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# Treball Final de Grau

**Characterization and classification of musts, wines and sparkling wines by their elemental composition determined by ICP-OES and ICP-MS**

**Caracterització i classificació de mosts, vins i caves mitjançant la seva composició elemental determinada per ICP-OES i ICP-MS**

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*However difficult life may seem, there is always something you can do and succeed at. It matters that you don't just give up.*

Stephen Hawking

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**REPORT**



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# 1. SUMMARY

The control of the composition of food products with Protected Designation of Origin like wines and sparkling wines is essential to detect fraudulent practices and adulterations, and one of these controls corresponds to the determination of their elemental composition. To do so, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) have been found as the best techniques, with the possibility of carrying out multi-element measurements in a short period of time and with easy and straightforward sample pre-treatments.

This way, samples of must, wine and sparkling wine of two different varieties (Pinot Noir and Xarel·lo) have been characterized by ICP techniques, to determine their metal composition and classify them according to their quality, geographical origin and oenological practices. For this purpose, Principal Components Analysis, box plot diagrams and bar charts have been used. Different markers and trends among samples have been found and related to the changes resulting from the different steps of the sparkling wines production process.

Finally, the soils from where these samples come from have been extracted and characterized by ICP-OES and ICP-MS. Data processing has allowed to classify them according to their geographical origin, as well as to find some trends in their metal content connected to the musts.

**Keywords:** wines, sparkling wines, elemental composition, ICP-OES, ICP-MS, must, quality, geographical origin, Principal Components Analysis, soils.



## 2. RESUM

El control de la composició dels productes alimentaris amb Denominació d'Origen Protegida com els vins i caves és essencial per detectar pràctiques fraudulentess i adulteracions, i un d'aquests controls correspon a la determinació de la seva composició elemental. Per fer-ho, l'Espectrometria d'Emissió Òptica amb Plasma Acoblat Inductivament (ICP-OES) i l'Espectrometria de Masses amb Plasma Acoblat Inductivament (ICP-MS) han estat considerades com les millors tècniques, oferint la possibilitat de realitzar mesures multi-elementals en un període curt de temps amb pretractaments de mostra fàcils i directes.

D'aquesta manera, mostres de most, vi i cava de dues varietats diferents (Pinot Noir i Xarel·lo) han estat caracteritzades mitjançant tècniques ICP, per determinar la seva composició en metalls i classificar-les segons la seva qualitat, regió d'origen i pràctiques enològiques. Per aquest propòsit, s'han utilitzat Anàlisi per Components Principals, diagrames de caixa i gràfics de barres. Diferents marcadors i tendències entre les mostres han estat trobats i relacionats amb els canvis resultants dels diferents passos del procés de producció del cava.

Finalment, els sòls d'on provenen les mostres han estat extrets i caracteritzats per ICP-OES i ICP-MS. El tractament de dades ha permès classificar-los segons el seu origen geogràfic, i a més trobar algunes tendències en el seu contingut de metalls relacionades amb els mostos.

**Paraules clau:** vins, caves, composició elemental, ICP-OES, ICP-MS, most, qualitat, regió d'origen, Anàlisi per Components Principals, sòls.



## **3. INTRODUCTION**

Nowadays, taking into account how easy it can be to modify the composition of food products, it is of great importance to control the authenticity of foods and beverages to guarantee that consumers are buying what is written in the product label. For this purpose, food controls pursue the objective of ensuring the origin and quality of the consumption products, and the achievement of these objectives falls on the use of different analytical techniques, such as High-Performance Liquid Chromatography (HPLC), Inducted Coupled Plasma Optical Emission Spectrometry (ICP-OES), Atomic Absorption Spectroscopy (AAS), Near Infrared (NIR), and many others depending on the object of study.

Moreover, in the case of products with Protected Designation of Origin (PDO), the control of possible frauds becomes even more important. The authenticity and geographical origin of products such as wines and sparkling wines are both factors influencing their overall perception in terms of quality and price, therefore being of great importance for consumers and commercial producers.

### **3.1. WINES AND SPARKLING WINES**

#### **3.1.1. Sparkling wines production process**

Considering that this project is focused on the study of different samples of soil, must, wine and sparkling wine, it is very important to know how they are produced, to understand where their composition comes from and how it is maintained or changed from the soil to the grape to the final product, in this case the sparkling wine. Knowing all the phases of the production of sparkling wines will be essential to extract the right conclusions to classify the samples.

Although there may be variations depending on the type of grape and wine to be produced, the winemaking process is generally made up of 6 main phases once the grapes have been packed and they arrive to the wine cellar: pressing, settling, alcoholic fermentation, malolactic fermentation, racking and aging <sup>(1)</sup>. The main difference between classes of wine is that white

wine is pressed without previous maceration, unlike rosé and red wine, which have more intense colours due to this maceration for a certain period of time.

Regarding sparkling wines, also known as cava wines, nowadays they are generally elaborated with the traditional *Champenoise* method <sup>(2)(3)</sup>, from a white or rosé wine which is called base wine. Therefore, once the grapes arrive to the cellar the steps are the following ones:

- 1) **Pressing:** The grapes are carefully pressed in batches separated by variety.
- 2) **First fermentation:** Must is fermented usually in stainless steel tanks at a controlled temperature between 15 and 18 °C. The yeasts convert the sugars from the must into alcohol and CO<sub>2</sub>, and from this fermentation the base wine is obtained.
- 3) **Blending:** Different base wines are blended depending on the sparkling wine that want to be produced. Then, the resulting wine is clarified by adding bentonite, a type of clay that removes the unclear and turbid appearance from the wine, caused by the suspended particles from fermentation. The wine is then stabilized in order to precipitate the tartaric acid from grapes in the form of salts, which are filtrated to remove them so they are not generated inside the bottle after bottling. Factors that enhance this precipitation are the low temperature, the presence of K<sup>+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup>, and a high alcohol content.
- 4) **Tirage:** This precipitation allows the obtention of the stabilized wine and it is followed by the tirage, which consists of filling the bottles with the wine, bentonite and tirage liqueur (a mixture of wine, sugars and yeasts to trigger the second fermentation inside the bottle). Subsequently, the wine is subjected to a second fermentation inside the bottle, which gives it its characteristic CO<sub>2</sub> content.
- 5) **Second fermentation and aging:** The bottles are covered with a shutter and a cap and are placed in a horizontal position known as rhyne position. The second fermentation begins and yeasts transform the alcohols and release CO<sub>2</sub>, which is trapped in the bottle and forms bubbles. This fermentation lasts about 30 days and is carried out in a cave with constant humidity and at low temperature (13-14 °C), to ensure that the bubbles are as thin and integrated in the wine as possible. Once the fermentation is finished, the yeasts die and are deposited in the bottom of the bottle. At this point aging begins, which consists of the time of contact of dead yeasts with the sparkling wine and is carried out for a minimum of 9 months, without a maximum limit.

Depending on aging time, sparkling wines can be classified as young (aging from 9 to 15 months), reserve (aging from 15 to 30 months), great reserve (aging of more than 30

months) and *paratge qualificat* (more than 36 months of aging, from grapes from low yield plantations with unique characteristics) <sup>(4)</sup>. In this project, sparkling wines of 3 and 7 months in rhyme were analysed, therefore they cannot even be qualified as young.

- 6) **Riddling:** After the corresponding time of aging of each sparkling wine, the riddling process is carried out for 2 or 3 weeks to move the sedimented remains of yeast to the neck of the bottle. As shown in *Figure 1*, the bottle is gradually turned and tilted (manually or automatically), changing the angle of the rhyme position to end up placing the bottle vertically with the neck pointing downwards.



Figure 1. Rhyme positions, from horizontal to vertical.

- 7) **Disgorgement:** The bottle is uncovered and a small amount of foam is let out and takes away the sediments. Usually, the neck is frozen at a temperature between -22 and -24 °C, so that when the cap is taken off, the solids come out easily.
- 8) **Expedition liqueur:** The original volume is reestablished by adding the expedition liqueur, which consists of a wine and sugar mixture that can be modified depending on the sparkling wine that wants to be obtained. This step is the best kept secret by winemakers in each wine cellar, because the expedition liquor allows to obtain certain and differentiated qualities regarding to the rest of the cellars.
- The amount of expedition liqueur added classifies sparkling wines according to sugar content in brut nature, extra brut, brut, extra dry, dry, semidry, and sweet (from the lowest to the largest amount of sugar) <sup>(5)</sup>.
- 9) **Finishing touch:** Finally, the bottle is “dressed” and labelled, ready to be distributed and sold to the consumers.

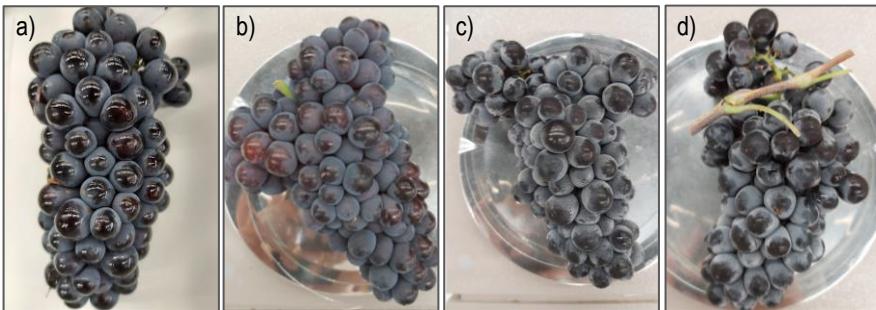
### 3.1.2. Grape varieties

The grape is the fruit of the vine and depending on the type, grapes are generally classified in white or black, essentially differing in their water content and organic and mineral composition. Besides this general classification, there are many varieties of grape such as Chardonnay, Pinot Noir, Sauvignon Blanc, Macabeu, Xarel·lo, Parellada, Concord, Valiant, and much more depending on the region of origin.

As it would be very long to explain the main characteristics of each one, only the two grape varieties studied in this project will be introduced:

- Pinot Noir <sup>(6)</sup>: Variety of black grape originating from France, small and round, with thick skin and an intense violet colour. It gives aroma and colour to the product and promote the formation of bubbles in the sparkling wine cup. Its variety Blanc de Noirs consists of a type of wine produced by quickly removing the skin of the black grape just after the pressing, so as not to transmit the colour to the must and having less aromas.

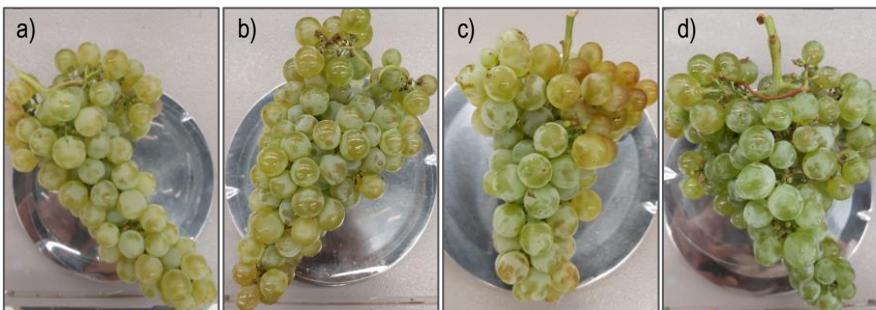
In *Figure 2* can be seen the Pinot Noir grapes from the four different qualities (from A to D) used in this project, showing the mentioned characteristics about their appearance.



**Figure 2.** Pinot Noir grapes of quality A (a), quality B (b), quality C (c) and quality D (d); from the 2019 grape harvest.

- Xarel·lo <sup>(6)</sup>: Variety of white grape originating from different regions of Catalunya, big and round, with thin skin, rich in sugars and with high alcohol content. It offers freshness and promotes aging, making it an excellent variety of grape for long-aged wines.

*Figure 3* shows the four types of Xarel·lo grapes from the different qualities (from A to D) used in this project.



**Figure 3.** Xarel·lo grapes of quality A (a), quality B (b), quality C (c) and quality D (d); from the 2019 grape harvest.

The variety of grape is the most influential factor to determine the type of wine or sparkling wine produced, as well as its quality and the variations in the procedure followed. This direct relation between the grapes and the sparkling wines is shown in *Figure 4*.



**Figure 4.** Sparkling wines of 7 months in rhyme, from the varieties Pinot Noir (a) and Xarel·lo (b), both ordered by quality from A to D.

In the case of the sparkling wines of Pinot Noir, two groups can be distinguishable looking at their colour. The qualities A and B correspond to the variety Blanc de Noir, which make them more acid and with less colour intensity; while the qualities C and D correspond to the original variety of Pinot Noir, making them stronger and darker.

On the other hand, the sparkling wines of Xarel·lo show very similar colours among all the qualities, but they differ a lot in the aromas, texture in mouth and acid content.

### 3.1.3. Protected Designation of Origin

Wines and sparkling wines composition and characteristics are affected by the region of the grapes, the climate, the soil and the intervention of the producer. The combination of these factors is known as Protected Designation of Origin (PDO), which is essential to regulate the quality and origin of the products to prevent fraud.

Apart from guaranteeing the authenticity of wine products, the PDOs also regulate the different phases of their production and verify the quality of the vintages and the wine. For a wine having a specific PDO, it must be produced, processed and bottled in the production area, being made of certain varieties of grapes and obtained in certain areas <sup>(7)</sup>.

### 3.2. METAL CONTENT IN WINES AND SPARKLING WINES

Wines and sparkling wines are made up of 85-90% water, ethanol, organic acids, carbohydrates, sugars and other substances like vitamins, minerals or inorganic compounds <sup>(8)</sup>. Therefore, their metal content can be seen as very little, but they are of great importance.

Commonly, elemental composition of wines and sparkling wines is mainly influenced by two primary sources <sup>(9)</sup>:

- Natural or endogenous sources, that represent the uptake of minerals from soil, through roots and plant into the grapes. Due to the composition of the soils from the vineyards, usually calcareous and clayey, grapes are rich in metals like Ca, Mg, Fe, Al or Mn, which are transferred to the musts, wines and sparkling wines.
- Interventions of winemakers in the vineyard and during winemaking processes. The addition of fertilizers to the vines increases its content in Cu, S, K or Zn, while the variations in the metal content during the winemaking process are more complex, due to the addition of different substances such as yeasts, bentonite, additives or the expedition liqueur, which are different for each company and added in variable proportions depending on the product.

The combination of these factors allow the definition of a representative “fingerprint” of the product, which is especially important for quality wines and sparkling wines with PDO. This means that their features could be related to their geographical origin, and the study of their elemental composition, among other procedures, could allow to distinguish and classify them. This is also a good way to afterwards prevent fraud and guarantee the origin and quality of the bottles <sup>(10)</sup>.

Apart from preventing fraud, the analysis of food products is also important to ensure the quantities of different metals are within the limits set by regulation (see *Table 1*), especially those that suppose a health risk.

**Table 1.** Maximum acceptable limits applicable to base wines for the production of sparkling wines.

| Element | Maximum amount                               | Element | Maximum amount |
|---------|--|---------|----------------|
| As      | 0.2 mg/L                                     | Pb      | 0.10 mg/L      |
| B       | 80 mg/L (as H <sub>3</sub> BO <sub>3</sub> ) | Na      | 80 mg/L        |
| Cd      | 0.01 mg/L                                    | Zn      | 5 mg/L         |
| Cu      | 1 mg/L                                       |         |                |

The maximum amounts of these metals in base wines are established by the *International Code of Oenological Practices* <sup>(11)</sup>, which also regulates other factors like the pH, the quantity of ashes or the concentration of relevant organic compounds such as tartaric or gluconic acid.

### 3.3. ANALYTICAL PROCEDURE FOR THE DETERMINATION OF METALS

#### 3.3.1. Instrumental techniques

A wide variety of analytical techniques can be used for the qualitative and quantitative determination of metals in wine and sparkling wine samples, such as Inducted Coupled Plasma Mass Spectrometry (ICP-MS), Isotope Ratio Mass Spectrometry (IRMS), Fourier Transform Infrared (FTIR), Site-specific Natural Isotope Fractionation studied by Nuclear Magnetic Resonance (SNIF-NMR), and so on.

Multi-elementary techniques are an excellent choice that offer accurate results, with good Limits of Detection (LODs) and fast and straightforward measurements. Therefore, to try to find the best option, *Table 2* summarizes papers previously published in the scientific literature between 2011 and 2020, which study the elementary composition of wines and sparkling wines from different regions around the globe.

ICP-OES and ICP-MS are widely used for these types of measurements. Techniques like IRMS or SNIF-NMR are more laborious, expensive and complicated to perform than multi-element analysis by ICP-MS and ICP-OES. That makes ICP techniques the most recommendable ones for the determination of geographical origin of wines and sparkling wines, being multi-elemental techniques suitable for accurate and fast determination of trace and ultra-trace elements in the samples, both able to determine and quantify most of the elements of the periodic table. In addition, they are frequently used with subsequent multivariate analysis to classify the samples.

To decide between them, it has to be considered that ICP-MS is more sensitive but limited to samples with low total dissolved solids content, while ICP-OES is less sensitive but more robust, with possibility to analyse complex matrix samples with high total dissolved solids content <sup>(9)</sup>. Wine is a high salt content sample with a complex matrix, so matrix effects affect more to ICP-MS, while ICP-OES allows to determine less elements due to its higher LODs, which will affect the subsequent chemometric study. However, these two ICP techniques are not excluding and could be complementarily performed to obtain better and more comprehensive results.

**Table 2.** Summary of papers previously published from 2011 to 2020 (chronologically). The last two papers correspond to the analysis of sparkling wines, while the rest correspond to the analysis of regular wines.

ICP-MS: Inducted Coupled Plasma Mass Spectrometry, ICP-OES: Inducted Coupled Plasma Optical Emission Spectrometry, PCA: Principal Components Analysis, LDA: Linear Discriminate Analysis, DA: Discriminant Analysis, CPANN: Counter-Propagation Artificial Neural Networks, NN: Nearest Neighbour, NB: Naïve Bayes, SVM: Support Vector Machine, LR: Logistic Regression.

| Origin of the samples                         | Instrumental methods | Sample pre-treatment                      | Dilutions  | Measured elements   | Chemometric treatment | Ref. |
|---|----------------------|---|--|---|-----------------------|------|
| Japan, Chile, United States, France and Italy | ICP-OES              | Acid digestion                            | 2.5:1 (v/v) with 60% HNO <sub>3</sub> and 30% H <sub>2</sub> O <sub>2</sub><br>Final 1:5 dilution with 1% HNO <sub>3</sub>             | Al, B, Ba, Ca, Cd, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, V, Y and Zn  | PCA and LDA           | (12) |
| Argentina                                     | ICP-MS               | Mineralisation followed by acid digestion | 5:6 (v/v) with HNO <sub>3(cc)</sub><br>Final 1:5 dilution with H <sub>2</sub> O  | Al, B, Ba, Ca, Cd, Ce, Co, Cs, Cu, Eu, Ga, K, La, Li, Lu, Mg, Mn, Mo, Na, Ni, Nd, Pb, Rb, Se, Sm, Sr, Ti, U, V, Yb, and Zn                                  | DA                    | (13) |
| Romania                                       | ICP-MS               | Acid digestion                            | 1:1 (v/v) with HNO <sub>3(cc)</sub><br>Final 1:20 dilution with H <sub>2</sub> O   | Ag, Be, Co, Cr, Cu, Mn, Ni, Pb, Rb, Sr, V and Zn  | PCA                   | (14) |
| Slovenia                                      | ICP-OES              | Only dilution                             | 1:3 (v/v) with 2% HNO <sub>3</sub>   | Al, B, Ca, Cu, Fe, K, Mg, Mn, Na and Zn   | PCA and CPANN         | (9)  |
|   | ICP-MS               | Only dilution                             | 1:10 (v/v) with 2% HNO <sub>3</sub>  | Ag, Au, As, Ba, Be, Bi, Ce, Co, Cr, Dy, Ga, Gd, Ge, Hg, Ir, La, Li, Mo, Nb, Nd, Ni, Os, Pb, Pd, Pt, Re, Rh, Ru, Sb, Sc, Se, Sn, Ta, Ti, Tl, V, W, Y, and Zr | PCA and CPANN         |      |
| Argentina                                     | ICP-MS               | Only dilution                             | 1:10 (v/v) with 1% HNO <sub>3</sub>  | As, Ba, Be, Bi, Cd, Co, Cu, Hg, Li, Mn, Mo, Ni, Pb, Rb, Sr, Tl and V  | PCA and LDA           | (15) |
| Argentina, Brazil, Chile and Uruguay          | ICP-OES              | Acid digestion                            | 1:3 (v/v) with 2% HNO <sub>3</sub> , followed by 1:25 dilution with H <sub>2</sub> O, and final 1:10 dilution with 5% HNO <sub>3</sub> | Al, Ba, Ca, Fe, K, Mg, Mn, Na, P, Rb, Sr, Ti, and Zn  | NN, LDA, NB and SVM   | (16) |
|   | ICP-MS               | Acid digestion                            | 1:3 (v/v) with 2% HNO <sub>3</sub> , followed by 1:25 dilution with H <sub>2</sub> O, and final 1:2 dilution with 5% HNO <sub>3</sub>  | Ag, As, Be, Bi, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Gd, Ho, La, Li, Lu, Mo, Nd, Ni, Pb, Pr, Sb, Se, Sm, Sn, Tb, Tl, Tm, U, V and Yb                             | NN, LDA, NB and SVM   |      |
| Argentina, Brazil, France and Spain           | ICP-OES              | Degasification                            | 1:10 (v/v) with 1 M HNO <sub>3</sub> for major elements, while minor elements are non-diluted  | Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, and Sr  | LDA and NB            | (17) |
| Brazil, Argentina, Spain and France           | ICP-OES              | Degasification using ultrasonic bath      | 1:10 (v/v) with 1 M HNO <sub>3</sub> for major elements, while minor elements are non-diluted  | Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na and Sr   | LDA, PCA and LR       | (18) |

### 3.3.2. Preparation of the samples

In the first part of the project, musts, wines and sparkling wines were analysed by ICP-OES and ICP-MS. These samples can be analysed after applying multiple types of pre-treatment, but they can also be analysed without any kind of preliminary treatment, only diluting them (see *Table 2*), which is simpler, quicker and minimizes the possibilities of sample contamination.

Hence, for the preparation of these samples they must be diluted in ultrapure  $\text{HNO}_3$  with ethanol content non exceeding 4% to minimize matrix effect, especially for ICP-MS. In addition, internal standards have to be added to correct for instrumental drift, and external calibration solutions for each metal have to be prepared to calculate their concentrations.

Depending on the dilution chosen, LODs may be modified and the obtained results for some metals may differ, making it necessary to find the best dilution ratio to achieve a balance between little matrix effect and having good LODs, to get the maximum amount of information from the samples for their subsequent classification.

### 3.3.3. Extraction of metals from soil samples

The other type of samples to analyse in this project were the soils from the different fields where the musts, wines and sparkling wines come from. For the extraction of metals from soil samples, different reagents and proportions of solvent/soil can be used, depending on the type of soil and especially on the metals to be extracted.

Taking into account that in this case only a general extraction was wanted to be achieved and any specific type was wanted to be used,  $\text{HNO}_3$  and  $\text{NH}_4\text{NO}_3$  in a proportion of 50 mL of solvent for each 20 g of soil were selected <sup>(19)</sup>. Extraction with  $\text{NH}_4\text{NO}_3$  consists of a mild attack of the soils by cations exchange with little mobilisation of their metals, while extraction with  $\text{HNO}_3$  consists of a strong attack of the samples, easily mobilising elements bound to acid soluble compounds and attacking labile complexes, therefore having greater extraction power. This way, using both extractants separately allows the extraction of the majority of metals and to compare results between methods.

After the metal extraction from the soils, the pre-treatment and analytical procedures were similar to those given by the must, wine and sparkling wine samples.

### 3.4. DATA TREATMENT

Principal Component Analysis (PCA) is one of the most used chemometrics methods in multivariate analysis, because it is useful to represent multivariate data in a small number of dimensions, reducing the dimensionality of large datasets by increasing their interpretability and minimizing information loss <sup>(20)</sup>. It converts the large number of variables (in this case metal concentrations) into few Principal Components (PCs), to visualize similarities and trends among samples and correlations among variables in order to reveal the most significant descriptors (metals up- or down-expressed in the different types of samples).

Unfortunately, with PCA there is always the risk that the models will not be effective in classifying the samples if too many PCs are needed, or if they do not explain enough variance from the samples. In this case, other chemometric methods which classify the samples into pre-established categories such as Linear Discriminant Analysis (LDA) or which correlate them like Partial Least Squares (PLS) could be considered. Nevertheless, it has been proved in different studies that characterization of wines based on analytical methods combined with chemometric data treatment provides excellent robustness and efficiency, also reducing costs compared with sensory tests performed by experts and wine-tasters <sup>(21)</sup>.

Apart from this, considering that PCA models do not explain the 100% of the variance of the samples, the information provided by the chemometric treatment must be checked with the raw data obtained, analysed using box plot diagrams and other type of graphics to define their final classification and characterization according to the metal content.

## **4. OBJECTIVES**

The main objective pursued by this project is to characterize and classify different musts, wines and sparkling wines according to their elemental composition, trying to find indicators and trends in their metal content which allow to discriminate among them by regions of origin and quality. With the aim of classifying them, the path has been the following one:

- To find the better preliminary treatment, experimental techniques and working conditions to determine the metal content of the samples.
- To characterize the elemental composition of various musts, wines and sparkling wines from Catalunya, determining the concentration of different metals by ICP-OES and ICP-MS.
- To authenticate and classify the studied musts, wines and sparkling wines according to their quality and region of origin, based on a subsequent chemometric study using PCA, box plot diagrams and bar charts.
- To relate the composition of these samples with the one of the soils where their grapes come from, also studying their metal composition by ICP-OES and ICP-MS and classifying them using PCA and box plot diagrams.

## 5. EXPERIMENTAL SECTION

### 5.1. REAGENTS, STANDARDS AND SOLVENTS

All reagents used were of analytical grade. The reagents and solvents needed for the dilutions and extractions were:

- Nitric acid (133255, 65% (w/w), PanReac AppliChem, Castellar del Vallès, Spain)
- Ammonium nitrate (09889, Sigma-Aldrich, Saint Louis, MI, USA)

For sample dilution and preparation of the extraction solvents, Milli-Q water (Elix3, Millipore, Bedford, MA, USA) was used. The solution for the dilutions consisted of 1% (v/v) HNO<sub>3</sub>, while the extractions solutions consisted of 20% (v/v) HNO<sub>3</sub> and 1 M NH<sub>4</sub>NO<sub>3</sub>, separately.

The preparation of the calibration standards for the ICP-MS and ICP-OES measurements was performed by technicians from the CCiT-UB (*Centres Científics i Tecnològics – Universitat de Barcelona*), by mixing of certified ICP grade single 1000 ppm element standards (Inorganic Ventures, traceable at National Institute of Standards and Technology (NIST)) that were subsequently diluted in 1% (v/v) HNO<sub>3</sub> for the analysis.

### 5.2. SAMPLES

#### 5.2.1. Must, wine and sparkling wine samples

A preliminary study was performed to determine the best dilution for the samples and if a previous filtered was necessary (see *Section 6.1.1.*). For this study, 4 samples of sparkling wine were provided by a Catalan company producer of wines and sparkling wines. From these samples, 2 of them were predominantly from the variety Pinot Noir, 1 Chardonnay, and the last one a Macabeu-Xarel·lo-Parellada blend.

For the final study, a total of 40 samples of musts, wines and sparkling wines were provided by the same Catalan company. From all 40 samples, half of them were Pinot Noir and the other half Xarel·lo; having for each variety 4 musts, 8 wines (4 monovarietal and 4 stabilized) and 8 sparkling wines (4 of 3 months in rhyme and 4 of 7 months). Moreover, for each one of the 10 types of samples (5 different groups from the two varieties of wine), there were 4 samples corresponding to the different qualities: A, B, C and D each. To make it clear, *Table 3* summarizes the variety of samples mentioned and the nomenclature used to classify them during the study.

**Table 3.** Classification of the 40 samples provided for the study.

Sample acronyms are based on the following codes. M: must, BW: base wine, SW: stabilized wine, C3: 3 months in rhyme cava wine (sparkling wine), C7: 7 months in rhyme cava wine (sparkling wine), P: Pinot Noir, X: Xarel·lo, A: quality A, B: quality B, C: quality C, D: quality D.

|            | Quality | Must | Base wine | Stabilized wine | 3 months sparkling wine | 7 months sparkling wine |
|------------|---------|------|-----------|-----------------|-------------------------|-------------------------|
| Pinot Noir | A       | MPA  | BWPA      | SWPA            | C3PA                    | C7PA                    |
|            | B       | MPB  | BWPB      | SWPB            | C3PB                    | C7PB                    |
|            | C       | MPC  | BWPC      | SWPC            | C3PC                    | C7PC                    |
|            | D       | MPD  | BWPD      | SWPD            | C3PD                    | C7PD                    |
| Xarel·lo   | A       | MXA  | BWXA      | SWXA            | C3XA                    | C7XA                    |
|            | B       | MXB  | BWXB      | SWXB            | C3XB                    | C7XB                    |
|            | C       | MXC  | BWXC      | SWXC            | C3XC                    | C7XC                    |
|            | D       | MXD  | BWXD      | SWXD            | C3XD                    | C7XD                    |

### 5.2.2. Soil samples

Apart from the mentioned samples, with the aim of correlating the composition of the musts, wines and sparkling wines with their soils of origin, the same company also provided soil samples from where the grapes of these samples come from. Therefore, 11 soil samples were provided, 4 of the Xarel·lo and 7 of Pinot Noir. *Table 4* and *Table 5* summarize the type of soils given and the main characteristics of the vineyards.

**Table 4.** Characteristics from the soils of Pinot Noir samples.

| Vineyard classification according to quality | Pinot Noir soils  |  |  |  |
|--|---|--|--|--|
|  | A   |  | B  |  |
| Reference/Name                               | DOC-PN-15/La Pleta  | POB-PN/Tros nou  | COD-PN-30  | SRA-PN-132   |
| Main characteristics                         | Very balanced old vineyard, where all is done manually is removing the excess of sprouts and grapes | Very balanced dryland vineyard, where the excess of sprouts and grapes are removed so that the remnant are well placed | Young vine in which during the spring the excess of sprouts and grapes are removed | Young vine in which during the spring the excess of sprouts and grapes are removed |

Table 4 (continued)

|  |   |  |   |  |
|--|---|--|---|--|
| Type of soil                             | Frank and granular texture, consisting of lutites between 1-2 m | Clayey, well drained with high content of slates | Frank and granular texture, consisting of lutites between 1-2 m | Calcareous soil with a sandy texture, well drained |
| Agricultural practices                   | Conversion  | Conversion                                       | Conversion  | Integrated production                              |
| Geographical location                    | Raimat  | Riudabella (Vimbodi i Poblet)                    | Raimat  | Raimat   |
| Climate                                  | Continental, semiarid   | Continental with Mediterranean influence         | Continental, semiarid   | Continental, semiarid                              |
| Vineyard altitude                        | 280 m   | 560 m  | 270 m   | 310 m  |
| Conduction system                        | <i>Royat Doble</i>  | <i>Royat Doble</i>                               | <i>Royat Doble</i>  | <i>Royat Doble</i>                                 |
| Plantation year                          | 1991  | 2005   | 2015  | 2010   |
| Yield obtained in the 2019 grape harvest | 5.600 kg/ha   | 6.160 kg/ha                                      | 10.030 kg/ha  | 10.380 kg/ha                                       |

| Vineyard classification according to quality | B  | C  | D  |
|--|--|--|--|
| Reference/Name                               | <b>SAL-PN-F3</b>   | <b>SAL-PN-84</b>   | <b>VAL-PN-SAIRA</b>  |
| Main characteristics                         | Young vine in which during the spring the excess of sprouts and grapes are removed                     | Young vine in which during the spring the excess of sprouts are removed to homogenize the remaining grapes | Old vine but very heterogeneous, in which only the necessary actions to guarantee the health of the grapes are carried out |
| Type of soil                                 | Limestone soil, frank and clayey, with large calcareous rocks on the surface and lutites between 1-2 m | Limestone soil, frank and clayey, with large calcareous rocks on the surface and lutites between 1-2 m     | Frank and granular texture, consisting of lutites between 1-2 m  |
| Agricultural practices                       | Ecological   | Integrated production  | Integrated production  |
| Geographical location                        | Raimat   | Raimat   | Raimat   |
| Climate                                      | Continental, semiarid  | Continental, semiarid  | Continental, semiarid  |
| Vineyard altitude                            | 280 m  | 280 m  | 315 m  |
| Conduction system                            | <i>Royat Doble</i>   | <i>Royat Doble</i>   | <i>Royat Doble</i>   |
| Plantation year                              | 2010   | 2014   | 1999   |
| Yield obtained in the 2019 grape harvest     | 7.800 kg/ha  | 12.520 kg/ha   | 6.620 kg/ha  |

**Table 5.** Characteristics from the soils of Xarel-lo samples.

| Vineyard classification according to quality | Xarel-lo soils  |   |  |  |
|--|---|---|--|--|
|  | A   | B   | C  | D  |
| Reference/Name                               | AK627/La Fideuera   | AB264/Peixo-Montagull   | IB657/Pla-Les Cabanyes   | AU095/Mateu-Refugi vell  |
| Main characteristics                         | Homogeneous vineyard, good transparency of vegetation (40%), grapes do not touch between vines, breezy and slightly shaded, 15 sprouts/m, length of 80-100 cm | Homogeneous vineyard, with good transparency of vegetation, grapes are breezy and slightly shaded, 15 sprouts/m, length of 80-100 cm. | Homogeneous vineyard, with good transparency of vegetation, grapes are breezy and slightly shaded, some vines touch each other but they are not packed, 20 sprouts/m, length of 110 cm | No homogeneous vineyard with an excess of vegetation, grapes hidden from the Sun, no breezy, over 20 sprouts/m, length over 150 cm |
| Type of soil                                 | Calcareous/clayey   | Calcareous/clayey   | Calcareous/clayey  | Calcareous/clayey  |
| Agricultural practices                       | Ecological farming  | Ecological farming  | Conventional farming   | Ecological farming   |
| Geographical location                        | Olèrdola (Penedès)  | Subirats (Penedès)  | Les cabanyes (Penedès)   | Santa Margarida i els Monjos (Penedès)   |
| Climate                                      | Mediterranean   | Mediterranean   | Mediterranean  | Mediterranean  |
| Vineyard altitude                            | 210 m   | 225 m   | 270 m  | 180 m  |
| Conduction system                            | Arbor – <i>Cordó Royat</i>  | Traditional vessel  | Arbor – <i>Cordó Royat</i>   | Arbor – <i>Cordó Royat</i>   |
| Plantation year                              | 1993  | 1965  | 2003   | 1990   |
| Yield obtained in the 2019 grape harvest     | 8.000 kg/ha   | 10.000 kg/ha  | 12.000 kg/ha   | 12.000 kg/ha   |

### 5.3. INSTRUMENTATION

For the determination of the elemental composition of musts, wines, sparkling wines and soil samples, the measurements were performed by ICP-OES using a Perkin Elmer Optima 3200RL and by ICP-MS using a Perkin Elmer Nexlon 350D instrument.

Both instruments used an Ar plasma. ICP-OES operated under standard conditions, while for ICP-MS measurements Rh was added as internal standard to correct for instrumental drift, and an He collision cell was used to avoid interferences (He flow: 4 mL/min).

## 5.4. ANALYTICAL PROCEDURE

### 5.4.1. Musts, wines and sparkling wines metal content measurements

From the preliminary study showed on *Section 6.1.1.* was concluded that the best dilution ratio was 1:10 (1 mL of sample for 9 mL of 1% HNO<sub>3</sub>) and that the samples were only going to be diluted, without filtering.

To prepare them for the ICP measurements, the 40 musts, wines and sparkling wines (see *Section 5.2.1.*) were unfrozen and 1 mL of each one was transferred to a 15 mL ICP tube using an electronic micropipette. Then the samples were frozen for three weeks, and two days before the measurements they were unfrozen and 9 mL of 1% HNO<sub>3</sub> were added to each one. In that point, the must samples were not totally clear and some sort of jelly had been formed in the bottom of 6 of them, so the 8 musts were filtered with syringe and 0.45 µm filters.

Apart from the 40 samples, 2 blanks with 10 mL of 1% HNO<sub>3</sub> and 4 different Quality Controls (QCs) were prepared. The mixture for the controls was prepared taking 1 mL of each one of the 40 samples, but as it was prepared before freezing them for three weeks the mixture had the unfiltered musts.

The 40 samples, 4 QCs and 2 blanks were taken to the CCiT-UB to perform ICP-OES and ICP-MS measurements in 2 consecutive days, one for each technique.

### 5.4.2. Soils metal content measurements

The 11 soil samples (see *Section 5.2.2.*) arrived at the laboratory with a lot of moisture, so first of all they needed to be dried to subsequently be sieved properly. For this purpose, they were left to dry in a fume hood for four weeks, during which they were constantly split manually to help remove the moisture.

Once the 11 soils were dry, they were lightly smashed to help divide the grains and break the remaining conglomerations. To do so, a wooden roller and a hammer were used, trying not to modify the composition of the samples. After smashing, they were sieved with a 2 mm sieve, separating the smaller particles from the unwanted bigger ones, rocks and branches.

For the mild extraction of metals from soil samples, 500 mL of 20% HNO<sub>3</sub> and 500 mL of 1 M NH<sub>4</sub>NO<sub>3</sub> were prepared. Two aliquots of 5 g of each soil were put in 50 mL plastic tubes, and afterwards 12.5 mL of the HNO<sub>3</sub> and NH<sub>4</sub>NO<sub>3</sub> solutions were added separately. The addition of

HNO<sub>3</sub> had to be carried out adding little amounts of the extractant, because big effervescence was produced due to the carbonate composition of the soils, which could lead to the loss of part of the sample.

Once all the gas was released, the tubes were covered with a cap and shaken in an end-over-end shaker overnight at 50-60 rpm. Then the extracts were separated from the solid residue by centrifugation for 30 minutes at 3000 rpm.

Extracts were further filtered with syringe and 0.45 µm filters. 0.5 mL of each extract was put in an ICP tube with a micropipette, and then 9.5 mL of 1% HNO<sub>3</sub> were added to each tube to dilute the samples in a 1:20 ratio.

Apart from these 22 samples, 2 different Quality Controls and 2 blanks were prepared. One of the blanks was prepared with 10 mL of 1% HNO<sub>3</sub>, and the other one with 0.5 mL of 1 M NH<sub>4</sub>NO<sub>3</sub> and 9.5 mL of 1% HNO<sub>3</sub>. For the first QC, 0.5 mL of a mixture of all 11 extracts from the extraction with 20% HNO<sub>3</sub> in equal content and 9.5 mL of 1% HNO<sub>3</sub> were added, while the second one was prepared with 0.5 mL of a mixture of all 11 extracts from the extraction with 1 M NH<sub>4</sub>NO<sub>3</sub> in equal content and 9.5 mL of 1% HNO<sub>3</sub>.

The 22 samples, 2 QCs and 2 blanks were taken to the CCiT-UB to perform the last set of ICP-OES and ICP-MS measurements of the project.

## 6. RESULTS AND DISCUSSION

### 6.1. ICP-OES AND ICP-MS MEASUREMENTS

#### 6.1.1. Preliminary study

Here, a preliminary study of multi-elemental measurements by ICP was performed to decide the best dilution for the samples, whether a previous filtering was necessary and also which could be the most relevant and discriminant elements for wine and sparkling wine classification.

Therefore, as a first approximation it was decided to simply dilute the samples in 1% HNO<sub>3</sub> to evaluate the accuracy of the results and the possible occurrence of matrix effects. This strategy was obviously more straightforward compared to acid digestion with HNO<sub>3</sub>, which could be performed as a second option. Both preliminary treatments could be performed with or without filtration, neither for musts nor for wines nor for sparkling wines.

To find the balance between minimizing matrix effect and obtaining low detection limits, different dilutions were tested. For this purpose, 4 different sparkling wines previously degassed (see *Section 5.2.1.*) were diluted in 1% HNO<sub>3</sub> in 3 different ratios (1:5, 1:10 and 1:20), preparing one set of samples without filtering and another one with a previous filtering with syringe and 0.45 µm filters. This way, there was a batch of 16 samples without filtering (4 samples with each one of the 3 dilutions and their corresponding blanks) and another batch of 16 samples with pre-filtering.

For the determination of their metal content, semi-quantitative measurements were performed by ICP-MS and ICP-OES in the CCiT-UB. The measurements of the 1:5 dilution by ICP-OES switched off the plasma, hence the ethanol content was too high and these diluted samples could not be properly measured. The results from 1:10 and 1:20 dilutions did not significantly differ, so it was decided that the best option was to choose the 1:10 dilution (1 mL of sample for 9 mL of 1% HNO<sub>3</sub>) for posterior must, wine and sparkling wine samples, thus having higher detection limits.

On the other hand, any relevant difference was observed between filtered and non-filtered samples, so the rest of the samples were only going to be diluted, without filtering.

28 elements were semi-quantitatively measured by ICP-OES, obtaining the results summarized in the *Table 6*.

**Table 6.** Experimental results from the semi-quantitative multi-element determination by ICP-OES.

| Element | Concentration range [ppm] | LOD [ppm] | Element | Concentration range [ppm] | LOD [ppm] |
|---------|---------------------------|-----------|---------|---------------------------|-----------|
| Al      | 0.125-0.175               | 0.5       | As      | -                         | 0.5       |
| B       | 0.4-0.5                   | 0.19      | Ba      | 0.025-0.050               | 0.005     |
| Be      | -                         | 0.1       | Ca      | 9-12                      | 0.1       |
| Cd      | -                         | 0.01      | Co      | -                         | 0.01      |
| Cr      | -                         | 0.02      | Cu      | 0.06-0.12                 | 0.02      |
| Fe      | 0.12-0.16                 | 0.01      | K       | 34-40                     | 0.5       |
| Li      | -                         | 0.1       | Mg      | 8-11                      | 0.1       |
| Mn      | 0.08-0.16                 | 0.005     | Mo      | -                         | 0.1       |
| Na      | 2-7                       | 15        | Ni      | -                         | 0.1       |
| P       | 12-23                     | 1         | Pb      | -                         | 0.2       |
| S       | 24-47                     | 0.5       | Sb      | -                         | 0.1       |
| Se      | -                         | 0.5       | Si      | 1.2-1.8                   | 0.25      |
| Sr      | 0.1-0.2                   | 0.005     | Ti      | -                         | 0.02      |
| V       | -                         | 0.05      | Zn      | 0.07-0.12                 | 0.02      |

The elements marked with darker blue were the ones that offered results above the detection limits of the technique and could be helpful to characterize and authenticate the samples in the following measurements.

On the other hand, 52 elements were semi-quantitatively measured by ICP-MS, obtaining the results summarized in the *Table 7*.

**Table 7.** Experimental results from the semi-quantitative multi-element determination by ICP-MS.

| Element | Concentration range [ppb] | DL [ppb] | Element | Concentration range [ppb] | DL [ppb] |
|---------|---------------------------|----------|---------|---------------------------|----------|
| Al      | 150-180                   | 5        | Ag      | -                         | 0.1      |
| As      | 0.6-0.9                   | 5        | Au      | -                         | 0.1      |
| B       | 750-1000                  | 100      | Ba      | 2.3-3.0                   | 0.1      |
| Be      | 0.07-0.12                 | 1        | Bi      | -                         | 0.1      |
| Ca      | 12000-16000               | 100      | Cd      | 0.025-0.050               | 0.1      |

Table 7 (continued)

|    |             |     |    |             |     |
|----|-------------|-----|----|-------------|-----|
| Ce | 0.04-0.07   | 0.1 | Co | 0.3-0.4     | 0.1 |
| Cr | 200-250     | 5   | Cs | 0.3-0.6     | 0.1 |
| Cu | 55-110      | 1   | Dy | 0.004-0.010 | 0.1 |
| Er | 0.004-0.008 | 0.1 | Eu | 0.001-0.003 | 0.1 |
| Fe | 230-310     | 10  | Ga | 0.15-0.18   | 0.2 |
| Gd | 0.003-0.011 | 0.1 | Ge | -           | 0.2 |
| Hf | 0.004-0.012 | 0.1 | Hg | -           | 0.2 |
| Ho | 0.001-0.004 | 0.1 | In | -           | 0.1 |
| Ir | -           | 0.1 | K  | 12000-40000 | 50  |
| La | 0.02-0.04   | 0.1 | Li | 3.0-4.5     | 0.5 |
| Lu | 0.001-0.003 | 0.1 | Mg | 11000-14000 | 5   |
| Mn | 100-180     | 0.1 | Mo | 0.3-1.8     | 0.1 |
| Na | 5000-9000   | 50  | Nb | 0.002-0.014 | 0.2 |
| Nd | 0.015-0.040 | 0.1 | Ni | 1.2-2.1     | 1   |
| Os | -           | 0.1 | P  | 9000-12000  | 500 |
| Pb | -           | 0.1 | Pd | 0.006-0.014 | 0.1 |
| Pr | 0.004-0.010 | 0.1 | Pt | -           | 0.1 |
| Rb | 40-50       | 0.5 | Re | -           | 0.1 |
| Rh | -           | 0.1 | Ru | -           | 0.1 |
| S  | 15500-18000 | 500 | Sb | -           | 0.1 |
| Sc | 1.4-1.7     | 1   | Se | -           | 5   |
| Si | 4500-6000   | 200 | Sm | 0.002-0.008 | 0.1 |
| Sn | 0.10-0.25   | 1   | Sr | 100-190     | 0.2 |
| Ta | 0.005-0.009 | 0.1 | Tb | 0.001-0.002 | 0.1 |
| Te | -           | 0.1 | Th | -           | 0.1 |
| Ti | 40-60       | 0.2 | Tl | -           | 0.1 |
| Tm | 0.001-0.002 | 0.1 | U  | 0.02-0.06   | 0.1 |
| V  | 0.8-1.4     | 5   | W  | 0.04-0.14   | 0.2 |
| Y  | 0.05-0.10   | 0.5 | Yb | 0.004-0.012 | 0.1 |
| Zn | 80-120      | 5   | Zr | 0.5-0.9     | 0.5 |

Following the same strategy, the elements marked with darker blue were the ones that offered results above the detection limits of the instrument and could be useful to characterize the samples in the following measurements.

This way, studying deeply the data obtained from these semi-quantitative measurements of sparkling wine samples by ICP-OES and ICP-MS, the following conclusions were extracted:

- Almost no difference was observed between filtered and non-filtered samples, therefore upcoming musts, wines and sparkling wines were going to be measured without filtering, only diluting them with 1% HNO<sub>3</sub> in a 1:10 ratio.
- Regarding the techniques used, ICP-OES measurements allowed to properly determine 13 elements (B, Ba, Ca, Cu, Fe, K, Mg, Mn, P, S, Si, Sr and Zn), while ICP-MS allowed to determine 24 (B, Ba, Ca, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, P, Rb, S, Sc, Si, Sr, Ti, Zn and Zr) over the detection limits of the instrument. In addition, ICP-MS offered lower detection limits and all thirteen elements determined by ICP-OES were also among the ones determined by ICP-MS, making it possible to use only this technique for the rest of the project. Even so, ICP-OES is less affected by matrix effect and generally a better technique to measure elements with higher concentrations like Ca, K, Na, Mg, P, S and Si, therefore it is recommended to perform both techniques in a complementary way.
- Apart from the 24 elements mentioned before, considering that this was a semiquantitative analysis, it was determined that some other elements with signals under the detection limits but showing significant results and differences among the sparkling wine samples could provide important information for the classification of the samples in quantitative measurements. This way, it was decided to include 14 more elements for following determinations (Al, As, Cd, Ce, La, Mo, Nd, Pb, Sb, Sn, U, V, W and Y), making it up to 38.

### **6.1.2. Must, wine and sparkling wine samples**

The preliminary study allowed to decide that the best dilution ratio was 1:10, with higher LODs than the 1:20 dilution and without the problems presented by the 1:5. In addition, must, wine and sparkling wine samples were not filtered, and considering that ICP-OES and ICP-MS provided complementary information, subsequent measurements were performed using both.

As it has been already mentioned, after transferring 1 mL of each one of the 40 samples to a 15 mL ICP tube, they had to be frozen for 3 weeks due to the lack of availability of the instruments.

Two days before the measurements, they were unfrozen and 9 mL of 1% HNO<sub>3</sub> were added to each one. It was decided that musts should be filtered due to the appearance of some sort of jelly in 6 of them, but there was an accident filtering one of the samples (Pinot Noir, quality A) and it had to be prepared again, taking more sample from the original jar which was also frozen.

From the list of 38 elements provided to the CCIT-UB, initially only 36 of them could be measured because there was a problem with the ICP-OES instrument so K and Na could not be analysed. Finally, another set of measurements could be performed six weeks later to determine K and Na in the samples, which had been kept in the fridge during this period.

12 elements were determined by ICP-OES (Al, Ca, Cu, Fe, K, Mg, Na, P, S, Si, Sr and Zn) and 31 by ICP-MS (As, Al, B, Ba, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, La, Li, Mn, Mo, Nd, Ni, Pb, Rb, Sb, Sc, Sn, Sr, Ti, U, V, W, Y, Zn and Zr). Therefore, 5 elements were measured by both techniques and results from the concentration of 38 elements in the different musts, wines and sparkling wines were obtained.

Previously to any type of chemometric data processing by PCA and box plot diagrams, the results showed the following:

- 26 elements had signals over the detection limits of the instrument and the method: Al, As, B, Ba, Ca, Co, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Rb, S, Sb, Si, Sr, Ti, V, Zn and Zr.
- 9 elements had signals under the detection limit of the instrument (Cd, Ce, Ga, La, Nd, Sc, U, W and Y), plus other 3 under the quantification limit of the method (Cr, Pb and Sn).
- Elements with greater variation in the concentration among the 40 samples (considering RSD > 100%) were further studied in a first approximation, because they could be the ones which provide more relevant information: As, Cu, Sb, and V.
- Elements with a big variation between the 4 Quality Controls (considering RSD > 20%) were not considered for sample classification, because they mainly contributed to noise.
- Metals like Cu, Zn and Ti showed very different values between the two blanks measured, probably due to instrumental inaccuracy. On the other hand, Fe and Al had very high values for the blanks (more than 6 ppb), in this case coming from the HNO<sub>3</sub> used for their dilution.
- The sample MPA (Pinot Noir must of A quality) had much higher values than expected of Cu and Zr, and a lower one of Sb. Knowing that this sample had to be prepared and filtered again, it could be the reason why the results differ from the expected, and the chemometric study would show whether it could be treated as anomalous or not.

### 6.1.3. Soil samples

The same list of 38 elements to measure was given to the CCiT-UB. All of them could be successfully measured, even though the samples extracted with 20% HNO<sub>3</sub> needed to be more diluted due to their high concentration of Ca, which affected the LODs of some metals.

13 elements were determined by ICP-OES (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si and Sr) and 29 by ICP-MS (As, Al, B, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, La, Li, Mn, Mo, Nd, Ni, Pb, Rb, Sb, Sc, Sn, Ti, U, V, W, Y, Zn and Zr). Therefore, 4 of them were measured by both techniques because depending on the type of extraction and the element to determine, one ICP instrument offered better results than the other one. This allowed to obtain the concentration of 38 elements in the 11 soil samples, extracted separately with 20% HNO<sub>3</sub> and 1 M NH<sub>4</sub>NO<sub>3</sub>.

Before starting with any type of chemometric data processing by PCA, the results gathered in the spreadsheet showed the following:

- For the 20% HNO<sub>3</sub> extraction, 31 elements had signals over the detection limits of the instrument and the method (Al, As, B, Ba, Ca, Cd, Ce, Co, Cr, Cu, Fe, Ga, K, La, Li, Mg, Mn, Nd, Ni, P, Pb, Rb, Sc, Si, Sr, Ti, U, V, Y, Zn and Zr), while 7 elements had signals under the detection limit of the instrument (Cs, Mo, Na, S, Sb, Sn and W).
- For the 1 M NH<sub>4</sub>NO<sub>3</sub> extraction, 16 elements had signals over the detection limits of the instrument and the method (As, Ba, Ca, Co, Cs, Cu, K, Li, Mg, Mn, Mo, Na, Rb, Sr, U and V), while 13 elements had signals under the detection limit of the instrument (Cd, Ce, Ga, La, Nd, P, S, Sb, Sc, Si, W, Y and Zn), plus other 9 under the quantification limit of the method (Al, B, Cr, Fe, Ni, Pb, Sn, Ti and Zr).
- As it was noticed for the must, wine and sparkling wine samples, Fe and Al had very high values for the blanks (more than 7 ppb) coming from the HNO<sub>3</sub> used for the extractions and dilutions, and this affected to the LODs of the method for the NH<sub>4</sub>NO<sub>3</sub> extraction.
- The Pinot Noir soil of D quality and the Xarel·lo of A quality had much lower concentrations than expected for the majority of measured elements in the HNO<sub>3</sub> extraction, possibly due to an experimental mistake during the addition of HNO<sub>3</sub> and the resulting effervescence or during the measurements. Hence, the chemometric treatment will show if they had to be treated as anomalous or not.



Moreover, the samples of the higher quality (quality A) can be distinguishable from the other qualities in wines and sparkling wines, being the difference greater in the case of Pinot Noirs.

Regarding this general classification, with the help of previously built models with Pinot Noir and Xarel-lo separated in groups and also looking at the spreadsheet where all the data is collected, the following conclusions were drawn:

- **Cu** and **K** are in high concentrations in the musts, lower in the base wines and in very low ones in the stabilized wines and sparkling wines, both for 3 and 7 months in rhyme.
- Musts and base wines have low concentrations of **Na**, **V** and **Zr**. Musts have low concentrations of **Al**, **Fe**, **Mn**, **S** and **Ti**; and much greater of **Sb**.
- The concentration of **K**, **Mg**, **Mn**, **Ni**, **Sr** and **Zn**, and in the A qualities of wines and sparkling wines is lower than in the rest of qualities. As opposed to this, the concentration of **Ba** and **Cs** in the higher quality of all Pinot Noir samples is greater than in the rest.

All these conclusions were thoroughly contrasted with the raw data from the ICP measurements because it is very important to recall that this PCA model only showed half the information (51.84% of the variance).

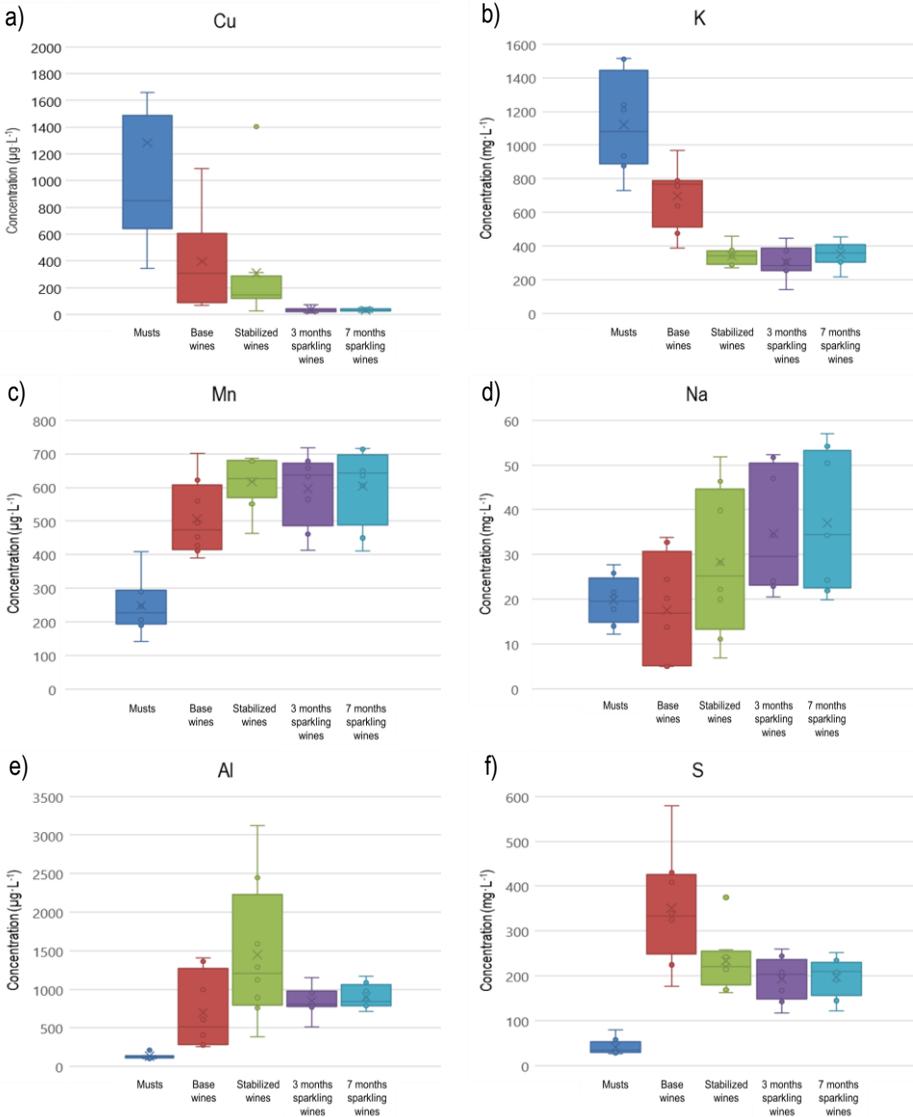
#### 6.2.1.2. Classification of the samples

Box plot diagrams and bar charts helped to complement the characterization of the samples according to their metal composition, showing the variability in metal content from the chosen groups of samples, and confirming the conclusions extracted from PCA.

This way, *Figure 6* shows the variation among the five general types of samples (without distinguishing between Pinot Noir and Xarel-lo) according to their quantity of different metals, with the aim of relating these variations in the metal content with the different steps of the procedure.

In general, some metals such as Cu or Fe join to the yeast walls and are deposited as sediments during the fermentation processes, thus decreasing their content. On the other hand, the addition of different additives, yeasts, bentonite and the expedition liquor during specific steps of the procedure also affects the concentration of some metals, making it higher. The type of yeasts and expedition liquor added depends on the company, so their effect in the metal content is difficult to predict and extrapolate. As for bentonite, it is a hydrated aluminosilicate that can also contain Na, Ca, Fe and Mg, added as a clarifying agent after the alcoholic fermentation to remove the excess of unstable proteins by their absorption.

However, some of the observed trends are difficult to explain due to the influence of organic compounds in the metal content and the usage of secret blends and yeasts by each company.



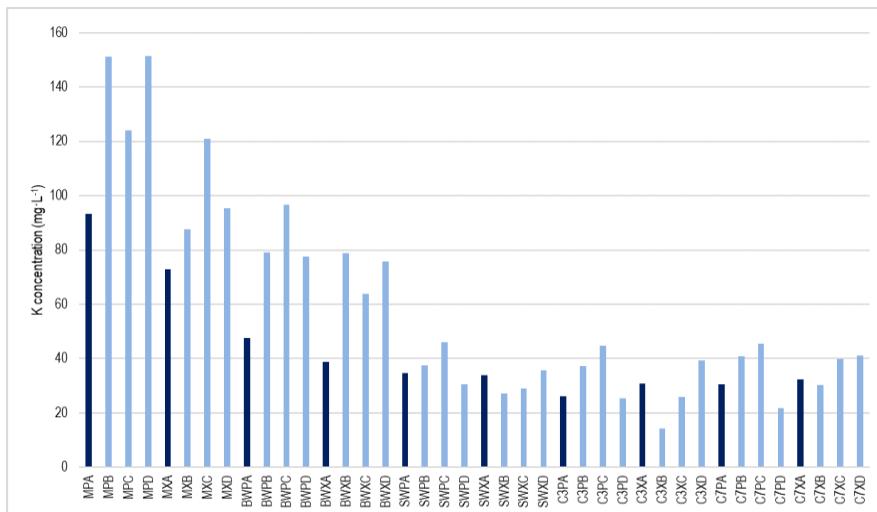
**Figure 6.** Box plot diagrams of the concentrations of Cu (a), K (b), Mn (c), Na (d), Al (e) and S (f), divided by groups of samples. Sb has a very similar behaviour to Cu(a), Fe and Ti follow the same trend than Mn (c), and V and Zr have similar progressions than Na (d).

Cu is in great concentration in the musts due to the sulfidation of the vines with  $\text{CuSO}_4$  to prevent illnesses like mildew, and its following decrease by precipitation and deposition helps to assure it is under the legislation limit of 1 ppm in wines and sparkling wines.

After the clarification, the base wine is stabilized through the precipitation of tartaric acid from grapes as potassium and sodium tartrate, explaining the big decrease in K content from base wines to the stabilized ones. This does not apply for Na, probably due to the addition of bentonite and other components that make it be in greater amounts in stabilized wines and sparkling wines.

For the rest of metals, a similar increasing trend as the one for the Na can be observed. Their behaviour also comes from the addition of bentonite, the expedition liqueur and other additives during the process. The big increase of Al from base wines to stabilized wines comes mainly from the bentonite. In the case of S, its big increase from the must to the base wine is due to the addition of  $\text{SO}_2$  to prevent the activation of acetic bacteria and other undesired reactions, while the stabilized wine has a lower content because it is deposited to allow the second fermentation.

On the other hand, as the PCA models showed a clear difference between the metal composition from A qualities and the lower ones (B, C and D qualities), bar charts were built for relevant elements to clearly see the main variations. As shown in *Figure 7*, this difference was evinced in a lower content of some metals for A qualities.



**Figure 7.** Bar chart of the potassium (K) concentration in the different samples, in  $\mu\text{g/L}$ . Quality A for each type of sample has been marked in darker blue to be distinguishable. Mg, Mn, Na, Ni, Sr and Zn follow similar progressions regarding the difference between A quality and the lower ones.

For the production of wines and sparkling wines of A quality (the higher one), only a 40-45% pressing is applied so that K, Ca and other minority ions are not extracted, thus explaining their minor metal content. Moreover, the base wines go through an electrodialysis machine before the tirage to remove the maximum possible amount of these ions, while the lower varieties follow a different procedure. Even so, this behaviour was not observed for Ca.

These products of A quality also follow a more “purist” process than the lower ones, to maintain their essence and search the nuances and aromas that make them a premium product. The procedure is often more manual, using the less possible additives and without blending them with other varieties; while varieties B, C and D follow more automated processes, more additives are added and usually their base wines are mixed between them to give rise to the stabilized wine, for this reason named blended wine.

## 6.2.2. Soil samples

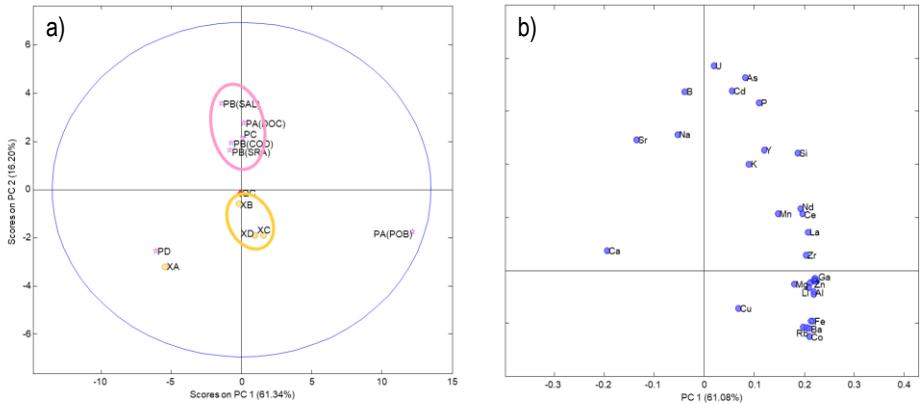
### 6.2.2.1. Chemometric evaluation by Principal Components Analysis

The extraction with 1 M  $\text{NH}_4\text{NO}_3$  is milder than the one with 20%  $\text{HNO}_3$ , so that smaller amounts of metals were extracted with  $\text{NH}_4\text{NO}_3$  and many of them had signals under the detection limits of the instrument and/or the method when measured by ICP. On the contrary, with  $\text{HNO}_3$  the extraction was greater and signals from 31 elements were obtained over the detection limits.

This way, the results from the  $\text{HNO}_3$  extractions were used to build different PCA models with the programme SOLO, to classify the soils by their geographical origin and metal composition. The global classification of the 11 samples and the QC was represented in the graphics showed in *Figure 8*. *Autoscale* was chosen as pre-processing and the model was built with 2 Principal Components, which retain a 77.54% of the total variance of the soil samples.

The model showed a clear compositional difference between soils from Pinot Noir and Xarel·lo, which allowed to classify them by their geographical origin. As an exception, the Pinot Noir soil of A quality from Vimbodí i Poblet (pointed as PA(POB) in the *Scores* graphic from the PCA) had different composition than the rest of soils of Pinot Noir, which are from Rimat.

In addition, the model also showed that the Pinot Noir sample of D quality (PD) and the Xarel·lo of A quality (PA) differed in composition from their varieties, hence they were finally classified as anomalous samples and were not considered for the final classification.



**Figure 8.** Graphics of Scores (a) and Loadings (b) from the PCA model. The pink ellipse encompasses the soils of Pinot Noir, except for the one of A quality from Vimbodí i Poblet and the one of D quality. The yellow one encompasses the soils of Xarel·lo, except the one of A quality.

Regarding the metal composition of the soils and the main differences between varieties, with the help of the spreadsheet where all the data is collected, the following conclusions were drawn:

- **Ca** is the major component in all soils (over 90% in all of them except for the Pinot Noir from Vimbodí i Poblet, which has 62%), followed in order by **Mg, Al, Fe, K** and **Si**.
- Soils of Pinot Noir from Raimat are richer in **As, B, Ca, Cd, Na, P, Sr** and **U**.
- Soils of Xarel·lo have greater concentrations of **Cu, Co, Fe, Rb** and **Sc**.
- The Pinot Noir soil from Vimbodí i Poblet has much less concentration of **Ca**, while is richer in **Al, Fe** and **Mg** than the other ones.

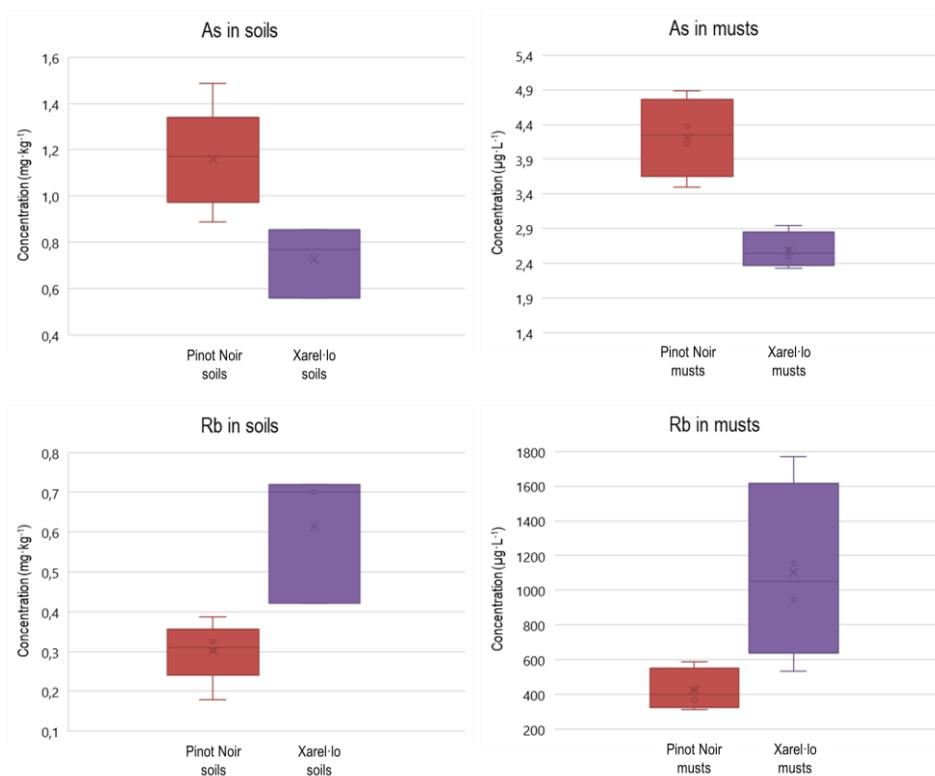
PCA models were also built with soil and must samples together to see if the composition between varieties and qualities was maintained. But must and soils are different type of samples with very different matrix, and moreover only 21 elements were successfully measured for both, so the models did not have complete information for any of the groups, almost any relationship was observed and no general trends were found.

Hence, following the same strategy than in *Section 6.2.1.*, the information concerning to the metal content of each variety extracted from the PCA had to be contrasted with the raw data from the ICP measurements, because the model only showed part of the information (77,54% of the variance).

### 6.2.2.2. Classification of the samples

Box plot diagrams were used to contrast the information provided by PCA and complement the characterization of the soils according to their metal composition, to classify them by region of origin and define their main similarities with the musts.

For this purpose, *Figure 9* shows two examples of the comparison between the variation in metal content of soils from Raimat (Pinot Noir) and Penedès (Xarel-lo) with their corresponding musts. Anomalous soil samples were not considered, as well as the Pinot Noir soil from Vimbodí i Poblet due to its different location and composition.



**Figure 9.** Comparison between box plot diagrams of the concentrations of As (a) and Rb (b) in soils and musts of Pinot Noir and Xarel-lo varieties. Ca, P and Sr have the same behaviour than As.

Soils from Penedès and Raimat are mainly calcareous and clayey (see *Table 4* and *Table 5*), therefore the data confirmed that Ca is their major component, followed by Al, Fe, K, Mg and Si.

This was the expected results as calcareous rocks are formed by  $\text{CaCO}_3$  and traces of  $\text{MgCO}_3$  and other carbonates, while clay rocks are aluminosilicates with cations like Ca, Fe, K, or Mg.

To differ between varieties and explain their distinction in the PCA model, it was found that soils of Pinot Noir (only those from Raimat vineyards) are richer in As, B, Ca, Cd, Na, P, Sr and U. These greater quantities of metal are also seen in their musts, which have high content of As, B, Ca, P and Sr (Cd and U were under the LODs).

On the other hand, soils of Xarel·lo have greater concentrations of Cu, Co, Fe, Rb and Sc. But these trends have only been observed in the musts for Rb, because Sc was not properly measured and there were not big differences for the other elements.

Finally, the Pinot Noir soil from Vimbodí i Poblet is mainly clayey and rich in slates, which make it have much less concentration of Ca (around 62%) and be richer in Al, Fe and Mg than the rest of the soils.

This way, the different composition between soils of Pinot Noir from Raimat and from Vimbodí i Poblet and soils of Xarel·lo from Penedès that was firstly seen in the PCA model (see *Figure 8*) was confirmed. Hence, the soils were successfully classified by region of origin and also related to the composition of the musts. Even so, the differences observed for the A qualities in musts, wines and sparkling wines were not observed in the soils, because the soil of Xarel·lo turned out to be an anomalous and the samples from A quality of Pinot Noir are a mixture of the grapes from both vineyards, Raimat and Vimbodí i Poblet.

## 10. CONCLUSIONS

The main objective set when starting this project was to characterize and classify samples from two varieties of must, wine and sparkling wine according to their metal content, finding trends and characteristics which allow to discriminate among them by regions of origin and quality. After performing ICP-OES and ICP-MS measurements of the mentioned samples and from the soils where their grapes come from, the following conclusions have been drawn:

- ICP-OES and ICP-MS measurements were successfully carried out, finding the appropriate pre-treatments and dilutions for each type of sample to obtain relevant data.
- Results from the musts, wines and sparkling wines measurements with their subsequent data treatment allowed to classify them according to the type of sample. A qualities were distinguishable from the lower ones, as well as must were distinguishable between the two varieties analysed and also with the rest of the samples. Nevertheless, qualities B, C and D did not show significant trends or differences to separate them by metal content.
- The measurements of the elemental composition of soils also allowed to classify them and discriminate among regions of origin. In addition, some trends and similarities were found between the must and their soils, which allowed to correlate them.

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## 12. ACRONYMS

AAS: Atomic Absorption Spectroscopy

CCiT-UB: *Centres Científics i Tecnològics – Universitat de Barcelona*

FTIR: Fourier Transform Infrared

HPLC: High-Performance Liquid Chromatography

ICP-MS: Inducted Coupled Plasma Mass Spectrometry

ICP-OES: Inducted Coupled Plasma Optical Emission Spectrometry

IRMS: Isotope Ratio Mass Spectrometry

LOD: Limit of Detection

NIR: Near Infrared

NIST: National Institute of Standards and Technology

PC: Principal Component

PCA: Principal Components Analysis

PDO: Protected Designation of Origin

QC: Quality Control

SNIF-NMR: Site-specific Natural Isotope Fractionation studied by Nuclear Magnetic Resonance

