359.3	11	34.9	13	76.1	8	134.3	19	197.6	13	215.5	20	216.0	28
vt		vt								vt		vt		vt	
354.1	4	259.2	8	24	6	64.5	6	113.8	11	170.5	29	199.2	22	192.5	11
665.2		618.5		58.9		140.6		248.1		368.1		414.7		408.5	
15.3	20	16.4	11	19.4	19	20.7	12	16.8	6	16.3	18	20	11	16.3	17
10.3	5	0	0	20.1	12	21.3	8	29.6	22	24.5	11	0	0	0	0
0	0	0	0	11.4	20	10.3	10	17	20	19	20	27.9	10	19.7	20
22.3	19	17.1	11	16.7	28	18.8	10	20.4	18	27.6	5	22.6	16	16.6	7
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.3	15	9.2	12	39.5	47	30.2	9	29.4	3	30.4	13	25.9	16	16	29
148.8	13	108.8	11	109.1	3	107.6	11	117.4	7	88.4	9	78.2	10	59.2	12
20.5	22	18	10	29.1	6	23.8	15	37.2	11	21	12	18.7	21	12.5	22
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65.9	7	59.4	2	96.5	14	90.6	4	78.2	7	55.2	11	35.6	15	43.1	13
60.4	3	46.6	16	148.2	15	178.3	19	152.5	19	137.6	22	89.2	15	117	14
48.2	3	37.7	21	0	0	0	0	0	0	0	0	0	0	0	0

7.9	9	9.5	5	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
524.5	13	740.7	7	148.2	19	292.4	15	304	14	344.3	14	229.3	26	657.4	16
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
364.6	17	370.2	15	98.2	5	415.7	2	467.8	9	619.1	8	333.1	10	346	22
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45.8	3	30.1	19	16.1	14	20.2	20	43.8	17	43.7	21	32.5	13	30.8	19
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32.3	4	25.9	22	34.1	18	42.3	6	29.1	15	28.8	25	17.1	26	37.8	10
69.3	6	31.6	26	21.8	21	38.1	13	49.7	14	45.5	16	29.4	12	38.2	13
39.1	15	20.3	6	33.9	16	31.4	14	40.6	6	36.4	19	35.8	12	35.8	12
17.8	21	16.5	10	0	0	0	0	13.3	11	14.4	13	10.7	9	9.6	12
223.9	10	241.5	12	80.3	6	103.3	6	189.2	26	171.3	15	159.5	15	104.1	17
0	0	0	0	37.3	18	48.1	8	41.9	22	35.1	4	33.9	17	14	26
0	0	0	0	0	0	11.7	12	16	28	22.2	14	15.2	14	11.6	9
0	0	0	0	vt		30.2	14	18.8	13	21.1	26	38.6	10	25.8	10
100.9	20	104.0	21	vt		30.5	20	50.5	3	139	27	119.9	26	97.2	15
588.3	11	594.2	6	268.9	23	294.1	10	366.5	24	400.5	26	434.2	22	496.1	13
43	12	71	12	vt		38.2	9	34.3	20	38.2	20	40.6	19	16.9	17
0	0	0	0	vt		64.9	12	19.8	15	33.3	6	22.8	11	15.4	19
113.2	10	149.9	13	vt		67.4	10	34.7	10	22.5	12	35.9	13	24.4	22
2559.3		2702.2		1209.4		2009.4		2201.7		2419.1		1886.6		2245.2	
0	0	0	0	15.3	32	37.3	7	18.3	20	10.2	6	11	14	10.1	29
0	0	0	0	103.8	28	98	3	79.4	11	68.0	7	51.2	11	49.0	9
0	0	0	0	89.9	3	102.3	6	88.6	3	74	25	71.1	2	27.3	10
0	0	0	0	70.6	8	66.8	11	66.3	9	47.4	19	52.2	5	54.4	11
122.8	22	86.3	10	139.6	23	251.5	13	213.6	23	152.1	10	151	16	148.6	14
111.3	17	139.2	10	111.7	12	152.3	18	136	11	111.7	19	76.1	9	44.6	15
83.2	11	82.7	1	0	0	0	0	0	0	8.2	4	6.7	12	0	0
0	0	0	0	33.9	23	43	8	19.4	13	12.6	19	13.4	23	11.1	11
0	0	0	0	21	22	27.9	22	16	18	10.2	9	32.9	2	29.7	16
21.1	4	0	0	18.6	21	20.6	15	18	12	15.8	32	16	10	14.3	19
42.7	8	38.9	7	0	0	0	0	11.5	9	48.9	29	44.8	28	47.3	34
381.1		347.1		604.4		799.7		667.1		559.1		526.4		436.4	
18.5	18	14.3	15	0	0	0	0	0	0	5.4	12	8.1	4	10.2	14
22.4	7	25.9	11	21.2	10	27.2	19	33.8	18	41.9	24	47.9	20	41.2	4
18.8	12	20.1	13	10.4	13	18.5	22	16.3	21	20.3	15	22.2	24	20.3	22

37.3	18	33.1	9	10.0	5	29.2	9	29.4	17	31.4	18	33.8	14	35.1	18
19.5	6	17.6	17	8.1	8	19.9	8	20.9	14	24.3	21	26.4	22	27.7	9
6.4	9	8.3	11	5.4	7	5.0	5	7.3	8	8.3	14	9.3	12	12.5	9
12.8	14	14.3	9	21.2	13	29.9	16	22.9	27	19.8	6	12.8	19	20.9	19
40.1	16	38.3	12	65.3	16	68.4	13	59.0	9	54.2	12	53.9	13	52.3	10
25.3	18	26.2	13	22.7	8	30	8	31.4	6	26.4	10	26.7	15	17.6	6
11.4	7	10.6	8	0	0	0	0	9.5	13	10.4	12	11.5	10	12.1	12
28.6	13	20.1	17	43.1	17	89.1	10	86.3	7	81.3	8	78.0	19	87.9	15
39.5	14	38.1	20	17.6	4	53.6	29	42.2	27	40.9	24	38.2	12	27.8	14
280.6		266.9		225		370.8		359		364.6		368.8		365.6	
30.3	12	30.5	9	0	0	0	0	0	0	19.2	6	22	4	22.3	2
21338.9		*22938.1*		*13800.6*		*16205.4*		*18710.3*		*16751.3*		*13635.3*		*22080.1*	

Table S14

Volatile components determined for *Vitis vinifera* L. cv. Sousão variety obtained from one parcel (SO-SM1), at Bairrada Appellation, from 2010, during maturation and for 2011 and 2012 harvests, only maturity was considered.

		GC×GC peak area x 10^4 (arbitrary units)											
		SO-SM1											
		2010											
		20/08/2010		27/08/2010		03/09/2010		10/09/2010		17/09/2010**		24/09/2010	
[1]Dtr(s),[2]Dtr(	Compound	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
C_6 compounds													
194, 0.640	Hexanal	2069.8	6	4278.9	13	4652.4	2	4518.1	12	4847	9	6155	4
206, 0.630	3-Hexenal	391.9	11	1789.4	15	1982.7	3	291.5	11	630.7	10	917.6	7
230, 0.651	2-Hexenal	2232.5	7	5390.2	3	3736.3	5	4910.1	13	6101.1	13	5698.3	6
242, 0.630	3-Hexen-1-ol	1047.3	13	1280.9	16	1242.4	19	1392.1	2	2044.9	9	1338.5	8
248, 1.076	2-Hexen-1-ol	1059.3	10	4254.8	4	4543.6	11	6607.7	18	4650	5	4065.3	6
266, 0.903	1-Hexanol	1002	3	2640.3	13	2202.6	16	2529.3	12	2879.6	14	1558.2	19
296, 0.930	2,4-Hexadienal	194.9	10	1919.4	12	1288.3	16	698.9	14	801.1	9	959.9	4
	Sub-Total	*7997.7*		*21553.9*		*19648.3*		*20947.7*		*21954.4*		*20692.8*	
Aromatic alcohols													
420, 3.014	Benzyl Alcohol	59.5	3	72.8	14	109.4	8	152.0	16	221.1	3	241.3	6
470, 1.960	2-Phenylethanol	25.5	21	98.5	17	114.1	3	125.2	5	211.4	19	202.3	10
	Sub-Total	*85*		*171.3*		*223.5*		*277.2*		*432.5*		*443.6*	
Monoterpenic compounds													
314, 0.440	α-Pinene	34.2	12	22.8	17	11.9	9	26.3	20	19.9	7	20.1	16
338, 0.480	Dehydroxylinalool	21.3	12	37.1	16	vt		0	0	0	0	0	0
344, 0.457	β-Pinene *	32.1	5	22.3	13	13.6	13	12.7	9	10	2	0	0
392, 0.405	*m*-Cymene	21	9	32.3	1	16.1	7	15.1	8	12.9	6	14.7	13
398, 0.476	Limonene *	64.1	3	64	6	58.2	6	46.9	9	46.3	6	53.8	9
404, 0.476	1,8-Cineole	0	0	16.8	30	36.4	6	38.8	6	53.3	4	21.1	7
428, 0.678	Linalool oxide (iso	28.9	16	19.2	13	34.6	6	41.8	10	29.3	1	24.8	8
446, 0.700	Dihydrolinalool	0	0	15.8	16	17	18	13.6	15	11.5	5	0	0
452, 0.746	Linalool *	82.3	22	270.3	15	227.2	19	287.1	10	235.9	7	218.4	11
464, 0.844	Fenchol	84.6	10	164.7	17	248.0	14	242.2	15	238.3	23	192.7	9
470, 0.646	Hotrienol	0	0	24.7	16	21.5	19	22.2	14	25.7	4	19	6
506, 1.190	Pinocarvone	26.7	5	88.2	3	28	13	22.9	7	vt		0	0
518, 0.834	Ocimenol	0	0	16.5	18	15.0	19	19.6	8	20.0	18	12.7	5
530, 0.984	Menthol *	21.3	13	91.1	5	141.8	15	137.8	20	177.3	23	210.3	23
542, 0.835	α-Terpineol *	17.2	12	30.1	1	38.5	9	43.4	3	40	11	43.8	8

566, 0.703	Menth-1-en-9-al	10.5	23	91.7	7	68.3	3	26.3	36	40.4	9	43.1	7
584, 0.873	Geraniol (isomer)	0	0	vt		54.2	7	41.8	2	36.6	10	24.7	2
596, 0.976	Citral (isomer)	vt		58.2	6	145.9	8	92.5	6	121.1	16	117.9	14
602, 0.815	Carvone *	vt		22.6	16	19.9	15	14.7	19	14.7	11	16.2	11
	Sub-Total	*444.2*		*1088.4*		*1196.1*		*1145.7*		*1133.2*		*1033.3*	
C_{13} Norisoprenoids													
566, 0.532	*m/z* 159, 91, 131	42.6	10	139.6	5	46.1	15	59.9	14	45.6	7	22.0	7
620, 0.595	Vitispirane	646.9	22	509.5	6	412.1	2	363	10	211.5	8	123.6	9
674, 0.681	β-Damascenone (is	28.1	18	116	6	66.1	4	40.5	7	36.8	7	38.4	9
700, 0.702	β-Damascenone (is	300.4	15	444.5	16	459.8	8	337.0	5	206.4	9	228.2	12
736, 0.648	Geranylacetone *	253.3	12	717	9	361.5	12	310.3	12	237.3	4	146	8
900, 0.894	Methyl dihydrojas	vt		29.7	6	137.1	16	81.2	6	29.2	21	32.2	13
	Sub-Total	*1271.3*		*1956.3*		*1482.7*		*1191.9*		*766.8*		*590.4*	
Sesquiterpenic compounds													
756, 0.630	Aromadendrene	28.9	6	44	13	56.2	8	49.8	5	41	4	41.9	13
790, 0.660	α-Farnesene	vt		97.4	1	51.1	3	44.7	4	42	6	55.1	7
808, 0.630	Calamenene	26.6	21	10.1	8	8.3	9	8.1	1	5.4	2	vt	
912, 0.654	m/z 119, 91, 191,	74.1	12	210	3	158.9	5	183.8	15	228.7	5	171.2	16
	Sub-Total	*129.6*		*361.5*		*274.5*		*286.4*		*317.1*		*268.2*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

** Data obtained at maturity

vt - vestigial

2011		2012	
19/09/2011**		02/10/2012**	
Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
2408.1	21	1944.7	13
299.8	10	1017	17
2043	6	2652.4	15
482.5	8	1684.4	20
2588.4	17	2449.8	9
2718.1	20	2565.5	13
213.2	11	541.4	14
10753.1		*12855.2*	
112.1	13	133.0	16
132.5	10	261.1	16
244.6		*394.1*	
5.8	19	5.7	5
0	0	0	0
vt		11.8	23
12.7	17	25	23
21.3	9	23.3	23
32.2	5	vt	
vt		127	15
vt		vt	
35.6	20	213.6	18
19.5	11	86.0	8
27.8	13	54.7	21
vt		86.9	23
vt		25.9	7
17.3	11	137.6	2
4.5	17	59.7	3

vt		vt	
17.6	16	28.8	19
0	0	119.8	4
0	0	13.9	15
194.3		*1019.7*	
12.4	10	50.8	29
74.2	14	126.7	22
9.3	21	24.5	21
96	11	190.6	2
94.9	16	136.9	20
10.4	19	46.1	25
297.2		*575.6*	
20.9	14	13.7	23
29.1	17	42.9	3
vt		3.2	15
27.1	13	134.4	9
77.1		*194.2*	

Table S15

Volatile components determined in the glicosidically-linked fraction of mature grapes of *Vitis vinifera* L. cv. Arinto variety obtained from 3 parcels and 3 harvests under study, grouped by chemical classes.

		Concentration (μg L^{-1}) *																	
		AR-VA1						**AR-VA2**						**AR-SM1**					
		2010		**2011**		**2012**		**2010**		**2011**		**2012**		**2010**		**2011**		**2012**	
^{1}Dtr(s),^{2}Dtr(	Compound	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)	Media	RSD (%)
Monoterpenic compounds																			
314, 0.440	α-Pinene	6.7	6	2.9	21	2.1	10	4.5	10	3.7	4	5.2	5	9.1	1	3.7	16	3.1	22
344, 0.457	β-Pinene	1.3	12	1.0	8	1.2	11	2.7	0	1.8	12	1.5	9	1.6	13	1.5	9	1.6	12
362, 0.520	3-Carene	7.3	9	1.2	10	1.4	9	14.2	9	3.1	15	1.7	14	10.2	8	1.7	23	1.7	15
398, 0.476	Limonene	21.6	14	5.6	2	9.1	4	30.5	12	6.2	3	10.8	7	24.8	13	5.9	13	10.6	5
404, 0.476	1,8-Cineole	6.0	13	2.6	12	2.0	23	5.6	8	3.9	2	2.2	11	5.9	20	3.3	8	2.0	10
428, 0.678	Linalool oxide	1.1	11	1.2	9	1.2	14	1.8	10	1.4	14	2.1	7	1.5	8	1.2	14	1.3	7
452, 0.746	Linalool	17.5	9	10.3	16	11.5	11	36.0	10	11.0	19	17.4	3	22.3	12	12.1	9	15.2	13
614, 0.775	Citral (isomer	1.3	15	1.0	8	1.1	8	2.4	13	2.1	23	1.7	12	1.7	8	1.3	12	1.5	6
	Sub-Total	*62.8*		*25.8*		*29.5*		*97.7*		*33.3*		*42.7*		*77.1*		*30.7*		*37.2*	
C13 Norisoprenoids																			
736, 0.648	Geranylaceton	3.0	9	2.0	21	3.1	4	11.1	7	4.3	26	6.5	14	8.0	9	3.4	18	4.0	9
900, 0.894	Methyl dihydr	5.6	11	5.0	11	4.9	16	13.8	12	7.6	28	7.6	9	14.2	8	5.7	5	6.5	13
	Sub-Total	*8.6*		*7.0*		*8.0*		*24.9*		*12.0*		*14.1*		*22.2*		*9.2*		*10.5*	
Sesquiterpenic compounds																			
796, 0.525	α-Farnesene	2.4	9	1.5	13	1.9	2	5.8	12	3.0	12	3.5	5	4.3	11	2.3	12	2.6	10
1044 , 0.510	Ledene oxide	4.3	13	1.3	8	2.4	11	9.6	17	8.5	6	3.3	18	7.8	12	1.7	17	2.7	21
	Sub-Total	*6.7*		*2.8*		*4.3*		*15.4*		*11.5*		*6.8*		*12.1*		*4.0*		*5.3*	
	Total	*78.1*		*35.6*		*41.9*		*138.0*		*56.7*		*63.7*		*111.3*		*43.9*		*52.9*	

^{a} 1Dtr (s), 2Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

^{b} Concentration expressed as equivalentes of 3-octanol (μg L-1).

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

Table S16

Volatile components determined in the glicosidically-linked fraction of mature grapes of *Vitis vinifera* L. cv. Bical variety obtained from 3 parcels and 3 harvests under study, grouped by chemical classes.

		Concentration (μg L^{-1}) *																	
		BI-VA1						BI-VA2						BI-VA3					
		2010		2011		2012		2010		2011		2012		2010		2011		2012	
[1]Dtr(s),[2]Dtr(	Compound	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	Media	RSD (%)
Monoterpenic compounds																			
314, 0.440	α-Pinene	2.9	9	2.0	3	2.8	13	4.3	4	2.6	12	2.8	17	3.7	12	2.7	0	4.2	12
344, 0.457	β-Pinene*	6.4	3	2.8	6	1.8	17	7.4	4	3.6	14	2.6	20	3.6	4	4.8	8	2.9	12
356, 0.570	β-Myrcene	3.2	10	2.6	13	2.8	11	4.2	12	3.9	10	4.2	9	5.6	12	3.2	10	4.7	6
362, 0.520	3-Carene	2.3	0	2.1	15	2.1	23	3.1	9	2.7	14	3.0	13	5.0	10	4.3	12	3.9	13
392, 0.405	*m* -Cymene	1.4	13	1.2	9	1.3	12	2.4	11	2.1	10	2.1	12	2.5	4	2.1	8	2.3	12
398, 0.476	Limonene	2.2	18	5.1	11	2.8	10	4.3	8	6.2	11	3.5	16	9.5	11	7.1	8	3.9	17
404, 0.476	1,8-Cineole	9.6	5	4.5	8	2.4	15	13.0	3	6.4	7	2.8	6	12.4	8	6.4	12	3.1	14
428, 0.678	Linalool oxide (is	2.4	8	2.1	13	2.3	9	4.3	21	3.1	19	2.1	18	5.5	5	3.2	6	1.8	22
440, 0.560	α-Terpinolene	3.3	12	2.4	9	2.7	13	5.3	14	4.9	8	4.2	12	7.0	13	3.5	11	4.7	11
440, 0.790	Linalool oxide (is	1.3	9	1.1	16	1.2	21	2.7	11	1.8	14	1.7	14	3.2	16	1.5	9	1.6	12
452, 0.746	Linalool *	7.1	17	2.7	13	1.6	13	6.0	6	3.8	11	3.0	6	6.7	6	3.4	13	3.0	15
470, 0.646	Hotrienol	5.3	15	3.2	9	3.9	19	7.0	13	4.2	13	5.0	12	6.8	5	4.6	15	5.3	9
542, 0.835	α-Terpineol	2.5	9	1.7	12	2.0	15	2.4	9	1.8	16	2.0	15	2.5	8	2.0	10	2.3	12
548, 0.850	Dihydrocarvone	2.3	13	1.8	10	1.9	8	2.4	12	1.9	8	1.9	6	2.5	15	1.7	0	2.0	10
560, 0.850	Verbenone (tran	4.8	19	4.1	13	3.9	10	6.2	14	4.3	14	5.8	13	5.8	17	4.7	13	6.1	8
566, 0.703	p-Menth-1-en-9-	2.7	11	2.3	10	2.4	15	3.0	10	2.5	14	2.6	10	2.6	17	2.3	16	2.5	11
578, 0.700	m/z 93, 121, 119	1.9	14	1.8	13	1.6	9	2.1	13	1.9	9	1.9	12	2.0	14	1.6	11	1.8	14
590, 0.737	Geraniol (isomer	4.3	17	2.6	15	1.2	17	5.3	9	3.2	16	1.7	15	6.0	11	3.5	13	1.5	24
596, 0.976	Citral (isomer cis)	3.0	9	2.1	12	2.4	14	3.1	15	2.4	14	3.1	9	3.0	12	2.6	9	2.8	13
626, 0.775	Citral (isomer tra	2.1	10	1.8	12	1.7	12	2.6	14	2.3	10	1.6	19	4.3	10	2.2	15	1.7	18
	Sub-Total	*71.1*		*50.1*		*45.0*		*91.1*		*65.7*		*57.6*		*100.2*		*67.4*		*62.0*	
C13 Norisoprenoids																			
566, 0.532	m/z 159, 91, 131	8.4	12	4.1	0	5.3	9	13.3	11	9.5	15	14.6	10	22.8	16	12.3	8	16.3	15
736, 0.648	Geranylacetone	10.7	4	1.6	15	3.2	13	11.2	13	1.7	10	6.6	17	15.8	11	3.3	12	6.8	15
784, 0.717	β-Ionone*	5.7	13	2.2	8	4.6	15	7.9	9	3.6	13	5.1	12	9.9	12	4.6	8	7.5	9
900, 0.894	Methyl dihydroja	9.5	6	4.0	13	6.5	8	16.0	4	8.0	21	8.1	16	12.4	13	9.3	5	8.8	4
	Sub-Total	*34.3*		*11.9*		*19.6*		*48.5*		*22.8*		*34.4*		*60.9*		*29.5*		*39.3*	

Sesquiterpenic compounds																			
790, 0.660	α-Farnesene	3.3	14	2.1	10	2.4	16	4.0	7	2.7	11	3.0	9	4.7	10	3.0	7	3.6	13
912, 0.654	m/z 119, 91, 191	3.1	6	2.6	14	2.3	9	3.8	6	2.8	9	2.7	12	4.5	15	3.0	12	2.7	20
942, 0.820	Farnesal	2.3	13	1.2	8	1.0	12	2.9	11	1.0	14	1.3	2	4.0	4	1.3	15	1.6	18
10436, 0.67	Ledene oxide	4.2	8	1.4	2	2.8	18	8.0	7	2.6	13	3.1	31	11.3	2	4.4	7	4.7	9
	Sub-Total	*12.9*		*7.3*		*8.5*		*18.7*		*9.1*		*10.1*		*24.4*		*11.8*		*12.7*	
	Total	*118.3*		*69.3*		*73.1*		*158.3*		*97.6*		*102.1*		*185.5*		*108.6*		*114.1*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] Concentration expressed as equivalentes of 3-octanol (μg L^{-1}).

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

Table S17

Volatile components determined in the glicosidically-linked fraction of mature grapes of *Vitis vinifera* L. cv. Sauvignon Blanc variety obtained from 3 parcels and 3 harvests under study, grouped by chemical classes.

		Concentration (μg L^{-1}) *																	
		SB-SM1						SB-SM2						SB-SM3					
		2010		2011		2012		2010		2011		2012		2010		2011		2012	
^{1}Dtr(s),^{2}Dtr(	Compound	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%	lean (n=:	RSD (%)
Monoterpenic compounds																			
314, 0.440	α-Pinene	7.7	7	2.1	19	2.1	10	6.6	15	2.7	8	2.2	17	8.0	8	3.0	6	2.2	5
362, 0.520	3-Carene	3.7	14	vt		1.3	1	4.3	11	1.3	9	1.7	6	8.1	12	1.3	12	1.9	12
398, 0.476	Limonene *	3.3	9	2.1	15	1.5	9	4.4	4	2.1	12	1.9	16	6.9	17	2.0	8	2.0	12
404, 0.476	1,8-Cineole	12.0	14	4.3	0	5.0	16	12.3	10	4.3	15	6.6	15	14.3	6	5.1	11	6.7	8
428, 0.678	Linalool oxide (	1.3	6	1.5	12	2.0	9	3.5	16	1.6	8	1.2	11	3.8	11	1.9	6	2.0	11
452, 0.746	Linalool *	7.7	5	7.5	9	8.5	20	19.5	6	5.3	17	7.0	2	23.3	18	7.4	12	8.0	9
524, 0.860	Borneol	2.4	11	1.6	6	2.1	12	5.4	5	2.3	14	2.9	13	7.6	11	2.3	10	3.1	13
536, 0.715	Terpinen-4-ol	3.2	9	2.4	11	2.2	6	5.6	12	2.4	9	2.7	16	12.1	17	2.5	12	2.9	4
542, 0.835	α-Terpineol*	10.4	11	6.6	7	6.3	10	16.6	17	6.5	12	7.8	5	19.5	15	6.2	8	6.6	3
548, 0.850	Dihydrocarvon	5.6	7	5.0	16	5.3	7	8.5	11	5.4	7	6.6	11	12.9	4	5.5	12	6.3	14
560, 0.850	Verbenone	7.4	12	1.2	9	1.2	14	11.1	7	1.3	10	1.1	13	11.3	16	1.2	17	1.4	8
584, 0.873	Geraniol (isom	30.0	9	17.4	15	18.3	11	45.1	6	18.7	0	19.6	5	54.2	5	20.1	0	20.8	17
584, 0.943	β-Citronellol*	1.0	16	1.3	11	1.0	13	1.1	9	1.0	14	1.0	11	1.4	4	1.0	11	1.2	7
590, 0.737	Geraniol (isom	20.0	12	16.5	13	15.7	9	33.0	13	17.4	8	19.5	16	39.9	14	18.5	0	20.1	15
596, 0.976	Citral (isomer)	1.0	13	1.1	6	1.1	12	1.3	8	1.0	13	1.2	14	1.4	15	1.2	12	1.2	7
602, 0.815	Carvone *	1.2	11	1.0	7	1.0	10	1.3	10	1.1	3	1.4	8	1.6	8	1.2	6	1.5	16
626, 0.775	Citral (isomer)	1.6	5	1.4	12	1.7	13	2.2	9	1.5	0	1.6	7	2.3	21	2.1	0	1.6	16
	Sub-Total	119.6		73.0		76.3		181.9		75.9		86.0		228.5		82.5		89.4	
C$_{13}$ Norisoprenoids																			
900, 0.894	Methyl dihydro	4.4	1	2.2	15	9.0	7	5.9	12	2.7	7	10.2	9	6.2	17	3.6	6	11.3	8
	Sub-Total	4.4		2.2		9.0		5.9		2.7		10.2		6.2		3.6		11.3	

Sesquiterpenic compounds																			
850, 0.751	Globulol	1.2	8	1.0	13	1.3	9	1.5	7	1.2	9	1.3	13	1.8	11	1.4	8	1.6	13
912, 0.654	m/z 119, 91, 19	1.0	16	vt		vt		1.4	10	1.0	20	1.2	9	2.0	8	1.1	5	1.9	10
942, 0.820	Farnesal	4.6	12	2.4	4	4.8	12	6.4	8	3.5	6	5.3	11	7.9	12	5.3	12	6.8	0
1036, 0.671	Ledene oxide	24.7	17	12.5	14	17.7	15	30.8	11	14.6	21	13.7	5	32.2	13	13.2	8	16.5	5
	Sub-Total	31.5		15.9		23.8		40.1		20.3		21.5		43.9		21.0		26.8	
	Total	155.5		91.2		109.1		227.9		98.9		117.7		278.6		107.1		127.5	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] Concentration expressed as equivalentes of 3-octanol (μg L-1).

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

vt - vestigial

Table S18

Volatile components determined in the glicosidically-linked fraction of mature grapes of *Vitis vinifera* L. cv. Baga variety obtained from 3 parcels and 3 harvests under study, grouped by chemical classes.

		Concentration (μg L^{-1}) *																	
		BA-VA1						**BA-VA2**						**BA-SM1**					
		2010		**2011**		**2012**		**2010**		**2011**		**2012**		**2010**		**2011**		**2012**	
^{1}Dtr(s),^{2}Dtr(	Compound	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)	ean (n=	RSD (%)
Monoterpenic compounds																			
314, 0.440	α-Pinene	1.5	7	vt		1.3	8	1.9	15	1.0	5	1.2	7	2.0	7	vt		1.0	9
344, 0.457	b-Pinene	2.3	7	3.4	6	1.4	8	2.5	20	3.8	18	1.4	16	1.6	10	1.1	3	1.6	18
356, 0.570	b-Myrcene	4.2	13	3.8	9	1.8	23	5.4	11	4.0	10	1.9	15	1.3	14	3.8	9	1.0	12
	Sub-Total	*8.0*		*7.2*		*4.5*		*9.8*		*8.8*		*4.5*		*4.9*		*4.9*		*3.7*	
C$_{13}$ Norisoprenoids																			
736, 0.648	Geranylacetone	2.8	4	6.8	6	4.6	18	2.9	14	6.9	15	6.2	11	2.8	11	3.4	18	4.6	11
900, 0.894	Methyl dihydroj	16.5	19	5.3	10	4.1	10	17	2	5.2	5	4.4	8	2.4	12	1.7	17	1.3	4
	Sub-Total	*19.3*		*12.1*		*8.7*		*19.9*		*12.1*		*10.6*		*5.2*		*5.1*		*5.9*	
Sesquiterpenic compounds																			
796, 0.525	γ-Cadinene	2.3	10	2.3	22	1.9	18	3.9	14	4.1	16	5.7	12	1.2	6	1.7	4	1.0	9
826, 0.629	α-Calacorene	8.2	11	5.8	13	5.6	13	13.4	5	5.3	26	6.2	7	5.6	20	4.9	25	5.1	15
912, 0.654	*m/z* 119, 91, 19	5.1	23	1.4	17	7.2	4	3.9	10	3.8	0	7.2	7	2.5	14	2.8	23	3.4	17
1036, 0.671	Ledene oxide	8.2	14	6.2	11	5.7	5	10.5	11	6.8	14	6.3	15	5.6	10	2.9	10	3.8	5
	Sub-Total	*23.8*		*15.7*		*20.4*		*31.7*		*20.0*		*25.4*		*14.9*		*12.3*		*13.3*	
	Total	*51.1*		*35.0*		*33.6*		*61.4*		*40.9*		*40.5*		*25.0*		*22.3*		*22.9*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] Concentration expressed as equivalentes of 3-octanol (μg L^{-1}).

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

vt - vestigial

Table S19

Volatile components determined in the glicosidically-linked fraction of mature grapes of *Vitis vinifera* L. cv. Castelão variety obtained from 3 parcels and 3 harvests under study, grouped by chemical classes.

		Concentration (μg L^{-1}) *											
		CA-SM1						**CA-SM2**					
		2010		**2011**		**2012**		**2010**		**2011**		**2012**	
								CA-SM3					
^{1}Dtr(s),^{2}Dtr	Compound	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%
Monoterpenic compoun													
314, 0.440	α-Pinene	4.5	9	2.6	12	3.5	11	3.2	14	1.3	7	2.3	12
344, 0.457	β-Pinene *	3.5	7	4.0	6	3.0	3	2.8	25	3.4	13	2.7	21
356, 0.570	β-Myrcene	4.1	15	2.0	16	4.2	13	3.9	11	1.7	13	3.6	23
	Sub-Total	*12.1*		*8.7*		*10.7*		*9.9*		*6.4*		*8.6*	
C_{13} Norisoprenoids													
736, 0.648	Geranylaceto	4.7	15	4.0	8	4.7	9	4.3	16	3.6	14	2.5	18
900, 0.894	Methyl dihyd	13.0	8	4.2	5	7.7	10	11.4	15	3.7	6	6.7	4
	Sub-Total	*17.7*		*8.2*		*12.3*		*15.6*		*7.3*		*9.1*	
Sesquiterpenic compou													
790, 0.660	α-Farnesene	7.6	16	5.2	16	6.7	12	6.1	8	4.5	5	5.8	8
912, 0.654	*m/z* 119, 91,	6.6	5	6.0	12	4.4	3	5.5	6	5.4	11	3.6	13
942, 0.820	Farnesal	12.3	8	9.5	9	8.9	17	11.7	12	8.1	18	8.0	5
	Sub-Total	26.5		20.7		19.9		23.3		18.0		17.4	
	Total	*56.3*		*37.6*		*43.0*		*48.8*		*31.7*		*35.1*	

		CA-SM3					
		2010		**2011**		**2012**	
^{1}Dtr(s),^{2}Dtr	Compound	ean (n=	RSD (%	ean (n=	RSD (%	ean (n=	RSD (%)
Monoterpenic compoun							
314, 0.440	α-Pinene	3.4	7	1.0	0	1.2	13
344, 0.457	β-Pinene *	2.0	13	1.4	14	1.4	5
356, 0.570	β-Myrcene	0.0	0	1.6	8	1.7	7
	Sub-Total	*5.4*		*4.0*		*4.3*	
C_{13} Norisoprenoids							
736, 0.648	Geranylaceto	4.8	9	2.1	18	1.8	24
900, 0.894	Methyl dihyd	8.7	4	1.5	8	3.0	28
	Sub-Total	*13.5*		*3.6*		*4.8*	
Sesquiterpenic compou							
790, 0.660	α-Farnesene	6.1	13	4.2	16	5.0	18
912, 0.654	*m/z* 119, 91,	5.2	4	5.5	11	3.2	3
942, 0.820	Farnesal	10.8	5	6.8	7	7.1	30
	Sub-Total	22.0		16.5		15.3	
	Total	*40.9*		*24.2*		*24.4*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] Concentration expressed as equivalentes of 3-octanol (μg L^{-1}).

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.

Table S20

Volatile components determined in the glicosidically-linked fraction of mature grapes of *Vitis vinifera* L. cv. Touriga Nacional variety obtained from 3 parcels and 3 harvests udy, under study, grouped by chemical classes.

		Concentration (µg L^{-1}) *															
		TN-SM1						**TN-SM2**						**TN-SM3**			
		2010		**2011**		**2012**		**2010**		**2011**		**2012**		**2010**		**2012**	
^{1}Dtr(s),^{2}Dtr(	Compound	lean (n=3	RSD (%)	lean (n=3	RSD (%)	lean (n=3	RSD (%)	lean (n=3	RSD (%)	lean (n=3	RSD (%)	lean (n=3	RSD (%)	lean (n=3	RSD (%)	lean (n=3	RSD (%)
Monoterpenic compoun																	
398, 0.476	Limonene *	3.6	4	2.4	1	1.7	11	7.1	14	2.9	15	2.2	16	1.4	6	1.4	5
452, 0.746	Linalool *	3.7	12	4.6	13	3.6	14	4.3	29	5.0	14	3.8	13	3.4	13	3.4	8
	Sub-Total	*7.3*		*7.0*		*5.3*		*11.4*		*7.9*		*6.0*		*4.8*		*4.8*	
C$_{13}$ Norisoprenoids																	
736, 0.648	Geranylacet	1.4	11	2.2	4	1.1	4	3.1	0	2.6	22	2.1	19	1.1	5	1.0	10
900, 0.894	Methyl dihy	14.5	19	7.6	17	9.3	11	18.6	15	8.2	5	10.8	7	10.5	19	5.5	15
	Sub-Total	*15.9*		*9.8*		*10.4*		*21.7*		*10.8*		*12.9*		*11.6*		*6.5*	
Sesquiterpenic compou																	
790, 0.660	α-Farnesene	4.1	28	2.6	26	4.1	5	4.4	2	3.1	21	4.9	9	2.6	14	4.7	7
912, 0.654	*m/z* 119, 91	4.2	6	1.3	0	3.6	16	5.3	20	2.3	10	4.3	3	3.5	19	2.2	22
1036, 0.671	Ledene oxid	19.3	14	17.5	24	17.0	11	25.0	5	19.2	19	18.5	22	12.1	15	10.9	12
	Sub-Total	*27.6*		*21.4*		*24.7*		*34.7*		*24.6*		*27.7*		*18.2*		*17.8*	
	Total	*50.8*		*38.2*		*40.4*		*67.8*		*43.3*		*46.6*		*34.6*		*29.1*	

[a] ^{1}Dtr (s), ^{2}Dtr(s): first and second dimension retention times (in seconds) of each compound determined.

[b] Concentration expressed as equivalentes of 3-octanol (µg L^{-1}).

* Compounds identified based on the comparison between the obtained mass spectra and mass spectra of high purity chemical standards.