

**UNIVERSITY OF SÃO PAULO**  
**FACULTY OF PHARMACEUTICAL SCIENCES**  
**DEPARTMENT OF FOOD AND EXPERIMENTAL NUTRITION**

**Chemical characterization of South American red wines**

Leonardo Antonio Valentin

Advisor: Prof. Dr. Inar Alves de Castro

**São Paulo**  
**2019**

**UNIVERSITY OF SÃO PAULO**  
Faculty of Pharmaceutical Sciences  
Graduate Program in Food Sciences

**Chemical characterization of South American red wines**

**Leonardo Antonio Valentin**

**Corrected Version**

Thesis presented to obtain the degree of

**DOCTOR IN PHILOSOPHY**

Area: Food Science

Advisor: Prof. Dr. Inar Alves de Castro

São Paulo

2019

Ficha Catalográfica elaborada eletronicamente pelo autor, utilizando o programa desenvolvido pela Seção Técnica de Informática do ICMC/USP e adaptado para a Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP

Bibliotecária responsável pela orientação de catalogação da publicação:  
Marlene Aparecida Vieira - CRB - 8/5562

VALENTIN, LEONARDO  
VValen Chemical characterization of South American red  
tinc wines / LEONARDO VALENTIN. - São Paulo, 2019.  
98 p.

Tese (doutorado) - Faculdade de Ciências  
Farmacêuticas da Universidade de São Paulo.  
Departamento de Alimentos e Nutrição Experimental.  
Orientador: Alves de Castro, Inar

1. Wine. 2. Phenolic. 3. South America. 4.  
Antioxidant. 5. Volatile. I. T. II. Alves de  
Castro, Inar, orientador.

Leonardo Antonio Valentin

CHEMICAL CHARACTERIZATION OF SOUTH AMERICAN RED WINES

Commission of Thesis for the degree of Doctor in Philosophy

Area: Food Science

---

1<sup>st</sup> Examiner

---

2<sup>nd</sup> Examiner

---

3<sup>rd</sup> Examiner

---

4<sup>th</sup> Examiner

---

5<sup>th</sup> Examiner

São Paulo, \_\_\_\_\_, 2019

## **ACKNOWLEDGMENT**

First of all, I would like to thank God for strength and health in order to complete this work.

I would like to thank my advisor Prof. Inar Castro for the guidance and support for this work. Thank you also for all the patience you had with me during this period, I'll always be grateful for that.. I really appreciate all of this.

I would like to thank very much for the Ladaf group for the partnership and support. Especially Marina who was very close in moments of difficulty.

Thanks to the members of the group, Bianca, Gabriela, Tayse.

Thanks to Agilent Technologies for the opportunity to do this work and for making their equipment structure available for the experiments.

I would like to thank the "Brazilian Association of Sommeliers" for his help in selecting the wines for this work, in special Dr. Gustavo A. de Paulo.

I also thank for all FBA employees and the postgraduate secretariat.

I thank my beloved wife for her encouragement, patience and partnership.

## RESUMO

VALENTIN, L. **Caracterização química de vinhos tintos sul americanos**. 2019. 98f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2019.

Neste estudo, 83 vinhos considerados por especialistas como representantes das seguintes categorias comerciais: “Malbec Argentino (AM)”, “Merlot Brasileiro (BM)”, “Tannat Uruguaio (UT)” e “Carménere Chileno (CC)” foram analisados de acordo com a sua composição de compostos fenólicos, voláteis e semi-voláteis. O objetivo foi identificar a combinação dos compostos químicos que caracteriza cada categoria comercial. De cerca de 600 picos obtidos por técnicas cromatográficas, 169 foram identificados e 53 deles foram selecionados para análise estatística multivariada. Quatro clusters, designados como “Categorias Químicas” foram obtidos. A Categoria Química 2 e a Categoria Química 4 foram compostas por 90% de CC e 68% de AM respectivamente, a Categoria Química 3 agrupou principalmente CC (50%) e BM (36%), enquanto a Categoria Química 1 mostrou predominância de AM (37%) e UT (30%). Com base na classificação química, os vinhos CC foram caracterizados por apresentarem maior teor de lactato isopentílico, álcool isobutílico, ionona, malvidina e ácido gálico e menor teor de rutina, quercetina e resveratrol. Por outro lado, os vinhos AM apresentaram maior concentração de miricetina. Os vinhos UT apresentaram a maior atividade antioxidante, maior teor de quercetina, epicatequina, rutina e também a maior quantidade de todos os semi-voláteis detectados no modo negativo, enquanto os vinhos BM apresentaram a maior concentração de catequina, petunidina e todos os semi-voláteis detectados no modo positivo. Considerando que compostos fenólicos e voláteis estão estritamente associados às características sensoriais dos vinhos, esses dados podem contribuir para o estabelecimento de critérios para posterior autenticação e tipificação de vinhos sul americanos.

**Palavras-chave:** vinho, fenólico, volátil, algoritmo, cluster, sul americano, antioxidante.

## ABSTRACT

VALENTIN, L. **Chemical characterization of South American red wines**. 2019. 98f. Thesis (PhD) – Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2019.

In this study, 83 wines considered by experts as representatives of the following commercial categories: “Argentinean Malbec (AM)”, “Brazilian Merlot (BM)”, “Uruguayan Tannat (UT)” and “Chilean Carménère (CC)” were analyzed according to their composition of phenolic, volatiles and semi-volatiles compounds. The objective was to identify the chemical compounds profile that characterized each commercial category. From about 600 peaks obtained by chromatographic techniques, 169 were identified and 53 of them were selected for multivariate statistical analysis. Four clusters, designated as “Chemical Categories” were obtained. Chemical Category 2 and Chemical Category 4 were composed by 90% of CC and 68% of AM respectively, Chemical Category 3 grouped mostly CC (50%) and BM (36%), while Chemical Category 1 showed predominance of AM (37%) and UT (30%). Based on the chemical classification, CC wines were characterized by higher content of Isopentyl lactate, Isobutyl alcohol, Ionone, malvidin and gallic acid and lower content of rutin, quercetin and resveratrol. On the other side, AM wines showed higher concentration of myricetin. UT wines had the highest antioxidant activity, higher content of quercetin, epicatechin, rutin and also the highest amount of all semi-volatiles detected in the negative mode, while BM wines only showed the highest concentration of catechin, petunidin and all semi-volatiles detected in the positive mode. Considering that phenolic and volatile compounds are strictly associated to the wines sensory characteristics, these data can contribute to establish criteria for further wines authentication and typification.

**Key-words:** wine, phenolic, volatile, algorithm, cluster, South American, antioxidant

## ABBREVIATIONS

AAPH, (2,2'-azobis(2-methylpropionamide) dihydrochloride)

ABS, Brazilian Association of Sommeliers

AM, Argentinean Malbec

ANOVA, Analysis of Variance

BM, Brazilian Merlot

CC, Chilean Carménère

CIE, International Commission on Illumination

D.O., Denomination of Origin

DPH, Direct Producer Hybrids

DPPH, Free Radical Scavenging Capacity

DVB-CAR-PDMS,  
Divinylbenzene/Carboxen/PolydimethylsiloxaneEU,  
European Union

FRAP, Ferric Reducing Ability of Plasma

FTIR, Fourier Transform Infrared

GC, Gas Chromatography

GC/MS, Gas Chromatography/ Mass Spectrometry

GF, Generate Formula

HPLC, High Performance Liquid Chromatography

HPLC/MS, High Performance Liquid Chromatography/Mass Spectrometry

HS, Headspace

IGP, Indicazione Geografica Protetta

MFE, Molecular Feature Extraction

MS, Mass Spectrometry

NIST, National Institute of Standards and Technology

NMR, Nuclear Magnetic Resonance

OIV, International Organization of Vine and Wine

ORAC, Radical Absorbance Capacity Assay

PC, Principal Component

PDO, Protected Designation of Origin

PGI, Protected Geographical Indication

PTFE, Polytetrafluoroethylene

QTOF, Quadrupole Time-of-Flight

S/SL, Split/Splitless

SIMCA, Soft Independent Modeling of Class Analogy

SPME, Solid Phase Micro Extraction

TPTZ, 2,4,6-Tris(2-pyridyl)-s-triazine

UT, Uruguayan Tannat



## **LIST OF TABLES**

Table 1. Some of the major grapes used to produce red wines.....	16
Table 2: Advantages and disadvantages of analytical techniques in wine analysis.....	44
Table 3. Validation data of phenolic method.....	96

## LIST OF FIGURES

Figure 1: Basic steps involved in the wine production.....	17
Figure 2: World wine production.....	20
Figure 3: Main varieties grown in Chile.....	24
Figure 4: Main varieties grown in Uruguay.....	25
Figure 5: Main varieties grown in Argentina.....	26
Figure 6: Main wine producing regions in Brazil.....	27
Figure 7: Main varieties grown in Brazil.....	29
Figure 8: Summary of major compounds present in each step of the wine production.....	30
Figure 9: Groups of phenolic compounds found in plants.....	33
Figure 10: Known grape compounds important for flavor.....	36

## SUMMARY

1. Introduction .....	12
2. Literature review .....	14
2.1 The history of wines .....	14
2.2 The production of wines in the world .....	19
2.3 The production of wines in South America .....	21
2.4 Important grape varieties in South America .....	22
2.5 Main wine producing countries in South America .....	23
2.5.1 Chile .....	23
2.5.2 Uruguay .....	24
2.5.3 Argentina .....	25
2.5.4 Brazil .....	26
2.6 Chemical characterization of wine .....	29
2.6.1 Total sugar .....	30
2.6.2 Alcohols .....	30
2.6.3 Organic Acids .....	31
2.6.4 Phenolic Compounds .....	32
2.6.5 Volatile Organic Compounds .....	35
2.6.6 Other Compounds .....	38
2.7 Wine typification .....	39
2.8 Instrumentation in wine analysis .....	41
2.9 Multivariate analysis in wine classification .....	44
3 Hypotheses and objectives .....	46
4 Manuscript .....	47
5 Additional considerations about manuscripts .....	83
6 Suggestions for future work .....	83
7 References .....	84
8 Attachments .....	96

*"I have no idols. I admire work, dedication and competence."*

*Ayrton Senna*

## 1. INTRODUCTION

Grapes and wines, especially the red ones, are considered quality-of-life items, due to the importance of their chemical constituents for human health. Studies have shown the benefits of these compounds as antioxidants and anti-inflammatory agents, protecting against cardiovascular diseases (Snopek et al., 2018).

Brazilian tropical viticulture was effectively developed from the 1960s, with the planting of commercial table grape vineyards in Vale do São Francisco region of northeastern. In the 1970s, the wine producing pole of the north of state in Paraná and in the 1980s, the regions of northwest of the state of São Paulo and Pirapora in the north of Minas Gerais developed, all aimed at producing fine grapes for in natura consumption (PROTAS, CAMARGO and MELLO, 2002). However, most of the activity's development is concentrated in Rio Grande do Sul, where it is responsible for 90% of production. Still, there is the emergence of new planting areas, indicating a trend of expansion of culture in the country. This evolution has supported the development and adoption of new technologies that contribute to the establishment of viticulture as an economically profitable activity in the country (EMBRAPA, 2012).

The market for *Vitis vinifera* wines in Brazil, including domestic production and imports, grew 12.47% in the last five years, with 80.19% being imported from other South American countries (Uvibra, 2016). This increase in wine consumption stimulated the wines commercialization by different channels, and the investment of South American wineries, including Brazil, in the production of high-quality sensorial wines. However, despite the significant increase in the quality standard, South American wines still do not have a defined identity. This identity, based on chemical composition and sensory properties, is extremely important because it is the way by which consumers recognize the product and then value it. Once defined, producers can invest in this standardization, thereby providing a guarantee that the wine will meet consumers' expectations. This is the philosophy that supports "controlled origin products" applied to various products, including cheeses, meats, oils and wines. According to Maitre et al. (2010), a wine can be

considered typical if the characteristics can be identified and make it recognizable as belonging to one type and unlike the others. It is important to emphasize that even in countries with "Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI)" consolidated systems, a scientific approach to typicality still represents a challenge in terms of concept and sensory methodologies (Maitre et al., 2010 ).

Another aspect that influences the increase in red wine consumption, not only in South America, but throughout the world, is the association between continuous and moderate wine consumption and the reduction of cardiovascular disease, resulting from the research originally developed by Renaud and De Lorgeril (1992), later consolidated by many other researchers. Basically, clinical and epidemiological studies suggest that the consumption of one or two glasses per day may reduce the risk of cardiovascular diseases, some cancers and diabetes, due to the presence of phenolic compounds capable of exerting an antioxidant action (Arranz et al., 2012). In this case, wines with different profiles in their phenolic composition exert a different antioxidant effect when regularly consumed.

In the previous studies carried out by our group, 666 South American red wines (2009-2010) were evaluated according to their phenolic compounds profile, sensory characteristics and antioxidant activity (Llobodanin et al., 2014a; Llobodanin et al., 2014b). The wines were produced in four countries using six grape varieties. From these combinations, four of them, here designed as "Argentinean Malbec", "Uruguayan Tannat", "Brazilian Merlot" and "Chilean Caménère" showed trend to separate from the others according to their chemical composition.

In these previous studies, just phenolic compounds were analyzed. It is known that volatile compounds are also important markers for geographical origin of the wines. For this reason, the objective of this study was to analyze authentic wines from these four groups before studied, in terms of phenolic and also volatile composition, aiming to identify which compounds would be associated with the typicality of these four important commercial categories.

## 2. LITERATURE REVIEW

### 2.1. The history of wines

Wine is a drink that comes almost along with civilization itself (Vidotti, 2010). Wine has an archeological record dating back more than 7.5 thousand years. The earliest suspected wine residues come from the early to mid-fifth millennium b.c. – Hajji Firuz Tepe, in the northern Zagros Mountains of Iran (McGovern *et al.*, 1996). Evidence from Neolithic pottery from Georgia suggests that contemporaneous wine production was dispersed throughout the region. Older examples of fermented beverages have been discovered, but they appear to have been produced from rice, honey, and fruit (hawthorn and/or grape) (McGovern *et al.*, 2004). The presence of wine residues is usually identified by the presence of tartaric acid residues, although additional procedures for identifying grape tannin residues are in development (Garnier *et al.*, 2003). Identification of red wine was made by the presence of syringic acid, an alkaline breakdown product of malvidin-3-glycoside. The same technique was used to establish the red grape origin of the ancient Egyptian drink – *Shedeh* (Guasch-Jané *et al.*, 2006). Although grapes readily ferment, due to the prevalence of fermentable sugars, the wine yeast (*Saccharomyces cerevisiae*) is not a major, indigenous member of the grape flora. The natural habitat of the ancestral strains of *S. cerevisiae* appears to be the bark and sap exudate of oak trees (Phaff, 1986). If so, the habit of grapevines climbing trees, such as oak, and the joint harvesting of grapes and acorns, may have encouraged the inoculation of grapes and grape juice with *S. cerevisiae*. The fortuitous overlap in the distribution of the progenitors of both *S. cerevisiae* and *V. vinifera* with the northern spread of agriculture into Anatolia may have fostered the discovery of winemaking, as well as its subsequent development and spread. It may

not be pure coincidence that most major yeast-fermented beverages and foods (wine, beer, mead, and bread) have their origins in the Near East (Cavaliere *et al.*, 2003).

According to archaeological studies (Vidotti, 2010), since the earliest times there are records of growing grapes for wine production. The indications of this production are in the Caucasus region, eastern Europe, and consist of a large quantity of seeds of cultivated grapes that indicate vinification, as well as the existence of vessels for the transportation and vinification of wines. According to Aguiar and Miwa (2009), it was through the ancient Greeks, who dominated the vinification techniques around 750 b.c., that the cultivation of grapes for wine production reached the countries where it would lay its roots such as Italy and France, besides Spain. Wine is also a drink that has always been linked to religious practice, from the earliest biblical accounts, until its production in monasteries (Vidotti, 2010).

In parallel with the expansion of European production, in the era of great navigation, in 15<sup>th</sup> century, the so-called "New World" emerges, covering the regions of the Americas, Africa and Oceania, where the cultivation of *Vitis Vinifera* was also started (Aguiar and Miwa, 2009). Viticulture in the South America had its origin in the missions (Franciscan or Jesuit), but only began to develop in the 19<sup>th</sup> century (Pitte, 2012). In recent years, South America has been the second most important wine producing continent in the world (Johnson and Robinson, 2008). The largest wine producer in terms of quantity in South America is Argentina, followed by Chile and Brazil. In Brazil, most of the wines are still produced with *Vitis labrusca*. It should be noted that the care in production regarding the management of the soil and maintenance of the temperatures in the vineyards and the advance of the technologies has allowed to maintain a regular quality standard of the wines produced in Brazil. In terms of wine production in South America, it is important to mention that Venezuela and Peru are also producers, but on a smaller scale than the other already mentioned countries in South America (Johnson and Robinson, 2008).

Regarding the production, the wine is a drink obtained from the yeast fermentation of grape juice sugars (Aquarone, 2001). *Vitis vinifera* is the most cultivated species of vine to wine production, counting with more than five thousands different varieties.



Basically, these grape varieties produce three types of wine designed as red, white and rose. **Table 1** presents some of the major grapes used to produce red wines according to the region.

**Table 1:** Some of the major grapes used to produce red wines.

Grapes	Region
Pinot Noir, Syrah, Malbec, Merlot, Grenache, Gamay, Carbernet Franc, Trebbiano Toscano	France
Nebbiolo, Sangiovese, Barbera, Cabernet Franc, Montepulciano	Italy
Tempranillo, Moristel, Provechon, Graciano, Airén	Spain
Toriga Franca, Tempranillo, Castelão, Santarem, Fernão Pires, Trincadeira, Baga, Syrah	Portugal
Cabernet Suvignon, Syrah, Merlot, Pinot Noir, Zweigelt, Malbec, Cabernet Franc, Petit Verdot	Australia/NZ
Cabernet Sauvignon, Zinfandel, Pinot Noir, Merlot, Malbec, Petit Verdot, Carignan, Sultanina, Chardonnay	USA
Cabernet Sauvignon, Carménère, Malbec, Merlot, Syrah, Bonarda, Tempranillo and Tannat	South America
Dornfelder, Gewurtraminer, Riesling, Muller, Pinot Noir, Rivaner, Pinot Gris	Germany
Kyoho, Red Globe, Cabernet Sauvignon	China

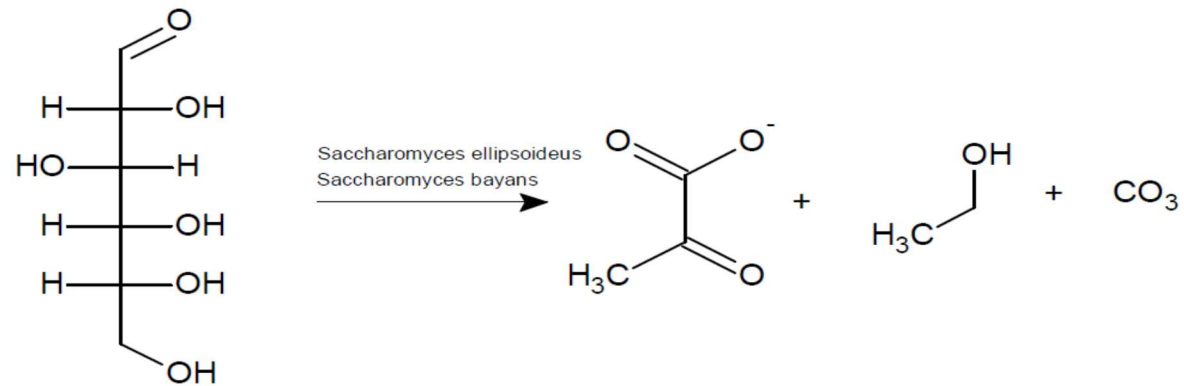
According to Fischer, et al. (1999), Campo et al. (2010) and Parr et al. (2010)

The red wines are produced by the fermentation of *Vitis vinifera* grapes, because these grapes can accumulate more sugar than other varieties of the *Vitis* genre (Sasso et al., 2004).

**Figure 1** presents a basic scheme of the wine production.

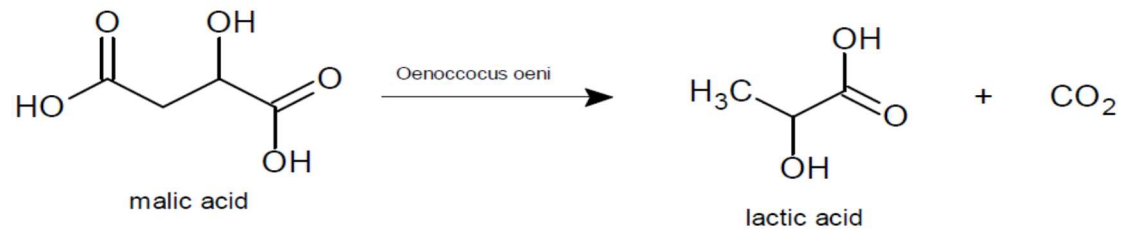
1 ➔ Destemming and crushing

2 ➔ Alcoholic fermentation by yeast



3 ➔ Pressing

4 ➔ Malolactic fermentation



5 ➔ Aging

6 ➔ Fining and clarification (gelatin, casein, egg whites )

7 ➔ Blending

8 ➔ Filtration

9 ➔ Bottling

**Figure 1:** Basic steps involved in the wine production (<http://www.espressocom.co.za/portifolio/white-wine-and-rose>)

During these steps, flavors and odors are formed according to the conditions established by the winemakers, which involve the mixture of different grapes, time, temperature and type of fermentation, time and type of storage among other factors (Góes, 2005). The final wine's quality and identity are influenced by factors that come from since the agriculture practices applied in the vineyards, climate changes, processing conditions carried out by wineries until the distribution and storage. For example, the variety of used grape, the direction in which the vines were planted, the elevation of the land and vines, soil fertilization, climatic conditions and seasonal of maturation time are inside the agricultural factors that influence wine characterization, while type of yeasts used in the fermentation process take part of the enological variations able to affect the wine's identity (Sasso et al., 2004). The combination of all these factors will determine the chemical and sensory characteristics of each wine. The final product will have its sensory properties evaluated by the wine expert tasters, who will contribute to define the wine prices. Therefore, it is clear the importance of the chemical composition and consequently sensory result for the wine's market around the world. One of the most interesting example is the sensory evaluation made each vintage by the famous expert Robert Parker, who defines the prices of the wines produced in Burgundy/France. Usually, sensory evaluation of the wines is carried out by one expert or a group of trained tasters, using different methodologies. Although each method or scale can vary, major sensory aspects evaluated include flavors, odors, alcohol, body, acidity and color (Maitre et al., 2010).

In general, wines have been priced according to their sensory characterization, which is a consequence of their chemical composition (Chira et al., 2011). Thus, when a consumer chooses a wine, there is a sensory expectation that must be achieved and kept by the winery. In the "Old World", this expectation is based on the region where the wine is produced, while in the "New World" the "varietal & region" combination is more used as a tool of choice (Parr et al., 2010). For example, the famous wine "Château d'Yquem" is identified by its geographical location, rather than by its grape varietal (Green et al., 2011). Considering the importance

of the region for “Old World” wines, some internationally recognized standards were implemented in different countries. Among them, there is the “Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI),” concepts. According to the “European Union” definition, PDO products are “produced, processed and prepared in a given geographical area, using recognized know-how”. Their quality and properties are significantly or exclusively determined by their environment. The category is named “Appellation d’Origine Protégée (AOP)” in France, “Denominazione di Origine Protetta (DOP)” in Italy and “Denominación de Origen Protegida (DOP)” in Spain. The “European Union” definition of a PGI product is one closely linked to the geographical area in which it is produced, processed or prepared, and which has specific qualities attributable to that geographical area. Unlike the PDO, it is sufficient that one of the stages of production has taken place in the defined area. The category is named “Indication Géographique Protégée (IGP)” in France, “Indicazione Geografica Protetta (IGP)” in Italy and “Indicación Geográfica Protegida (IGP)” in Spain (European Commission, 2004). It can be inferred that the regional classifications have the intention of not only separate the quality of wines from the regions of a country, but also differentiate them from other countries.

Once the PDO claim provides a guarantee of the expected characteristics of the wine, PDO wines can achieve higher prices in the market than non-PDO wines. In 2008, about 47% of France’s total wine volume was PDO and 29% PGI (Maitre et al., 2010). Thus, PDO are wines that can be recognized by their specific sensory characteristics. This concept is known as “typicality”. Actually, typicality in wine is difficult to define and even more difficult to study (King et al., 2014), and include sensory, technical and environmental dimensions (Cadot et al., 2010). According to Charters and Pettigrew (2007), a wine is considered to have typicality if it reflects both its origin and varietal purity, forming a template against which other wines are measured. It means, the concept of wine typicality concerns the degree by which a wine express not only its varietal purity but also its source of origin.

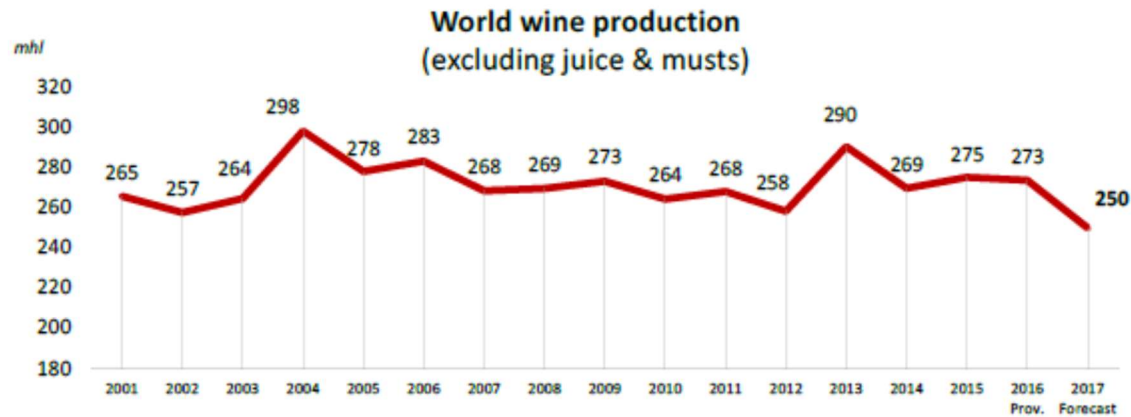
Although several studies have already been developed to typify “Old World” wines (Cadot et al., 2010; Ballester et al., 2008; Fischer et al., 1999; Perrin et al., 2008), there is little information about typification of “New World” wines, including the South American ones. Some research groups have reported studies involving typicality of wines produced in New Zealand (Parr et al., 2010;

Green et al., 2011), California (King et al., 2014) and South America (Fanzone et al., 2012; King et al., 2014; Llobodanin et al., 2014b). In a study reported by King et al. (2014), Malbec wines produced in Argentina were clearly separated from Malbec wines produced in the USA based on their chemical and sensory profiles. Thus, a chemical and sensory characterization of these “New World” wines, grouped according the “varietal & region” as they use to be chosen by consumers, is a necessary step aiming to offer a guarantee of quality or maybe to develop a further PDO/PGI process.

## 2.2. The production of wine in the world

Grape production began timidly to become one of the most important crops in the world. Worldwide grape production in 2015 was about 77 million metric tons. This compares with roughly 57, 50, and 43 million metric tons for oranges, bananas, and apples, respectively. Approximately 66% of the grape production is fermented into wine, 18.7% consumed as a fresh fruit crop, and the remaining 7.7% dried for raisins (OIV, 2005). The use of the grapes varies widely from country to country, often depending on the physical and politicoreligious (wine prohibition) dictates of the region. Despite its world importance, vines only cover about 0.5% of land, and its produce constitute about 0.4% of global household expenditure (Anderson, 2004). Grape production is largely restricted to climatic regions similar to those of the indigenous range of *Vitis vinifera*. This zone approximates the area between the 10 and 20 °C annual isotherms. Grape culture is further largely restricted to regions characterized by Mediterranean-type climates. Extension into cooler, warmer, or more moist environs is possible when local conditions modify the climate or viticultural practice compensates not ideal conditions. Commercial production even occurs in subtropical regions, where severe pruning stimulates nearly year-round vine growth (Jackson, 2008).

Since the 1970s, wine production has ranged from about 250 to 330 million hl (66 to 87 million gallons), with recent production (2015 and 2016), levels being about 270 million hl. The **Figure 2** presents a world wine production until 2017.



**Figure 2:** World wine production (OIV web site 2018)

Although Spain has the largest vineyard hectarage, France and Italy produce the largest volumes of wine. Together, France and Italy produce about 50% of the world's wine, and supply about 60% of world wine exports (OIV, 2018). The increasing economic significance of wine export is partially reflected in the marked increase in research conducted throughout the world (Glänzel and Veugelers, 2006). Several major wine-producing nations, such as Argentina and the United States, export a relatively small proportion of their production (Jackson, 2008). In contrast, countries such as Chile and Portugal export the majority of their production. Although Europe is the most important wine producing and exporting region, in terms of volume, it is also the primary wine-consuming region

(OIV, 2018). For centuries, wine has been a significant caloric food source in the daily diets of many workers in France, Italy, Spain and other Christian Mediterranean nations (Jackson, 2008).

### **2.3. The production of wines in South America**

The wine producing regions of South America are located in a large territorial area and have different climatic conditions within the same country. In grape growing areas, Brazil presents areas of humid climate and high rainfall, such as Serra Gaúcha, which has a rich soil in organic matter, passing through regions with drier climate mixed with high rainfall index, such as Campaign, to a semi-arid region with a relatively low rainfall index, such as São Francisco Valley (Johnson and Robinson, 2008). Soils also have their differences: in Serra Gaúcha there is a sandy and acid soil with poor drainage. In the Gaucho Campaign, the soil is sandy with low organic matter but good drainage. The São Francisco Valley presents fairly poor soil, which favors the quality of the grape (Farias, 2014). Brazilian producing regions have in common, besides the technology used in the cultivation of grapes, a good thermal amplitude, ranging from 28°C during the day to 13°C at night, which contribute for the grapes to develop better flavors (Farias, 2014). The wine region of Chile has also great thermal amplitude in the region near to Cordillera and its soil ranges from the lightest and most fertile to the poorest, as in the Colchagua region, and infertile with layers of clay and granite, as in Casablanca Valley. The climate can be warm in Aconcagua Valley or refreshed by the influence of the current of air that originates in Antarctica and the cold air that comes from the Andes every night (Johnson and Robinson, 2008). In Uruguay, the climate is sunny and humid, with decreasing temperatures at night in the producing regions. The afternoons are cold and windy. The soils are generally clayey and limestone, which favors the cultivation of grapes since the vine needs poor soils with stones, limestone and clay to produce higher quality fruits. Argentina has low rainfall index, extremely cold climate in winter and in some regions very hot in the summer. In the main Argentine producing region, Mendoza, due to the variation of altitude, there are several microclimates. In general the climate in Mendoza is temperate and the soils stony and

sandy. Each region of the countries presented has its own climate and soil type, although they have some similarities, each one has its characteristic which causes them to produce wines with their preserved particularities.

#### **2.4. Important grape varieties in South America**

South America has a large wine producing area and each one with its own *terroir*, which facilitates the adaptation of several species of *Vitis vinifera*, for the production of fine wines, used to manufacture the wines (Johnson and Robinson, 2008). It is noticed that there is no uniformity of production of a certain type of grape in South America. Each country developed according to its *terroir* a cultivation of its own. In Chile, although not the most planted, Carménère has become the iconic grape of the country. Dized by *Phylloxera*, a disease that attacked grape production in Europe, it was recently rediscovered by chance in Chile and Merlot grapevines. It produces a wine with enough color and pronounced tannins (Copello, 2013). In Argentina, the caste chosen as an icon is Malbec, also produced in France. Argentine Malbec gives rise to a wine of deep and brilliant color with soft tannins and excellent alcohol content (Johnson and Robinson, 2008). Tannat is the emblematic caste in Uruguay, originating in the southwest of France, has been in the country for more than 100 years. Its wines are potent, with alcohol content high around 12% to 13.5% (Farias, 2014).

Brazil still does not have an emblematic grape, but all efforts are concentrated to make Merlot, originated in the Bordeaux region of France, the iconic grape of the country. Less structured than Cabernet Sauvignon, Merlot produces soft wines and does not require much time in the bottle (Johnson and Robinson, 2008).



## 2.5. Main wine producing countries in South America

### 2.5.1 Chile

The Chilean modern viticulture was developed since from 1851 (Pitte, 2012). Due to a privileged geographic position (between the Pacific and the Andes Mountains) that provides insulation, sparing Chile from disease common to European vines, the country was free from the devastation caused by *Phylloxera vastatrix* between the 19<sup>th</sup> and 20<sup>th</sup> centuries. According to Copello (2013), this plague, that wiped out the wineries of France and Portugal in the 19<sup>th</sup> century, reaching Australia, Algeria, South Africa, Tunisia and Morocco, caused the extinction of several vineyards, by destroying the leaves of the plant. Some varieties were considered extinct due to the attack of the *Phylloxera*. The best known example was the Carménère caste mentioned previously. A solution for *Phylloxera* used until this moment was to use *Vitis labrusca* to support *Vitis vinifera*, in order to obtain a more resistant plant (Copello, 2013) Chile has as main grapes Cabernet Sauvignon, Chardonnay (white), Merlot and of course, Carménère. **Figure 3** presents the main varieties grown in Chile.

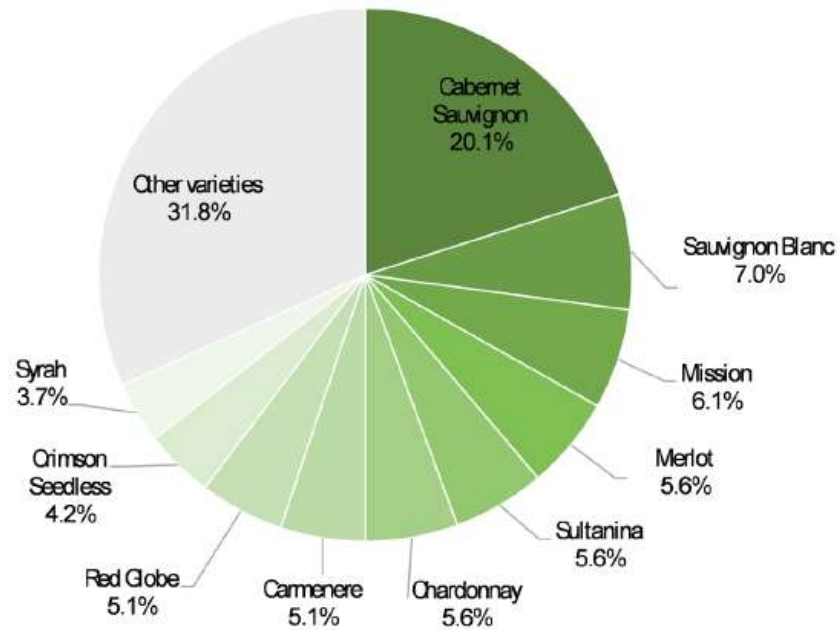
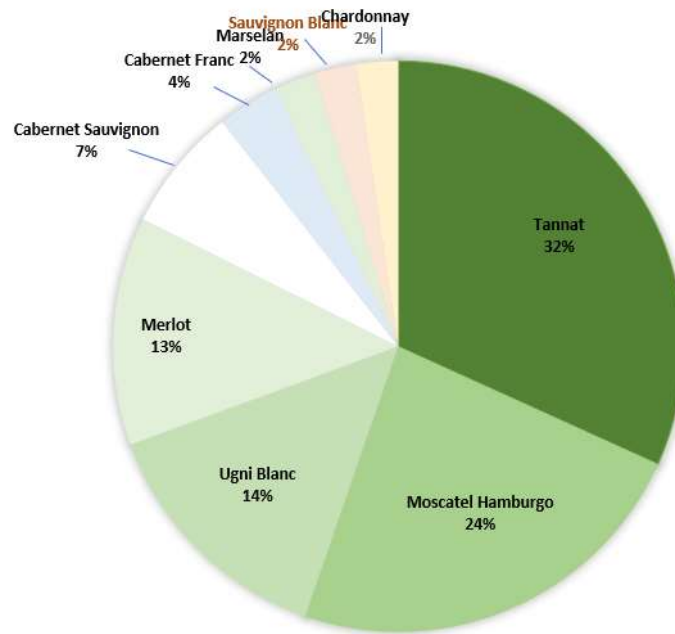


Figure 3: Main varieties grown in Chile (OIV web site 2017)

According to Johnson and Robinson (2008), the main producing regions of Chile are Aconcagua, Casablanca, San Antonio and Maipo Valley, also passing through the regions of Cachapoal, Colchagua and Maule, which has the largest area of grapevines in volcanic soil. Its annual production is 12,554,000 hl, corresponding to 5% of world production and per capita consumption was 12 liters in 2012 (Adega, 2013).

### 2.5.2 Uruguay

Uruguay began to produce wine in the 19<sup>th</sup> century, when Spanish emigrants arrived in the country, followed by Italians and French. In 1870 was inaugurated the first commercial viniculture, that is a public entity (Vinitude, 2014). European immigrants began planting grapes with the knowledge they acquired in their homeland, being 70% of the vineyards planted with Tannat and Folle Noire varieties. Tannat is a strain of French origin, but the wines of Tannat produced in Uruguay are distinct from those of France. They are less harsh, velveter, with less tannins and mature earlier. Tannat with its variety has been able to adapt the climatic conditions in the region of Salto (Northwest of Uruguay) and its harvest is manual. The internal consumption of wine is significant being the 8<sup>th</sup> placed worldwide in consumption per capita. Currently 50% of production is exported to Brazil. The largest concentration of wine producers and wine producers is in Montevideo, Canelones, San José and Colonia. **Figure 4** shows the main varieties grown in Uruguay.

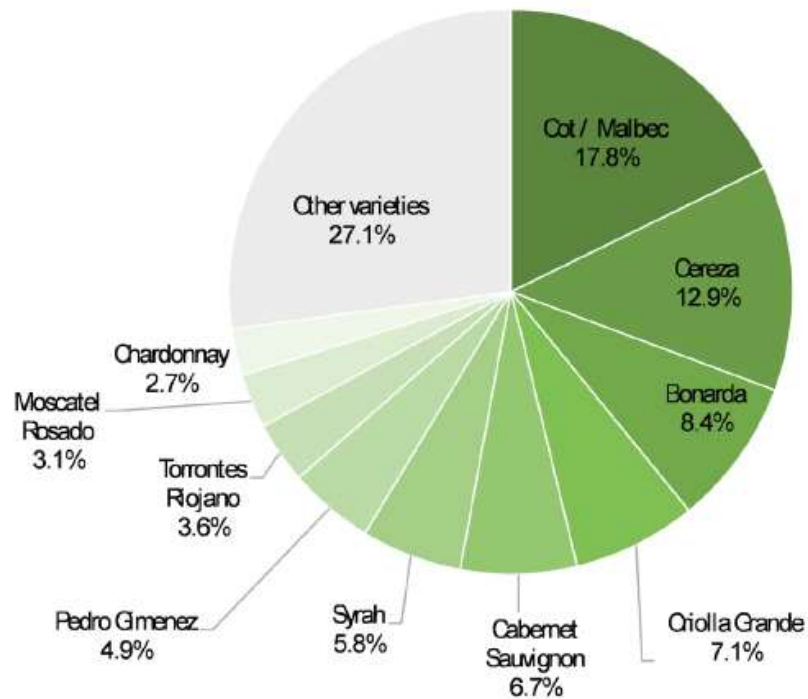


**Figure 4:** Main varieties grown in Uruguay (OIV web site 2017)

### 2.5.3 Argentina

Argentinean vineyards only gained importance at the end of 19<sup>th</sup> century with the Italian and Spanish immigrants. After, the country expanded its grapevines and implemented new winemaking techniques. These innovations have made the Malbec wine produced in Argentina known worldwide (Academy, 2014). Fifth largest wine producer in the world, Argentina began exporting wines from the 90's after a long period of economic instability. It has a planted area of 221,000 hectares, corresponding to 2.9% of the world total and a per capita consumption of 24.4 liters (OIV, 2018). Most of the country is located between the 31<sup>st</sup> and 38<sup>th</sup> parallels,

considered among the best areas for winemaking (Johnson and Robinson, 2008), being Mendoza the most important Argentine winemaking area. **Figure 5** shows the main varieties grown in Argentina.

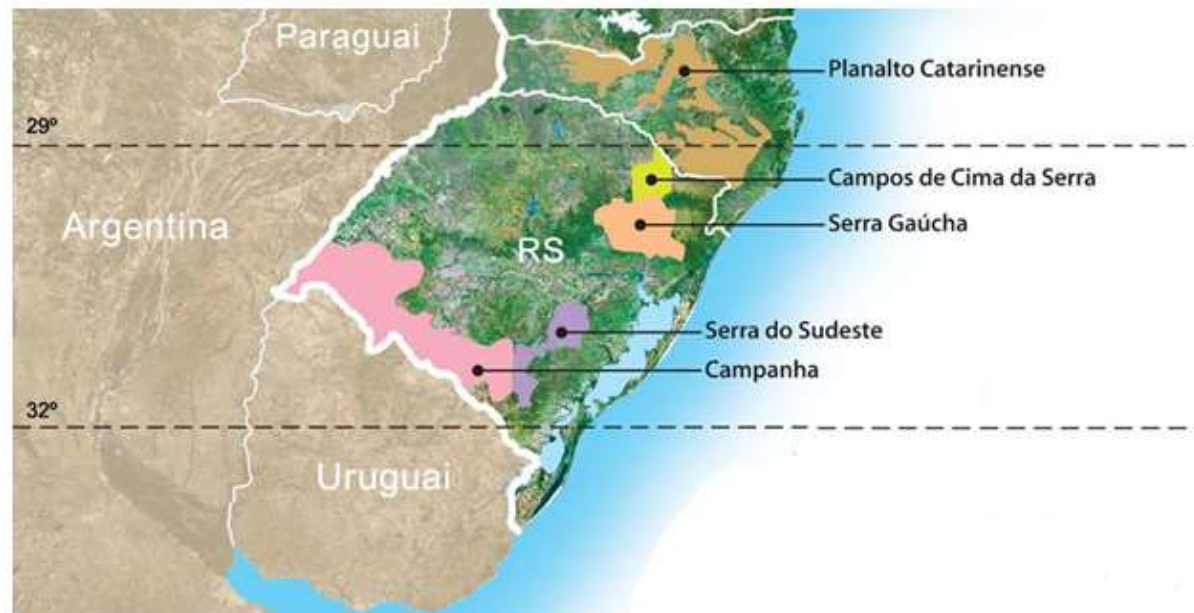


**Figure 5:** Main varieties grown in Argentina (OIV web site 2017)

#### 2.5.4 Brazil

Brazil is the third most important wine producer in volume in the South American continent. Only after the arrival of the first Italian immigrants to Serra Gaúcha around 1870s wine production began in the country (Pitte, 2012). Until 1970 the national

production was turned to elaboration of wines produced with the American species, like Isabella, that produces inferior wines popularly called "wines of bottle" and the grape juice. This fact can be justified since the annual rainfall index is very high and vines of the American species have greater resistance to rot (Johnson and Robinson, 2008). *Vitis vinifera* began to be introduced at that time, which improved the quality of wine produced along with the adoption of high technology and maintenance of a regular quality standard in national wines. Brazil has several wine producing regions, six of them most located in the southern region of the country. Serra Gaúcha is the main producing region, with soil and climate favorable for the production of grapes with high acidity, used in the preparation of base wine for elaboration of sparkling wine. Serra Gaúcha can be divided into three microregions: Vale dos Vinhedos, Pinto Bandeira and Altos Montes (IBRAVIN, 2013). Vale dos Vinhedos is the first Brazilian region to obtain the "Denomination of Origin" classification (D.O.) limiting production to Garibaldi, Bento Gonçalves and Monte Belo do Sul, which ensures the quality of the wines of that region. **Figure 6** shows the main wine producing regions in Brazil.



**Figure 6:** Main wine producing regions in Brazil (adapted from [sobrevinho](#) website)

The authorized vine varieties for cultivation in Vale dos Vinhedos are: Merlot and Cabernet Sauvignon as main, and Cabernet Franc and Tannat as auxiliaries. Chardonnay is main and Italic Riesling is auxiliary variety and for sparkling wines. Campanha, located in the border of Rio Grande do Sul with Uruguai, in the 31<sup>st</sup> parallel, is a region of relatively dry climate, its soils have low acidity, are sandy and clayey and have good drainage. It has a thermal amplitude considered good for the cultivation of grapes. The main grape varieties planted in the region are: Merlot, Cabernet Sauvignon, Tannat, Pinot Noir, Chardonnay, Sauvignon Blanc, Pinot Grigio and

Viognier. Serra do Sudeste, also on the 31<sup>st</sup> parallel, lies between Serra Gaúcha and Campanha region. With drier climate than the Serra Gaúcha, it has a good thermal amplitude. The producing region of the State of Santa Catarina is located mainly in São Joaquim and Vale do Rio do Peixe, where viticulture occupies about 2,200 hectares, with less than 5% dedicated to grapes. São Francisco Valley is located in the semi-arid region of the States of Bahia and Pernambuco, specialized mainly in the production of grapes for consumption *in natura*. The Valley of the São Francisco has been investing in high technology for the production of *Vitis vinifera* grapes and elaboration of wines with quality for exportation (IBRAVIN, 2013). The main red varieties planted in the region are: Syrah, Cabernet Sauvignon, Movèdre and Tempranillo. Among the white ones, varieties of Moscatel, Chenin Blanc, Sauvignon Blanc and Verdejo are planted. According to data from the Yearbook Wines of Brazil (Anuário, 2014), Brazil has just over 1% of the planted area in the world, an area of 81,600 hectares, with a production of 3,364,000 hectoliters (about 1.1% of world production). Brazilian per capita consumption was around 2 liters in 2013, of which 1.4 liters corresponds to table wines made with non-vinous grapes, while the consumption of fine wines produced with the grapevine varieties was around 0.6 liters. **Figure 7** shows the main varieties grown in Brazil.



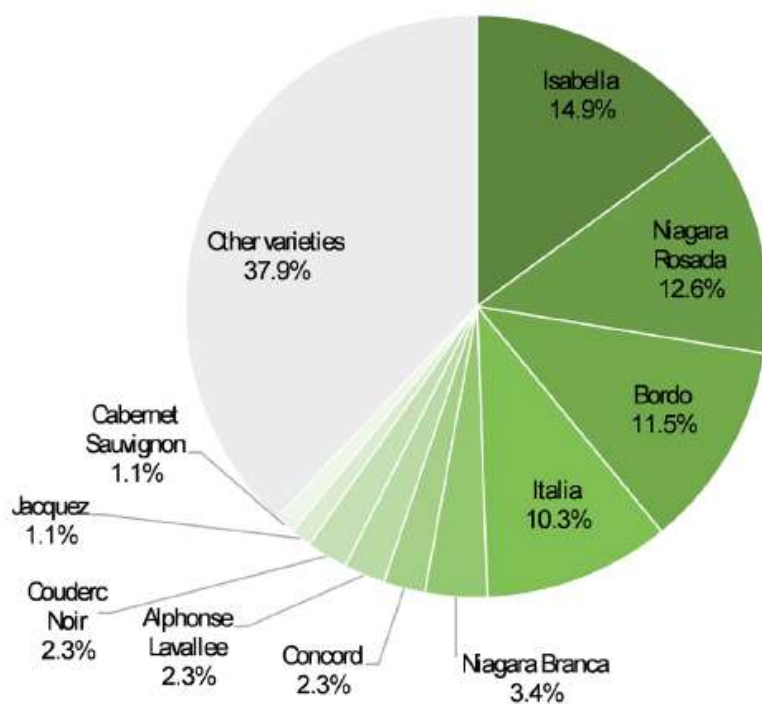
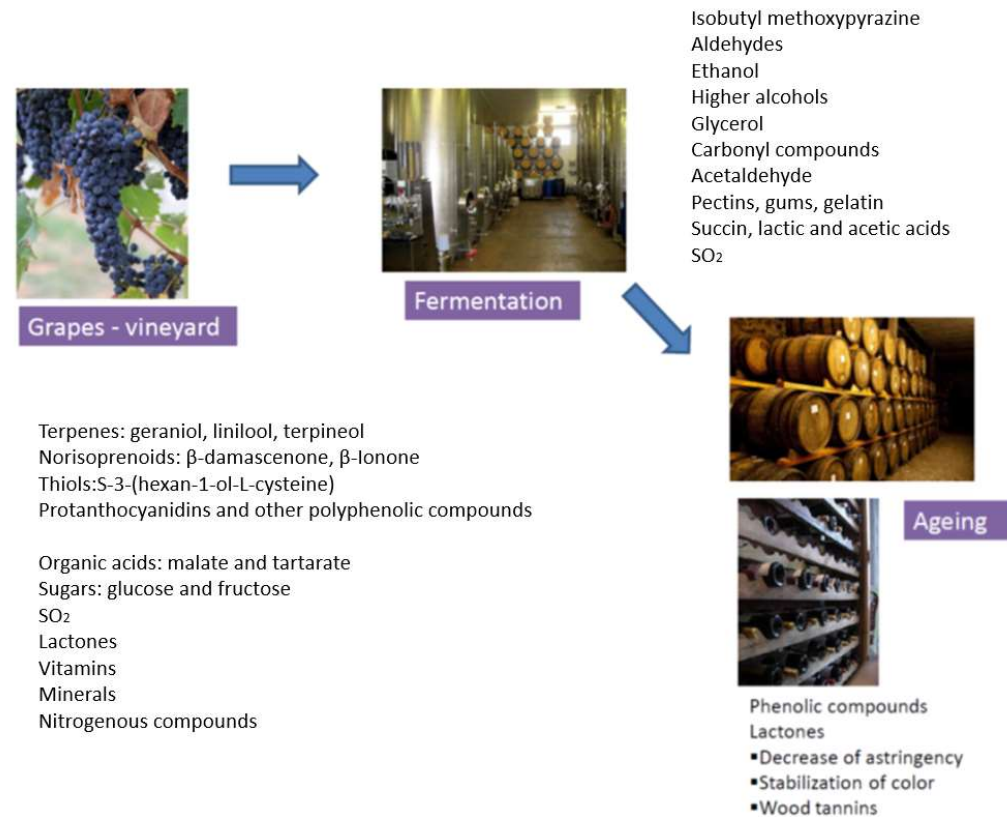


Figure 7: Main varieties grown in Brazil (OIV web site 2017)

## 2.6. Chemical characterization of wines

The most important chemical compounds in the wine are sugars, alcohols, organic acids, phenolic compounds, pigments, nitrogenous substances, pectins, gums, aromatic volatile compounds (esters, aldehydes and ketones), vitamins, minerals and sulfur dioxide (De Souza et al., 2006; Ali et al., 2010). Knowledge of the chemical composition of wine and its association with the grape

varietal is important in oenology and a necessary tool for marketing (Fanzone et al., 2012). **Figure 8** shows an overview of when such compounds are present from the vineyard to the wine storage.



**Figure 8:** Summary of major compounds present in each step of the wine production Adapted from Lund and Bohlmann (2006).

The fermentation step is an essential process for conversion of grape into wine, encompassing two major types of fermentation: alcoholic and malolactic (Styger et al., 2011). The former, common for all wines, are produced by the yeast, resulting in

ethanol and numerous byproducts (Styger et al., 2011). The malolactic fermentation is the transformation of malic acid into lactic acid by the action of lactic acid bacteria. This type of fermentation may occur spontaneously or through the inoculation of lactic acid bacteria in the wine, and begins at the end of alcoholic fermentation, when autolysis the yeast is intensified (Styger et al., 2011). In general, red wines are benefited by malolactic fermentation that promotes better aroma and softer taste (Clarke and Bakker, 2004).

### 2.6.1 Total sugar

The grape sugar content ranges from 15 to 30% depending on variety, maturation stage and climate (Ribéreau-Gayon et al., 2006). The grape sugars are almost entirely composed of D-glucose and D-fructose in equal proportions at the time of full maturation (**Figure 8**).

### 2.6.2 Alcohols

The major components in the wine are ethanol, water and glycerol. Ethanol and glycerol, in proportions of 5 to 10 g/L, are derived from the fermentation (**Figure 8**). Besides these two alcohols, butylene glycol, inositol and methanol are also usually present in wines (Ali et al., 2010). The ethanol content of wine is directly related to the perception of other volatile compounds related to aroma. For example, in 14-17% ethanol concentrations, Malbec wine flavor was described as more herbal and fruity than when the amount of ethanol was smaller (10 - 12%) (Goldner et al., 2009). The higher alcohols that have more than 2 carbons are produced from sugars and amino acids during the fermentation and include aliphatic and aromatic compounds. These alcohols can have both

positive and negative effects on the wine aroma. Aliphatic alcohols found in most wines are propanol (ripe fruit aroma), butanol (solvent aroma), hexanol (floral, grass), isobutanol and isoamyl alcohol (Garcia et al., 2011; Lilly et al., 2000; Swiegers and Pretorius, 2005). In another example, 2-phenylethanol is described as floral, being considered one of the most important aromatic alcohols to the sensory quality of the wine (Marti et al, 2003).

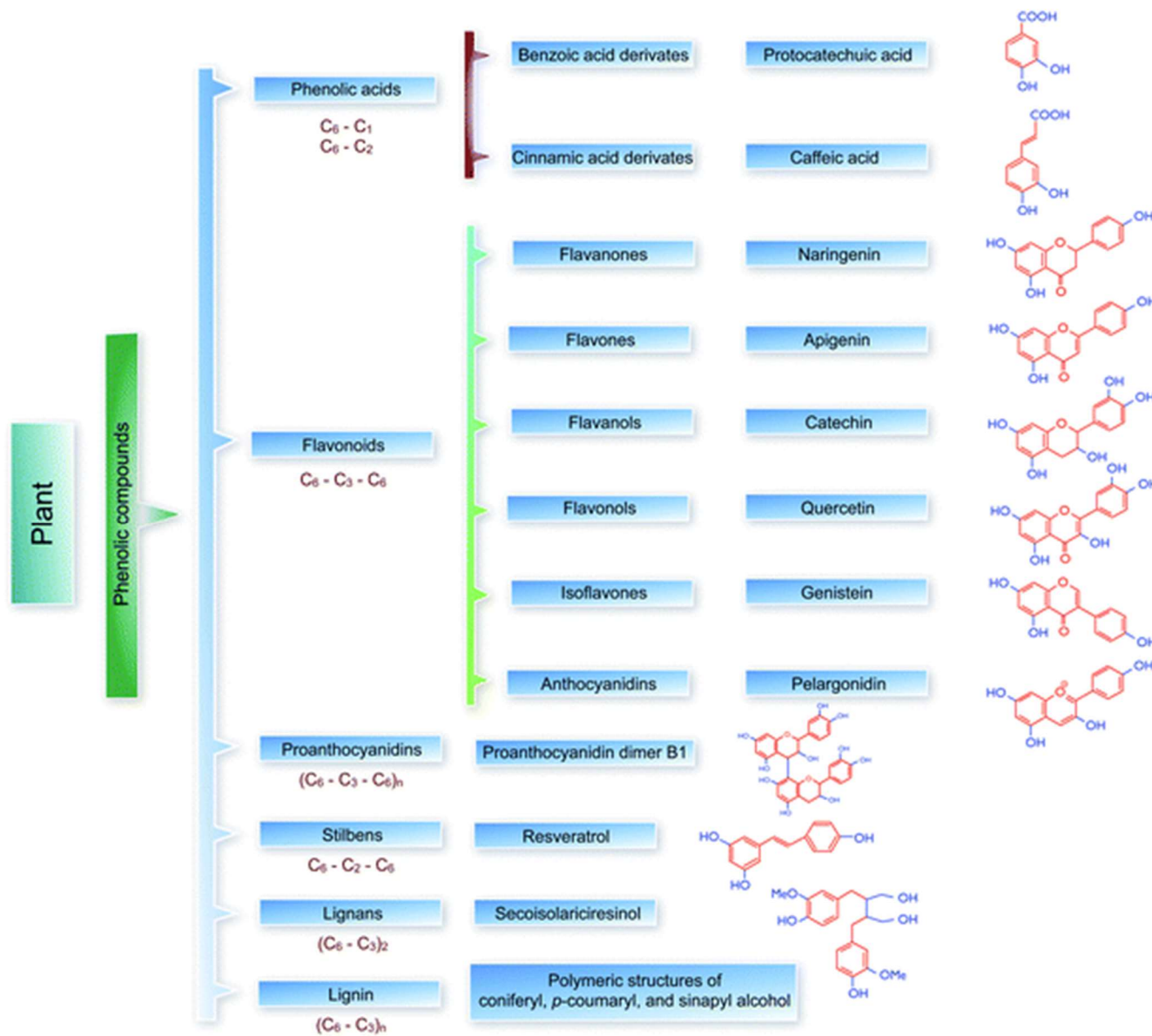
### 2.6.3 Organic acids

The main organic acids found in wines are: D-tartaric acid, L-malic and citric acids from grapes, besides succinic, lactic and acetic acid formed during the fermentation (**Figure 8**). Acetic acid, primarily responsible for volatile acidity of the wine (Ribéreau-Gayon et al., 2006), is present on the order of 0.6 g/L being produced by yeasts. However, higher concentrations are originated from microbial spoilage. The propanoic acid, 2-methyl-propanoic acid and 3-methyl-butanoic acid are produced by yeast during protein metabolism. Other important aliphatic compounds of wine, such as hexanoic, octanoic and decanoic acids are also metabolites from yeast activity (Clarke and Bakker, 2004). In the iron presence, tartaric acid can be oxidized giving glyoxilic acid, that reacts with two flavonol molecules to form a color dimer which suffers dehydration forming yellow pigments during wine ageing (Li et al., 2008).

### 2.6.4 Phenolic compounds

Polyphenol biosynthesis is strictly controlled by enzymes, thus reflecting to a great extent the genetic potential of grapes (Makris et al., 2006; Fanzone et al., 2012). However, as environmental changes play a critical role in regulating activities of enzymes, the climatic conditions occurring in different areas will also impact the polyphenols profile (Makris et al., 2006). The phenolic

compounds are of great importance in wine because confer colour, flavour and protect the grapes against attack by fungi, bacteria, viruses and solar radiation (Mazza and Miniati, 1993; Singletary et al., 2003). In grapes, polyphenols are found in peel, seed and pulp (Ribeiro and Manfroi, 2010). They have beneficial health action, mainly due to the antioxidant properties, helping to prevent cardiovascular disease (Ribéreau-Gayon et al., 2006; Singletary et al., 2003). Phenolic compounds are synthesized from phenylalanine resulting a phenyl propanol that can originate different structures, including coumarins, stilbenes, benzoic acid, cinnamic acid, lignins and lignans (**Figure 9**).



**Figure 9:** Groups of phenolic compounds found in plants: adapted from Vacek et al., (2010).

The most common flavonoids present in red wine are anthocyanins, flavanols and flavonols, differentiated by degree of unsaturation, which are responsible for the color characteristics and body (Zoecklein et al., 1995; Space et al., 2008). Grape phenolics are classified into two groups: flavonoids and non-flavonoids. The former includes flavan-3-ols, flavonols and anthocyanins. The later encompass hydroxybenzoates, hydroxycinnamates and stilbenes (Yang et al., 2009).

Anthocyanins, responsible for the huge diversity of colors in flowers and fruits (Dangles et al., 1992), are usually located in the epidermal cell vacuoles of plants, and can be found in fruits, flowers, roots, stems, leaves and seeds (Saona-Rodriguez et al., 1998). Anthocyanins are a family of phenolic compounds directly related to red wine color, which is stabilized during ageing by the complexation between anthocyanins and proanthocyanidins (Chira et al., 2012). They differ from the other flavonoids by their strong absorption in the visible region of the spectrum, and their ability to form resonant structures by varying the pH (Mazza and Miniati, 1993). Several factors can interfere with the biosynthesis of anthocyanins, including climate conditions, light, temperature, soil type and the applied agricultural practices (Roggero et al., 1986). For this reason it has been suggested the use of anthocyanins as chemical markers to differentiate grape varieties and red wines (Yang et al., 2009). Fanzone et al. (2012) evaluated the individual phenolic composition of six varieties used to produce wines in Mendoza (Argentina), and concluded that anthocyanins, flavonols and phenolic acids exerted a great influence on cultivar-based differentiation.

The anthocyanins found in grapes and wines are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, malvidin, etc. Only monoglycosidics and acylated anthocyanins have been identified in wine and grape varieties of *Vitis vinifera* (Ribéreau-Gayon et al., 2006; Somaatmadja and Powers, 1963; Burns et al., 2002). In addition to the coloring function, they also have beneficial health effects as antioxidant (Rivero-Pérez et al., 2007; Yang et al., 2009), raising up the scientific interest in these functional properties associated

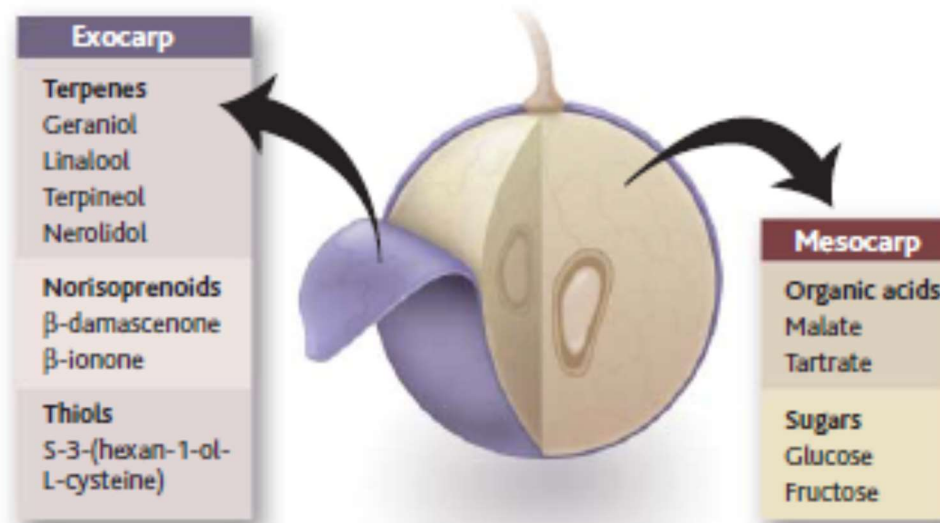
with this class of compounds (Kapadia et al., 1997). Pyranoanthocyanins show high stability during ageing and are more resistant to elevate pH than anthocyanins, expressing more color than other pigments at the typical wine pH (Fanzone et al., 2012).

Although found in lower concentrations, flavonols can exert an important role in the development of color in red wines by co-pigmentation with anthocyanins (Ribéreau-Gayon et al., 2006). Studies have shown that quercetin plays antioxidant activity *in vivo* and *in vitro*, which in turn reflects on potential health benefits (Edwards et al., 2007; Boots et al., 2008). Among the flavanols present in grapes, flavan3-ols, mainly represented by catechin and epicatechin, undergo structural transformation through oxidation and condensation reactions that affect the wine astringency and color (Mateus et al., 2003). Proanthocyanidins or condensed tannins are represented by procyanidins which are oligomers and polymers of catechin and epicatechin. In red wines, the majority procyanidin B1 has several chemical properties, such as metal chelator, antioxidant activity and capacity of complexation with macromolecules, reflecting in the wine stability (Moreno-Arribas et al., 2009). Based on high potential of phenol-based analysis for red wine differentiation, Fanzone et al. (2012) applied multivariate statistical techniques to separate wines produced in the same origin. The authors suggested that non-acylated delphinidin and petunidin, acetylated anthocyanins, pyranoanthocyanins, flavonols and phenolic acids were compounds that more influenced a cultivar-based differentiation. Jaitz et al. (2010), using canonical discriminant analysis, obtained a geographic discrimination of several grape varieties and also a grape variety based discrimination of four regions based on their polyphenolic spectrum. The authors showed that phenolic pattern including their isomeric pairs, can support the origin-and grape variety based classification.

### 2.6.5 Volatile Organic Compounds

Major volatile compounds present in grapes and wines are terpenes, norisoprenoids and thiols that contribute to flavor **Figure 10** (Lund and Bohlman, 2006).





**Figure 10:** Known grape compounds important for flavor. Adapted from Lund and Bohlman, (2016)

The volatile composition of the grape is related with varietal genotype, corresponding to the genetic characteristics of each cultivar grape, and the phenotype characterized by interactions between genes and environmental factors (Clarke and Bakker, 2004). Green et al. (2011) reported that geographical location influenced the volatile composition in Sauvignon blanc wines. The aroma of pre-fermentation origin are formed from the grape harvest until the beginning of the alcoholic fermentation (**Figure 8**), including the transport, destemming, pressing and maceration (Ribéreau-Gayon et al., 2000). During the mechanical processing, grapes are smashed

and the enzymes such as glycosidases and peptidases come in contact with their substrates (Lund and Bohlman, 2006). Moreover, these treatments have resulted in the incorporation of oxygen in the medium, resulting in enzymatic oxidation reactions (Ribéreau-Gayon et al., 2000). In addition to ethanol, other volatile compounds that are produced in smaller quantities during alcoholic fermentation, can contribute significantly to the overall aroma of the wine (Clarke and Bakker, 2004). Ethyl and acetate esters smell berry fruits, C6 alcohols smell vegetable, phenylacetaldehyde and ethyl cinnamate smell caramel and whiskey lactone smells woody (King et al., 2014). Other volatile compounds such as hydrogen sulfide, organic sulfides and some thiols can be present in wine and are responsible for some undesirable flavours (Clarke and Bakker, 2004). Dozen of different terpenoids contribute with nuances of floral or fruity aromas, depending on varietal. In general, monoterpenes such as geraniol, linalool and citronellol contribute to a distinctive floral character of the wines (Lund and Bohlman, 2006). Regarding the norisoprenoids,  $\beta$ -damascenone is detected as honey like fruity character at femtomolar levels (Lund and Bohlman, 2006).

Terpenes are formed by a basic structural unit containing five carbon atoms, the isopentenyl pyrophosphate. These molecules are classified according to the number of carbons: monoterpenes (10), sesquiterpenes (15), diterpenes (20), sesterpenes (25), triterpenes (30) and tetraterpenes (40) (McCaskill and Croteau, 1998). Most terpene compounds of grapes and wines are monoterpenes and sesquiterpenes that influence the wines aroma (Camara et al., 2007).

A large number of carbonyl compounds can be formed by decarboxylation of  $\alpha$ -keto acids. Among them are pyruvic acid,  $\alpha$ -ketolactic, ketobutiric- $\alpha$ ,  $\alpha$ -ketoisovaleric, ketoisocaproic,  $\alpha$ -keto-methyl-valeric and ketohydroxybutiric (Clarke and Bakker, 2004). The acetaldehyde, which is the largest amount of aldehyde formed during alcoholic fermentation, is highly volatile and described as pungent odor that resembles tint, bringing bitterness and oxidized flavour to the wine (Li et al., 2008; Morozova et al., 2015; Gürbüz et al., 2006). It can also be derived from oxidation of ethanol catalized by transition metals or through coupled oxidation of phenols, generating polycondensated flavanol polymers, which cleave into vinylflavonol oligomers and xanthylium pigments (Li et al., 2008). Acetaldehyde affects color development of red wines, promoting condensation reactions between anthocyanin, catechins and tannins,

forming stable polymeric pigments which resist to discoloration (Romero and Bakker, 2000). The enzymes involved in the formation of alcohols and aldehydes are acylhydrolase, lipoxygenase and alcohol dehydrogenase (Ribéreau-Gayon et al., 2000). Alcohol and aldehyde compounds have a grassy flavor and a relatively low perception threshold. For example, the thresholds of perception of cyclohexanol and 2-hexenal are about 1 µg/L and 17 µg/L, respectively (Clarke and Bakker, 2004). Hexanal and hexenal are considered enzymatic oxidation products of linoleic and linolenic acids, which occurs during the pressing of the grape and its aroma has been described as “green”. The 2,3-butanedione, commonly referred to as diacetylketone is the most important to the flavor of the wine. When it is present in wines in a concentration above 7.5 mg/L, is considered undesirable. However, when present at concentrations of 1-4 mg/L, contributes positively, with buttery or caramel flavour (Bartowsky et al., 2004).

#### **2.6.11. Other compounds**

The nitrogenous substances have less interference in the wine tasting, but are essential nutrient media for yeast and bacteria. The esters, when present in high amounts in wine, make it to become spoiled or with acidic character, but under low concentrations are considered favorable aroma constituents (Aqarone, 2001). Methoxypyrazine compounds, in special 2-methoxy-3-isobutylpyrazine, can be sensory detected as ppt levels (Lund and Bohlman, 2006).

Sulphur dioxide (SO<sub>2</sub>) is an antioxidant and inhibitor of microbiological activity widely used to prevent wine from browning (Li et al., 2008; Morozova et al., 2015). However, due its health-related objections such as allergic reactions, its use was regulated by the “World Health Organization (WHO)” (Li et al., 2008). In Brazil, it is allowed to use up to 350 mg/L as the total SO<sub>2</sub>. Sulfur avoids the spontaneous fermentation of the grapes and it is usually sprinkled during the grapes harvest. Sulfur can also be provided by the enzymatic amino acid degradation, other products from the fermentation process containing sulfur or some pesticide residue

degradation (Mestres et al., 2000). Sulphur dioxide reacts with  $H_2O_2$  resulting from Fenton reaction and also with quinones formed by oxidation of phenolic compounds (Morozova et al., 2015).

Lactones are formed by an esterification involving hydroxyl and carboxyl groups to form a cyclic ester, which in most cases has rings containing 4 carbons. Lactones can be originated from grapes, formed during the fermentation or can be extracted from the barrels used in the wine aging (Clarke and Bakker, 2004). Lactones from grapes usually are not involved with the development of the varietal aromas, except 2-vinyl-dihydrofuran-2-one, which is associated with the aroma of wines produced with Riesling and Muscat grapes, and 2,5-dimethyl-4-hydroxy-3 (2H) -furanone (furanol) which is related to Merlot wines (Clarke and Bakker, 2004). The sotolon (3-hydroxy-4,5-dimethyl-2 (5H) -furanone) contributes to the aroma of wines produced from grapes infected with *Botrytis fungi* and is also related to the typical aroma of aged wines, since its concentration increases during storage (Silva Ferreira et al., 2003). Other important lactones for flavoring wines are the isomeric forms, *cis* and *trans* 3-methyloctalactone, also known as oak whiskey lactone or just lactone. These compounds contribute to the woody aroma of the wine (Gómez García-Carpintero, 2012).

## 2.7 Wine typification

Wine production is one of the largest economic agricultural activity worldwide and demand for wine is steadily increasing in the last years (Perini and Camin, 2013). The information indicated on the wine label is connected to special consumer's expectations concerning the sensory and quality criteria. The label may indicate, therefore, characteristics such as brand, type, vintage, variety and origin of the product, which represents the wine identity. Due to its economic importance, wine is one of the most common beverages subject to fraud and mislabeling. Manipulations can be ascribed to both its intrinsic (e.g. dilution of wines with water, addition of alcohol, coloring or flavoring substances) and its extrinsic properties (e.g. fraudulent misrepresentation of the cultivar and geographic origin) (Holmberg, 2010). The European Union protects agricultural products strictly linked to the origin area and to specific methods

of production by conferring designations of origin, namely Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialties Guaranteed (TSG) (European Commission, 2003). In Europe, most wines of high economic value belong to the PDO category. Each PDO wine is produced according to a set of specific regulations governing such aspects as grape varieties to be used, cultivation and vinification methods, aging, physical/chemical parameters and sensory characteristics. Several PDO wines are produced by a single variety (monovarietal wines). European legislation is now in place to regulate and guarantee the authenticity and geographical traceability of food (Regulation (EU) No. 1151/2012; No. 664/2014; No. 668/2014), with the main instruments being analytical controls and analysis of mandatory documents related to PDO wine production.

Various examples of wine fraud and mislabeling have been recorded over the years, highlighting the need for improvements in authentication of labeling information. Cavicchi and Santini (2011) reported the case of the Brunello wine crisis that occurred in 2008 in Montalcino (Tuscany, Italy), when producers were accused of not adhering to the production standards established by the appellation system. Indeed, grape varieties other than Sangiovese (the only one admitted by the production regulations) were used to make a softer wine, more appreciated by American consumers. Another case occurred in France in 2010, when producers were convicted of replacing Pinot Noir wine with cheaper Merlot and Syrah wines (Takeoka & Ebeler, 2011). A well known example of an unintentional mislabelling of wine occurred in Chile up to the late 90's, when Carménère grapes were incorrectly confused with a special clone of Merlot (von Baer, Mardones, Gutiérrez, Hofmann, Hitschfeld, and Vergara, 2006). To prevent fraud, a critical aspect is the ability to assess whether the wine in question conforms with the label information in terms of grape variety and geographical origin. Fraud is often discovered through auditing or denunciation, but might also be detected (or confirmed) chemically. In the last few years, there has been growing interest in developing analytical methods for wine authentication. Stable isotopic ratio analysis of wine has been used for identify its geographical origin due to the existence of an Official European Database (EU-Wine DB). However, instrumentation and running costs, along with the requirement of highly trained analysts, make this investigation difficult (Danezis, Tsagkaris, Brusica, & Georgiou, 2016).

As regards varietal authentication, controls against counterfeiting are left to the analysis of mandatory documentation in the vineries and photographic records of the vineyards. The analytical parameters and sensory characteristics laid down in wine production regulations are not designed to identify the grape variety used. Indeed, chemical parameters for which a minimum value is established are not specifically linked to a grape variety (total alcohol degree, total acidity, non-reducing extract) and very generic sensory terms are used to describe the wine (e.g., fruity, fresh, characteristic, harmonic, dry). In addition, there are no accredited methods to evaluate whether a wine fulfills the legal requirements in terms of sensory characteristics, and evaluation relies on the opinion, training and experience of expert tasters (Etaio et al., 2010). In this scenario, since food authentication methods are in the phase of exponential growth, effective control for the varietal authenticity of a wine is an excellent opportunity, yet still a challenge.

## **2.8 Instrumentation in wine analysis**

The large amount of wine analytical targeted analyses have definitely contributed to a better knowledge of the wine chemistry (Flamini, 2013). This is especially true for the complex organic fractions of wine, such as the volatile compounds, phenolics and influential trace-level constituents. In this way, gas chromatography (GC) and liquid chromatography (LC), mainly coupled with mass spectrometry (MS), has greatly aided the determination of organic compounds in wines. Other techniques for sample preparation have also helped in the determinations, for example the headspace technique, SPME (Solid-Phase Microextraction), ITEX (In-Tube Extraction) and more recently SPME arrow. Alternatively, metabolomics approaches have shown great potential for the study of grape or wine (Gougeon et al., 2009). The concept of wine omics (Metabolomics: Wine- omics, 2008), for the untargeted wine analyses coupling NMR (Nuclear Magnetic Resonance) and GC-MS were followed by the oenomics approaches, described by Gougeon et al. (2009), exemplified by the revealing of the metaboledgeography expression of cooperage oak wood by FTICR-MS (Fourier-Transform Ion Cyclotron Resonance). The composition of wine is determined by a complex interplay between environmental factors, genetic

factors (grape varieties) and viticultural practices (Atanassov et al., 2009; Roullier-Gall et al., 2014a), and metabolomics offers the toolbox for integrated analyses of the wine composition resulting from all of these factors. Holistic analyses require access to sensitive and powerful instruments, therefore metabolomics typically employs high resolution techniques like FTICR-MS, LC-MS, GC-MS, and NMR spectroscopy combined with multivariate statistics.

The advantages and disadvantages in terms of selectivity, sensitivity, dynamic range, robustness, and accuracy of these different analytical tools have been well described (Heeren et al., 2004; Hertkorn et al., 2007; Cevallos-Cevallos et al., 2009; Hong, 2011; Theodoridis et al., 2012a). Vine and wine are well-documented fields of chemistry and various NMR-based or MS-based studies have been published in recent years. As examples, NMR now allows the differentiation of important parameters like the grape variety, geographical origin and year of vintage (Godelmann et al., 2013) whereas FTICR-MS enables the separation of wines according to the geographical origin inside proximate areas (Roullier-Gall et al., 2014a,b). FTICR-MS is a powerful technique for studying wine, mostly employed in direct infusion experiments to benefit from its ultra- high resolving power and mass accuracy (Gougeon et al., 2009; Liger-Belair et al., 2009; Roullier-Gall et al., 2014b). However, as a major disadvantage, direct infusion techniques do not allow to discriminate between isomeric and isobaric compounds (Forcisi et al., 2013). If a chromatographic separation is not applied at the first stage, a large number of molecules are subjected simultaneously to the ionization process causing ion suppression for numerous analytes and the inability to separate isobaric and isomeric substances (Forcisi et al., 2013; Gika et al., 2014). Due to this fact, chromatographic separation prior to MS-analysis is particularly important for both targeted and non-targeted metabolomics, and is already used for untargeted metabolomics analysis of grape to differentiate grape berry ripening and post- harvest withering (Toffali et al., 2011), for wine authentication (Rubert et al., 2014), or for the understanding of the terroir impact in wine (Tarr et al., 2013). LC/MS is designed for the analyses of non-volatile compounds present in complex matrices such as amino acids (Tolin et al., 2012), phenolic compounds (Jaitz et al., 2010), fatty acids (Della Corte et al., 2013).

A large number of LC/MS metabolite profiling studies have been realized using a combination of UPLC with Time-Of-Flight Mass Spectrometry (TOF-MS) (Grata et al., 2008; Fontana et al., 2011; Vaclavik et al., 2011; Forcisi et al., 2013; Rubert et al., 2014). LC-Q-TOF-MS was applied in many wine studies for targeted analyses such as the separation and detection of resveratrol (Wang et al., 2002), of phenolic compounds (Püssa et al., 2006; Sun et al., 2006; Prosen et al., 2007; Gruz et al., 2008; Jaitz et al., 2010) or toxins (Zöllner et al., 2000). In contrast, very few studies have used this technique for untargeted metabolomics analyses of grapes and wines, as shown for instance by Arapitsas et al. or Vaclavik et al. for the understanding of the mechanism of wine micro-oxygenation or to discriminate wines according to grape varieties (Vaclavik et al., 2011; Arapitsas et al., 2012). Such approach combines the highest chromatographic resolution with an excellent sensitivity, fast data acquisition and high mass accuracy (Forcisi et al., 2013; Gika et al., 2014). However, it does not refer to the highest resolution that can only be achieved by FTICR-MS, which typically requires higher time domains and thus do not fully exploit the potential of fast UPLC.

MS-based techniques are in perpetual development, improving the mass resolution, the precision and velocity of acquisition, thus enabling improved detection and identification (Allwood and Goodacre, 2010; Forcisi et al., 2013). The selection of the ionization mode and sorbent materials will have a significant effect on the obtained metabolic profile. Some molecules are ionized more efficiently in one ionization mode. In order to cover the widest range of compounds separated via LC, UPLC-Q-TOF-MS combined with both reversed phase and hydrophilic interaction chromatography represents a powerful analytical platform (Theodoridis et al., 2012a; Forcisi et al., 2013). Reversed-phase (RP) liquid chromatography has been highlighted as the most used separation mode for the metabolome analysis (Vaclavik et al., 2011; Theodoridis et al., 2012a,b; Gika et al., 2014). RP separation covers a large part of the metabolome, and at the same time provides the most reliable, robust and sophisticated LC stationary phases, while the number of available chemistries, geometrical characteristics of particles and columns surpasses the corresponding numbers for all other modes. Alternative separation mechanisms are required to separate polar analytes. Hydrophilic Interaction Chromatography (HILIC) provides



separations complementary to those obtained by RPLC–MS in that early eluting analytes in the RP mode are often well retained by HILIC (Forcisi et al., 2013; Müller et al., 2013; Gika et al., 2014).

The current bottleneck in MS based metabolomics is the structural identification of compounds associated with the detected masses. The high levels of resolution and mass accuracy make Q-TOF-MS appropriate for combined MS profiling and MS/MS analysis, thus allowing the complete metabolite discovery process from the untargeted analyses to the structural elucidation of markers (Allwood and Goodacre, 2010; Vaclavik et al., 2011; Rubert et al., 2014). Novel approaches for the analysis of MS/MS spectra are currently developed, e.g., *in silico* fragmentation, offering interesting alternatives for metabolite identification. The development of non-targeted combined metabolomics approaches have already demonstrated its importance for the metabolite coverage, validation of data and identification of biomarkers, meaning that no method can describe the whole metabolome alone (Theodoridis et al., 2012a; Forcisi et al., 2013; Müller et al., 2013). **Table 2** presents the main advantages and disadvantages of analytical techniques in wine analysis.

**Table 2:** Advantages and disadvantages of analytical techniques in wine analysis.

<b>Technique</b>	<b>Advantage</b>
NRM	Allows the molecular structure of a material to be analyzed
FTICR-MS	Ultra- high resolving power and mass accuracy
GC/MS	Separations and mass spectra for confirmation
LC MS/MS	Separations, large range for quantitation, MSMS spectra
LC QTOF	Separations, high resolving power, isotopic separations, mass accuracy
SPME	Excellent technique for extraction of volatiles with or without immersion in the sample
ITEX	Excellent for extracting volatiles more sensitive than HS and SPME
SPME Arrow	Larger sorption phase volume and robustness
<b>Technique</b>	<b>Disadvantage</b>
NRM	Direct infusion techniques do not allow to discriminate between isomeric and isobaric compounds
FTICR-MS	Direct infusion techniques do not allow to discriminate between isomeric and isobaric compounds
GC/MS	Low resolution and low isotope separations
LC MS/MS	Low resolution and low isotope separations
LC QTOF	Not an ideal technique for quantification
SPME	There is no fiber that is optimal for all classes of compounds
ITEX	There is no tube that is optimal for all classes of compounds
SPME Arrow	There is no fiber that is optimal for all classes of compounds

## 2.9 Multivariate analysis in wine classification

Multivariate data analysis was developed in the late 1960s and was used by a number of research groups in analytical and physical organic chemistry. Its development was due to the introduction of modern instrumentation, which gave multivariate responses for each sample analyzed (eg, wavelengths, ions, mass to charge ratios, chromatographic peaks) and to the wider availability of computers (Wold et al., 2001). In modern chemical measurements, we are often confronted with so much data that the essential information may be not readily evident (Otto 1999). Certainly that can be the case with chromatographic or spectral data for which many different observations (peaks or wavelengths) have been collected from a single sample. Each different measurement can be thought of as a different dimension. Traditionally, analysts strive to eliminate matrix interference in analytical methods by isolating or extracting the analyte in order to make the measurement itself apparently simple and certain. However, this approach ignores the possible effects of chemical and physical interactions between the different constituents present in the sample, which is something that is especially evident when complex materials like grapes and wine are analyzed (Arvantoyannis et al., 1999). Multivariate methods provide the means to move beyond the one-dimensional (univariate) world. In many cases, multivariate analysis can reveal constituents that are important through various interferences and interactions. This inductive rather than deductive reasoning needs a leap of imagination; it might be harder to accept and seemingly less certain and secure, but it opens a new chapter in the development of inferences from the analysis of the whole matrix (Wold et al., 2001). Therefore, multivariate data analysis is defined as the application of mathematical or statistical methods to chemical data. Multivariate data analysis, unlike classic statistics, considers multiple variables simultaneously. Although multivariate ANOVA (MANOVA) is available, other multivariate analytical methods have the advantage that they can take collinearity into account (Naes et. al., 2002). Multivariate analysis can take into account the variation in one variable, or a group of variables, in terms of co-variation with other variables (Naes et. al., 2002). The analysis can

mathematically describe the co-variation (degree of association) between variables, or find a mathematical function (regression model), by which the values of the dependent variables are calculated from values of the measured (independent) variables (Gelad 2003). Today, many food quality measurement techniques are multivariate and based on indirect measurements of chemical, physical, and sensory properties (Naes et. al., 2002). A typical characteristic of many of the most useful of these instrumental techniques is that, paradoxically, the measurement variable might not have a direct relationship with the property of interest, for instance the concentration of a particular chemical in the sample, that is, a correlative method. This often refers to chemical and physical interference (Gelad 2003).

Multivariate data analysis research spans a wide area of different methods which can be applied in different fields of science and technology. They include methods and techniques for collecting good data (optimization of experimental parameters, design of experiments, calibration, signal processing) and for getting information from these data (statistics, pattern recognition, modeling, structure– property–relationship estimations) (Naes et. al., 2002). The most commonly used multivariate data analysis techniques applied to grape and wine analysis are principal component analysis (PCA) and partial least squares (PLS) regression (Wold et al., 2001).

### 3. HYPOTHESIS AND OBJECTIVE

#### Hypothesis

Four groups of red wines from South America (Argentinean Malbec, Chilean Carménère, Brazilian Merlot and Uruguayan Tannat) can be grouped and characterized according to their composition in terms of phenolic and volatile compounds.

#### Main objective

To identify phenolic and volatile compounds that characterize four commercial categories of South American red wines.

#### Specific objectives

- Determine the fingerprint of the samples using a Quadrupole Time-of-Flight (QTOF) Liquid Chromatography–Mass Spectrometry.
- Identify which compounds differed among the four commercial categories and apply these compounds as variables to perform multivariate statistical analysis.
- Group the wines into “Chemical Categories” derived from multivariate statistical analysis and cross validate them using the commercial categories.
- Describe which compounds characterize each “Chemical Category” by HPLC/MS and GC/MS.
- Determine color, antioxidant activity, acidity, sulfites and tannins concentration to complete the “Chemical Categories” characterization.

<https://www.sciencedirect.com/science/article/pii/S0308814619314530>

## 5. ADDITIONAL CONSIDERATIONS ABOUT MANUSCRIPTS

- ✓ Ethanol was not considered in the determinations due to its high concentration.
- ✓ Spectral absorbance profiles of red wines across the visible spectrum from 400 to 700 nm were determined. The absorbance values at three wavelengths were used, (420, 520 and 620 nm).

## 6. SUGGESTIONS FOR FUTURE WORK

After the experience with this study I would like to present my considerations regarding the next steps that should contribute even more to these chemical characterizations of these wines or in other studies.

- ✓ Work with wines produced with a single grape. This will yield valuable information on the exact composition of wines produced with certain grape varieties. We have information from laboratories that make this production and that can be great partners.
- ✓ Create database for wine analysis for LC / MS. Thus, the determinations will be much faster without the purchase of all the standards. Databases are very common for veterinary drugs, pesticides, etc., but we do not have a specific one for wines.
- ✓ Work with new technologies for volatile determinations such as ITEX and SPME arrow. With these new technologies we will have the possibility of more efficient extractions of volatiles with levels lower than those achieved nowadays.
- ✓ Work with GC / MS QTOF for volatile determinations. With this we will have more precision in the results with the use of isotope separation and precision of masses.

## 7. REFERENCES.

ADEGA. Consumo de vinhos no Chile cai 15,3% em cinco anos: ainda assim, país é o terceiro maior mercado consumidor da América Latina. Disponível em: <[https://revistaadega.uol.com.br/artigo/consumo-de-vinhos-no-chile-cai-153-em-cinco-anos\\_9486.html](https://revistaadega.uol.com.br/artigo/consumo-de-vinhos-no-chile-cai-153-em-cinco-anos_9486.html)>. Acesso: 06 de Março de 2017.

AGUIAR, J.E.; MIWA, M. ADEGA. O vinho e sua história: do império Romano. Disponível em: <[http://revistaadega.uol.com.br/artigo/ovinho-e-sua-historia\\_1064.html](http://revistaadega.uol.com.br/artigo/ovinho-e-sua-historia_1064.html)>. Acesso: 06 de setembro de 2014.

AKASAKA, K. et al. Fluorometric determination of sulfite in wine by N-(9-acridinyl) maleimide. **Agricultural and Biological Chemistry**, v.54, n.2, p.501-504, 1990.

ALEIXANDRE, M. et al. Portable e-nose to classify different kinds of wine. **Sensors and Actuators**, v.131, p.71–76, 2008.

ALI, K. et al. Metabolic constituents of grapevine and grape-derived products. **Phytochemistry Reviews**, v.9, n.2, p.357-378, 2010.

ALLWOOD, J. W.; GOODACRE, R. An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. **Phytochemical Analysis : PCA**, v.21, n.1, p.33–47, 2010.

ANUÁRIO vinhos do Brasil 2014. [S.l.]: Baco Multimídia, 2014 ACADEMIA do vinho. Argentina. Disponível em:< [https://www.academiadovinho.com.br/\\_\\_mod\\_regiao.php?reg\\_num=AR](https://www.academiadovinho.com.br/__mod_regiao.php?reg_num=AR) >. Acesso: 5 de maio de 2018.

AQUARONE, E. **Biotechnologia Industrial**. 1. ed. São Paulo: Edgar Blücher, 2001.

ARAPITSAS, P. et al. A metabolomic approach to the study of wine micro-oxygenation. **PLoS ONE**, v.7, n. 5, p.1-11, 2012.

ARRANZ, S. et al. Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. **Nutrients**, v.4, n.2, p.759-781, 2012.

ARVANITOYANNIS, I.S. et al. Application of quality control methods for assessing wine authenticity: Use of multivariate analysis (chemometrics). **Trends in Food Science and Technology**, v.10, n.10, p.321-336, 1999.



ATANASSOV, I. et al. Wine metabolite profiling: Possible application in winemaking and grapevine breeding in Bulgaria. **Biotechnology and Biotechnological Equipment**, v.23, p.1449-1452, 2009.

BALLESTER, J. et al. Conceptual vs. perceptual wine spaces: Does expertise matter? **Food Quality and Preference**, v.19, n.3, p.267-276, 2008.

BARTOWSKY, E. J., HENSCHKE, P. A. The 'buttery' attribute of wine—diacetyl—desirability, spoilage and beyond. **International Journal of Food Microbiology**, v.96, n.3, p.235-252, 2004.

BOOTS, A.W.; HAENEN, G.R.M.M.; BAST, A. Health effects of quercetin: From antioxidant to nutraceutical. **European Journal of Pharmacology**, v.585, p.325-337, 2008.

BRAND-WILLIAMS, C.B. Use of a Free Radical Method to Evaluate Antioxidant Activity Lebensm.-Wiss. u.-**Technology**, 28.25-30, 1995.

BRERETON, R. G. Introduction to multivariate calibration in analytical chemistry. **The Analyst**. v.125, p.2125–2154, 2000.

BURNS, J. et al. Variations in the Profile and Content of Anthocyanins in wines made from Cabernet Sauvignon and Hybrid Grapes. **Journal Agriculture and Food Chemistry**, v.50, p.4096-4102, 2002.

CADOT, Y. et al. Sensory dimension of wine typicality related to a terroir by Quantitative Descriptive Analysis, Just About Right analysis and typicality assessment. **Analytica Chimica Acta**, v.660, n.1, p.53-62, 2010.

CAMARA, J. S. et al. Comparative study of the whisky aroma profile based on headspace solid phase microextraction using different fibre coatings. **Journal of Chromatography A**, v.1150, n.1, p.198-207, 2007.

CAMPO, E. et al. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odour profiling: An application to Burgundy Pinot noir wines. **Food Quality and Preference**, v. 21, n. 1, p. 44-55, 2010.

CAVALIERI, D. et al. Evidence for *S. cerevisiae* fermentation in ancient wine. **Journal of Molecular Evolution**, v.57, p.S226–S232, 2003.

CAVICCHI, A.; SANTINI, C. Brunellopoli: A wine scandal under the Tuscan sun. **Tourism Review International**, v.15, n.3, p.253-267, 2011.

CHARTERS, S.; PETTIGREW S. The dimensions of wine quality. **Food Quality and Preference**, v.18, n.7, p.997-1007, 2007.

CHIRA, K.; JOURDES, M.; TEISSEDE, P.L. Cabernet sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. **European Food Research and Technology**, v. 234, n. 2, p. 253-261, 2012.

CHIRA, K. et al. Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. **Food Chemistry**, v.126, n.4, p. 1971-1977, 2011.

CLARKE, R.J.; BAKKER, J. **Wine flavour chemistry**. 1. ed. Oxford: Wiley Online Library. 2004.

COPELLO, M. Filoxera, a praga do vinho. **Revista Baco**, v.2, n.9, p.30-32, 2014.

COZZOLINO, D.; SMYTH, E.; LATTEY, A. Combining mass spectrometry based electronic nose, visible-near infrared spectroscopy and chemometrics to assess the sensory properties of Australian Riesling wines. **Analytica Chimica Acta**, v.563, p.319–324, 2006.

CYNKAR, U. et al. Feasibility study on the use of a head space mass spectrometry electronic nose (MS e-nose) to monitor red wine spoilage induced by Brettanomyces yeast. **Sensor Actuators**, v.124, p.167–171, 2007.

DAMBERGS, R.G. et al. The determination of red-grape quality parameters using the LOCAL algorithm. **Journal of Near Infrared Spectroscopy**, v.14, p.71–79, 2006.

DANEZIS, G. P. et al. Food authentication: State of the art and prospects. **Current Opinion in Food Science**, v.10, p.22-31, 2016.

DANGLES, O.; WIGAND, M. C.; BROUILLARD, R. Anthocyanin anti-copigment effect. **Phytochemistry**, v.31, p.3811-3812, 1992.

DE SOUZA, G. G. et al. A uva roxa, *Vitis vinifera* L.(Vitaceae)–seus sucos e vinhos na prevenção de doenças cardiovasculares. **Natureza Online**, v.29102, p.770, 2006.

DELLA CORTE, A.; MATTIVI, F.; VRHOVSEK, U. Development of a Targeted LC-MS/MS Method for the Determination of Different Classes of Grape Lipids. Available online at: <http://openpub.fmach.it/handle/10449/22721>, 2013.

DI NATALE, C. et al. Application of metalloporphyrins-based gas and liquid sensor arrays to the analysis of red wine. **Analytica Chimica Acta**, V.513, p.49–56, 2004.

EDWARDS, R. et al. Quercetin reduces blood pressure in hypertensive subjects. **The Journal of Nutrition**, v.137, p.2405-2411, 2007.

EMBRAPA. O Brasil no Contexto do Mercado Vitivinícola Mundial: Panorama 2012. Empresa Brasileira de Pesquisa Agropecuária. Bento Gonçalves, RS, 2012 Disponível em < <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/961582/atuacao-do-brasil-no-mercado-vitivinicola-mundial-panorama-2012>> Acesso em 09 de Novembro de 2019.

ESLER, M.B.; GISHEN, M.; FRANCIS, I.L. Effects of variety and region on new infrared reflectance spectroscopic analysis of quality parameters in red wine grapes. In: Cho RK, Davies AMC, editors. *Proceedings of the 10th International Conference*. Chichester, UK: NIR Publications, 2002.

ETAIO, I. et al. Sensory quality control for food certification: A case study on wine. Method development. **Food Control**, v.21, n.4, p.533-541, 2010.

European Commission, European Union (EU) Agricultural Product. Quality Policy, Specific EU Quality Schemes-Guaranteeing Quality. (2003) [ec.europa.eu/agriculture/quality](http://ec.europa.eu/agriculture/quality)

FARIAS, R. Curso para profissionais ciclo I. Rio de Janeiro: Associação Brasileira de Sommeliers, 2014.

FANZONE, M. et al. Phenolic characterisation of red wines from different grape varieties cultivated in Mendoza province (Argentina). **Journal of the Science of Food and Agriculture**, v.92, n.3, p.704-718, 2012.

FISCHER, U.; ROTH, D.; CHRISTMANN, M. The impact of geographic origin, vintage and wine estate on sensory properties of *Vitis vinifera* cv. Riesling wines. **Food Quality and Preference**, v.10, n.4, p.281-288, 1999.

FLAMINI, R. Recent applications of mass spectrometry in the study of grape and wine polyphenols. **ISRN Spectroscopy**, v.1, p.1-45, 2013

FORCISI, S. et al. Liquid chromatography-mass spectrometry in metabolomics research: mass analyzer sinultra high pressure liquid chromatography coupling. **Journal of Chromatography A**, v.1292, p.51-65, 2013

GARCIA, C. V. et al. Characterization of the bound volatile extract from baby kiwi (*Actinidia arguta*). **Journal of Agricultural and Food Chemistry**, v.59, n.15, p. 8358-8365, 2011.

GIKA, H.G. et al. Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics. **Journal of Pharmaceutical and Biomedical Analysis**, v.87, p.12-25, 2014.

GLÄNZEL, W.; VEUGELERS, R. Science for Wine: A Bibliometric Assessment of Wine and Grape Research for Wine-Producing and Consuming Countries. **American Journal of Enology and Viticulture**, v.57, p. 23-32, 2006.

GARNIER, N. et al. Characterization of thermally assisted hydrolysis and methylation products of polyphenols from modern and archaeological vine derivatives using gas chromatography-mass spectrometry. **Analytica Chimica Acta**, v.493, p.137–157, 2003.

GELADI, P. et al. Chemometrics in spectroscopy. Part I. Classical chemometrics. **Spectrochimica Acta Part B**, v.58, p.767–782, 2003.

GODELMANN, R. et al. Targeted and nontargeted wine analysis by <sup>1</sup>H NMR spectroscopy combined with multivariate statistical analysis. Differentiation of important parameters: grape variety, geographical origin, year of vintage. **Journal of Agricultural and Food Chemistry**, v. 61, p.5610–5619, 2003.

GÓES, F. J. **Desenvolvimento e otimização do processo fermentativo para a produção do vinho branco a partir da uva Itália**. 2005. 157 f. Dissertação (Mestrado em Engenharia Química)—Universidade Federal de São Carlos, São Paulo, 2005. Disponível em: <https://repositorio.ufscar.br/handle/ufscar/4175?show=full>. Acesso: 20 de julho de 2017.

GOLDNER, M. C. et al. Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. **Journal of Sensory Studies**, v.24, n.2, p.243-257, 2009.

GÓMEZ GARCÍA-CARPINTERO, E. et al. Impact of alternative technique to ageing using oak chips in alcoholic or in malolactic fermentation on volatile and sensory composition of red wines. **Food Chemistry**, v.134, n.2, p.851-863, 2012.

GOUGEON, R.D. et al. The chemodiversity of wines can reveal a metabo-geography expression of cooperage oakwood. **Proceedings of the National Academy of Sciences of the United States of America**, v.106, p.9174–9179, 2009.

GRATA, E. et al. UPLC–TOF–MS for plant metabolomics: an sequential approach for wound marker analysis in *Arabidopsis thaliana*. **Journal of Chromatography B**, v.871, p.261–270, 2008.

GREEN, J. A. et al. Sensory and chemical characterisation of Sauvignon blanc wine: Influence of source of origin. **Food Research International**, v.44, n. 9, p. 2788-2797, 2011.

GUASCH-JANÉ, M. R. et al. Liquid chromatography with mass spectrometry in tandem mode applied for the identification of wine markers in residues from Ancient Egyptian vessels. **Analytical Chemistry**, v.76, p.1672–1677, 2004.

GUASCH-JANÉ, M. R. et al. The origin of the ancient Egyptian drink *Shedeh* revealed using LC/MS/MS. **Journal of Archaeological Science**, v.33, p.98–101, 2006.

GÜRBÜZ, O.; ROUSEFF, J. M.; ROUSEFF, R. L. Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography-olfactometry and gas chromatography-mass spectrometry. **Journal of Agricultural and Food Chemistry**, v.54, n.11, p.3990-3996, 2006.

HEEREN, R.M.A. et al. A mini-review of mass spectrometry using high-performance FTICR-MS methods. **Analytical and Bioanalytical Chemistry**, v.378, p.1048–1058, 2004

HOLMBERG, L. Wine fraud. **International Journal of Wine Research**, v.2, p.105-113, 2010.

IBRAVIN. Avaliação setorial 2013. Disponível em: < <https://www.ibravin.org.br/Dados-Estatisticos> >. Acesso em 26 de janeiro de 2015.

JAITZ, L. et al. LC–MS/MS analysis of phenols for classification of red wine according to geographic origin, grape variety and vintage. **Food Chemistry**, v.122, n.1, p. 366-372, 2010.

JACKSON, R. S. **Wine science. Principles and applications**, 3. ed. Elsevier, Amsterdam. Academic Press, 2008.

JAITZ, L. et al. LC–MS/MS analysis of phenols for classification of red wine according to geographic origin, grape variety and vintage. **Food Chemistry**, v. 122, p.366–372, 2010

JOHNSON, H.; ROBINSON, J. **The World Atlas of Wine**, 6. Ed. New York. Octopus Book, 2008.

KAPADIA, G. J. et al. Inhibition of 12-O-tetradecanoylphorbol-13-acetate induced Epstein virus early antigen activation by natural colourants. **Cancer Letters**, v.115, p.173-178, 1997.

KING, E. S. et al. Regional sensory and chemical characteristics of Malbec wines from Mendoza and California. **Food Chemistry**, v.143, p.256-267, 2014.

LAGUERRE, M.; LECOMTE, J.; VILLENEUVE, P. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. **Progress in Lipid Research**, v.46, n.5, p.244-282, 2007.

LI, H.; GUO, A.; WANG, H. Mechanisms of oxidative browning of wine. **Food Chemistry**, v.108, n.1, p.1-13, 2008.

LILLY, M.; LAMBRECHTS, M. G.; PRETORIUS, I. S. Effect of increased yeast alcohol acetyltransferase activity on flavour profiles of wine and distillates. **Applied and Environmental Microbiology**, v.66, n.2, p.744-753, 2000.

LLAUDY, M.C. et al. New method for evaluating astringency in red wine. **Journal of Agricultural and Food Chemistry**, v.52, n.4, p.742-746, 2004.

LLOBODANIN, L. G.; BARROSO, L. P.; CASTRO, I. A. Prediction of the functionality of young South American red wines based on chemical parameters. **Australian Journal of Grape and Wine Research**, v.20, n.1, p. 15-24, 2014.

LLOBODANIN, L. G.; BARROSO, L. P.; CASTRO, I. A. Sensory Characterization of Young South American Red Wines Classified by Varietal and Origin. **Journal of Food Science**, v.79, n.8, p.1595-1603, 2014.

LUND, S. T.; BOHLMANN, J. The molecular basis for wine grape quality-A volatile subject. **Science**, v.311, n.5762, p.804-805, 2006.

MAITRE, I. et al. Sensory typicality of wines: How scientists have recently dealt with this subject. **Food Quality and Preference**, v.21, n.7, p.726-731, 2010.

MAKRIS, D. P.; KALLITHRAKA, S.; MAMALOS, A. Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. **Talanta**, v. 70, n.5, p.1143-1152, 2006.

MARTI, M. P. et al. Solid-phase microextraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. **Journal of Agricultural and Food Chemistry**, v.51, n.27, p.7861-7865, 2003.

MATEUS, N. et al. A new class of blue anthocyanins-derived pigments isolated from red wines. **Journal of Agricultural and Food Chemistry**, v.51, p.1919-1923, 2003.

MAZZA, G.; MINIATI, E. **Anthocyanins in fruits, vegetables and grains**. 1. Ed. Florida. Boca Raton, CRC Press. p.362, 1993.

MCCASKILL, D.; CROTEAU, R. Some caveats for bioengineering terpenoid metabolism in plants. **Trends in Biotechnology**, v.16, n.8, p.349-355, 1998.

MESTRES, M.; BUSTO, O.; GUASCH, J. Analysis of organic sulfur compounds in wine aroma. **Journal of Chromatography A**, v.881, n.1, p.569-581, 2000.

MCGOVERN, P.E.; D.L. GLUSKER, L.J.; EXNE, R. Neolithic resinated wine. **Nature**, v.381, p.480-81, 1996.

MCKELLAR, R.C. et al. The electronic nose as a tool for the classification of fruit and grape wines from different Ontario wineries. **Journal of the Science of Food and Agriculture**, v.85, p.2391-2396, 2005.

MORENO-ARRIBAS, M. V.; POLO, M. C.; CARMEN, M. **Wine chemistry and biochemistry**. 1. Ed. Madrid. Springer, 2009.

MOROZOVA, K.; SCHMIDT, O.; SCHWACK, W. Effect of headspace volume, ascorbic acid and sulphur dioxide on oxidative status and sensory profile of Riesling wine. **European Food Research and Technology**, v.240, n.1, p.205-221, 2015.

MÜLLER, C. et al. Molecular cartography in acute *Chlamydia pneumoniae* infections—a non-targeted metabolomics approach. **Analytical and Bioanalytical Chemistry**, v.405, p.5119–5131, 2013.

NAES, T. et al. User-friendly guide to multivariate calibration and classification. **Chemometrics**, v.17, p.571-572, 2002.

NÚÑEZ, D. R.; WALKER, M. J. A review of paleobotanical findings of early *Vitis* in the Mediterranean and of the origins of cultivated grape-vines, with special reference to prehistoric exploitation in the western Mediterranean. **Review of Paleobotany and Palynology**, v.61, p.205–237, 1989.

OIV (2018) The world viticultural situation in 2002. Disponível em: <http://www.oiv.int/uk/statistiques/index.htm>. Acesso: 25 de setembro de 2018.

OTTO, M. Statistics and computer application in analytical chemistry. **Chemometrics**, v.51, n.6, p.1237-1238, 1999.

PARR, W. V. et al. Evaluation of French and New Zealand Sauvignon wines by experienced French wine assessors. **Food Quality and Preference**, v.21, n.1, p.56-64, 2010.

PERRIN, L. et al. Comparison of three sensory methods for use with the Napping® procedure: Case of ten wines from Loire valley. **Food Quality and Preference**, v.19, n.1, p.1-11, 2008.

PERINI, M.; CAMIN, F. d18O of ethanol in wine and spirits for authentication purposes. **Journal of Food Science**, v.78, n.6, p.839-844, 2013.

PHAFF, H. J. Ecology of yeasts with actual and potential value in biotechnology. **Microbial Ecology**, v.12, p.31–42, 1986.

PITTE, J. R. **O Desejo do Vinho Conquistando o Mundo**. 1. Ed. Trad. Carmen Ferrer. São Paulo. Editora Senac São Paulo, 2012.

PROTAS, J, F, S; CAMARGO, U, A; MELO, L, M, R. A vitivinícola brasileira: realidades e perspectivas. Empresa Brasileira de Pesquisa Agropecuária, Bento Gonçalves, 2002. Disponível em:

<<https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1052798/a-vitivinicultura-brasileira-realidade-e-perspectivas>> Acesso em 10 de Novembro de 2019.

PÜSSA, T. et al. Survey of grape vine *vitis viniferas* tempolyphenols by liquid chromatography-diode array detection- tandem mass spectrometry. **Journal of Agricultural and Food Chemistry**, v.54, p.7488–7494, 2006.

RAMISHVILI, R. New material on the history of viticulture in Georgia (in Georgian, with Russian summary) *Matsne. Hist. Archaeol. Ethnol. Art Hist. Ser.*, v.2, p.125–140, 1983.

RE, ROBERTA, et al. Antioxidant activity applying an improved ABTS radical cation decolourization assay. **Free Radical Biology and Medicine**, v.26, n.9, p.1231-1237, 1999.

RENAUD, S. D.; DE LORGERIL, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. **The Lancet**, v.339, n.8808, p.1523-1526, 1992.

RIBEIRO, M. E. M.; MANFROI, V. Vinho e Saúde: uma visão química. **Revista Brasileira de Viticultura e Enologia**, v.2, n.2, p.91-103, 2010.

RIBÉREAU-GAYON P.; GLORIES Y.; MAUJEAN A. **Handbook of Enology: The Chemistry of Wine and Stabilization and Treatments**. 2. Ed. Bordeaux. John Wiley & Sons, 2000.

RIBÉREAU-GAYON, P.; DUBOURDIEU, D.; LONVAUD, A. **Handbook of Enology, The microbiology of wine and vinifications**. 2. Ed. Bordeaux. John Wiley & Sons, 2006.

RIVERO-PÉREZ, M.D.; MUÑIZ, P.; GONZÁLEZ-SANJOSÉ, M.L. Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger activity and biomarkers of oxidative stress methodologies. **Journal of Agricultural and Food Chemistry**, v.55, p. 5476-5793, 2007.

ROGGERO, J.P.; COEN, S.; RAGONNET, B. High Performance Liquid Chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanins metabolism. **American Journal of Enology and Viticulture**, v.37, p.77-83, 1986.

ROULLIER-GALL, C. et al. GRAPE and wine chemodiversity - lations in Burgundy: vintage vs terroir effects. **Food Chemistry**, v.152, p.100–107, 2014.

ROULLIER-GALL, C. et al. How subtle is the “Terroir” Effect? Chemistry-related signatures of two “Climats de Bourgogne.” **PLoS ONE**, v.9, p.1-11, 2013.

ROMERO, C.; BAKKER, J. Effect of acetaldehyde and several acids on the formation of vitisin A in model wine anthocyanin and colour evolution. **International Journal of Food Science & Technology**, v.35, n.1, p. 129-140, 2000.



RUBERT, J. et al. Metabolic fingerprinting based on high resolution tandem mass spectrometry: are liable tool for wine authentication? **Analytical and Bioanalytical Chemistry**, v.406, p.6791–6803, 2014.

SAONA-RODRIGUEZ, L. E.; GIUSTI, M.; WROLSTAD, R. E. Anthocyanin pigment composition of red-fleshed potatoes. **Journal of Food Science**, v.63, p.458- 463, 1998.

SASSO, C.; BASSIN, J. P.; RONCHI, S. Vinhos. Documento Eletrônico. Disponível em: [www.enq.ufsc.br/labs/probio/disc\\_eng\\_bioq/trabalhos\\_grad2004/vinho/pagina\\_final.htm](http://www.enq.ufsc.br/labs/probio/disc_eng_bioq/trabalhos_grad2004/vinho/pagina_final.htm). Acesso: 28 Setembro de 2018.

SILVA FERREIRA, A. C.; BARBE, J. C.; BERTRAND, A. 3-Hydroxy-4, 5-dimethyl-2 (5 H)-furanone: a key odourant of the typical aroma of oxidative aged port wine. **Journal of Agricultural and Food Chemistry**, v.51, n.15, p. 4356-4363, 2003.

SINGLETARY, K. W. et al. Inhibition of mammary Tumorigenesis by concord grape juice constituents. **Journal of Agriculture and Food Chemistry**, v. 51, p. 7280-7286, 2003.

SOMAATMADJA, D.; POWERS, J. J. Anthocyanins pigments of Cabernet Sauvignon grapes. **Journal of Food Science**, v.28, p.617-622, 1963.

SNOPEK, L. et al. Contribution of red wine consumption to human health protection. **Molecules**, v.23, n. 7, p.1-16, 2018.

STRATIL, P.; KUBAN, V.; FOJTOVA, J. Comparison of the phenolic content and total antioxidant activity in wines as determined by spectrophotometric methods. **Czech Journal of Food Sciences**, v.26, n.4, p.242-53, 2008.

STYGER, G.; PRIOR, B.; BAUER, F. F. Wine flavour and aroma. **Journal of Industrial Microbiology & Biotechnology**, v. 38, n. 9, p. 1145-1159, 2011.

SWIEGERS, J. H.; PRETORIUS, I. S. Yeast modulation of wine flavour. **Advances in Applied Microbiology**, v. 57, p. 131-176, 2005.

TANNAT: la uva emblema de la vitivinicultura uruguaya. Disponível em: <http://www.winesofuruguay.com/#/Tannat>. Acesso: 19 de Agosto de 2018.

TAKEOKA, G.; EBELER, S. Progress in authentication of food and wine. Disponível em: <https://pubs.acs.org/doi/pdf/10.1021/bk-2011-1081.ch001?rand=5ao4tw0z>. Acesso: 15 de janeiro de 2017.

THEODORIDIS, G.A. et al. Liquid chromatography–mass spectrometry based global metabolite profiling: a review. **Analytica Chimica Acta**, v.711, p.7–16, 2012.

THEODORIDIS, G. et al. LC-MS based global metabolite profiling of grapes: solvent extraction protocol optimisation. **Metabolomics**, v.8, p.175–185, 2012.

TOFFALI, K. et al. Novel aspects of grape berry ripening and post-harvest withering revealed by untargeted LC-ESI-MS metabolomics analysis. **Metabolomics**, v.7, p.424–436, 2011.

TOLIN, S. et al. Analysis of commercial wines by LC-MS/MS reveals the presence of residual milk and egg white allergens. **Food Control**, v.28, p.321–326, 2012.

VACEK, J. et al. Analytical methods and strategies in the study of plant polyphenolics in clinical samples. **Analytical methods**, v.2, n.6, p.604-613, 2010.

VACLAVIK, L. et al. The use of high performance liquid chromatography–quadrupole time-of-flight mass spectrometry coupled to advanced data mining and chemometric tools for discrimination and classification of red wines according to their variety. **Analytica Chimica Acta**, v.685, p.45–51, 2010.

URBANO-CUADRADO, M. et al. Near infrared reflectance, spectroscopy and multivariate analysis in enology – Determination or screening of fifteen parameters in different types of wines. **Analytica Chimica Acta**, v.527, p.81–88, 2004.

UVIBRA (2016). Importation of Wines. Available at [http://www.uvibra.com.br/dados\\_estatisticos.htm](http://www.uvibra.com.br/dados_estatisticos.htm). Acesso 10 de janeiro de 2017.

VIDOTTI, E. **O serviço, acessórios e equipamentos do vinho**. São Paulo. Moderna. (Coleção folha o mundo do vinho), 2010.

Vitude. Uruguai: um pequeno país de grandes vinhos. [S.l.]. Disponível em: <https://www.clubedovinhos.com.br/uruguai-um-pequeno-pais-de-grandesvinhos/>.

VON BAER, D. et al. Relevance of chromatographic efficiency in varietal authenticity verification of red wines based on their anthocyanin profiles: Interference of pyranoanthocyanins formed during wine ageing. **Analytica Chimica Acta**, v.621, n.1, p.52-56, 2008.

YANG, J.; MARTINSON, T. E.; LIU, R. H. Phytochemical profiles and antioxidant activities of wine grapes. **Food Chemistry**, v.116, n.1, p.332-339, 2009.

WANG, Y. et al. An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. **Journal of Agricultural and Food Chemistry**, v.50, p.431–435, 2002

WOLD, S.; SJÖSTROM, M.; ERIKSSON, L. PLS-regression: a basic tool of chemometrics. **Chemometrics and Intelligent Laboratory Systems**, v.58, p.109–130, 2001.

WOLFFENBÜTTEL, P. Os vinhos produzidos em RS. Disponível em: <<http://www.sobrevinho.net/paises/brasil/regioes/vinhos-produzidos-rs>> Acesso: 13 de março de 2019.

ZOECKLEIN, B.W. et al. **Wine analysis and production**. 1. Ed. New York. The Chapman e Hall Enology Library. International Thompson Publishing, 1995.

ZOHARY, D.; HOPF, M. Domestication of Plants in the Old World, 3. Ed. Oxford. University Press (Clarendon), 2000.

ZÖLLNER, P. et al. Application of achromolith speed RODRP-18e HPLC column: determination of ochratoxin A in different wines by high-performance liquid chromatography-tandem mass spectrometry. **Chromatographia**, v.52, p.818–820, 2000.

## 8. ATTACHMENTS.

**Table 3.** Validation data of phenolic method

Compound	Conc Range mg/L	Regression Eq	R2	LOD	LOQ	Rec low	Rec mid
Gallic acid	0.25-20	$y=11271x-9213.1$	0.9934	0.01	0.05	98%	100%
Catechin	1.0-75	$y=5143.5x-5798.3$	0.9986	0.05	0.15	101%	101%
Caffeic acid	0.5-10	$y=4921.3x-3310.9$	0.9939	0.025	0.08	99%	102%
Epicatechin	0.5-40	$y=1118.5x-171.37$	0.9992	0.02	0.06	98%	98%
Rutin	0.5-20	$y=2479x-770.26$	0.9997	0.05	0.15	97%	102%
Ferrulic acid	0.5-10	$y=5232.8x-3463.6$	0.9968	0.01	0.06	98%	104%
Miricetin	1.0-50	$y=1374.7x-869$	0.9912	0.1	0.3	102%	100%
Resveratrol	0.05-5	$y=11599x-2107.7$	0.9949	0.003	0.01	101%	99%
Quercetin	0.5-25	$y=2661.2x-151.7$	0.9991	0.02	0.06	103%	98%
Kaempferol	0.5-10	$y=2056.3x-1140.1$	0.9961	0.03	0.08	99%	103%
Delphinidin	0.5-10	$y=386.53x-54.829$	0.9986	0.01	0.03	104%	101%
Cyanidin	0.5-10	$y=532.72x-58.522$	0.9966	0.04	0.1	102%	97%
Petunidin	0.5-15	$y=6152.6x-3724.6$	0.9972	0.02	0.06	99%	99%
Peonidin	0.5-20	$y=5200.2x-2630.5$	0.9991	0.02	0.06	97%	98%
Malvidin	5-200	$y=3998.5x-1999.2$	0.9991	0.05	0.15	98%	102%

LOD: limit of detection, LOQ: limit of quantitation, recovery low, recovery middle, recovery high

