Physicochemical and sensory analysis of Syrah wines from vineyards mechanically pruned treated with different organic amendments

Rafaela Alexandra Cerqueira Pardal

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Orientador: Professor Doutor Jorge Manuel Rodrigues Ricardo da Silva

Júri:

Presidente: Doutor Carlos Manuel Antunes Lopes, Professor associado com agregação, Instituto Superior de Agronomia da Universidade de Lisboa

Vogais: Doutora Sofia Cristina Gomes Catarino, Professora auxiliar convidada, Instituto Superior de Agronomia da Universidade de Lisboa

Doutor Jorge Manuel Rodrigues Ricardo da Silva, Professor catedrático, Instituto Superior de Agronomia da Universidade de Lisboa

Doutor Henrique Manuel Filipe Ribeiro, Professor auxiliar, Instituto Superior de Agronomia da Universidade de Lisboa

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Resumo

Para avaliar o efeito da poda mecânica e da aplicação de corretivos orgânicos na qualidade de vinhos da casta Syrah foram testadas três vinhas situadas em regiões vitivinícolas distintas – Quinta do Côro (Tejo), Quinta do Gradil (Lisboa) e Herdade de Rio Frio (Setúbal).

A avaliação da influência da técnica de poda e dos corretivos orgânicos foi feita com base em análises físico-químicas, bem como determinação das características cromáticas dos vinhos, dos seus teores de azoto e minerais, bem como metais pesados e outros elementos e ainda através da sua análise sensorial.

Os resultados mostram que a poda mecânica originou vinhos com menor teor alcoométrico volúmico, menor intensidade cromática com uma concentração inferior de antocianinas totais e taninos. Este tipo de poda também fez com que os vinhos tivessem um teor de minerais e metais pesados inferior, em geral. A análise sensorial mostrou que os vinhos provenientes de videiras podadas mecanicamente foram menos apreciados pelo painel de prova. O corretivo orgânico que originou vinhos com piores resultados nos parâmetros estudados foi a lama de Estação de Tratamento de Águas Residuais, que reduziu o teor alcoométrico volúmico dos vinhos bem como a intensidade da sua cor e teor de antocianinas, por outro lado o pó de carvão e as parcelas de controlo originaram vinhos com melhores resultados.

Palavras-chave: Poda, corretivos orgânicos, vinho

Abstract

In order to study the effect of mechanical pruning and the addition of organic amendments to the vineyard in Syrah wine quality, three vineyards from different viticultural areas were tested – Quinta do Côro (Tejo), Quinta do Gradil (Lisboa) and Herdade de Rio Frio (Setúbal).

In order to evaluate the influence of the treatments applied the wines were subjected to a physicochemical analysis as well as chromatic characteristics determination, total nitrogen content, mineral and heavy metals and other elements analysis and the tasting panel performed sensory analysis to determine if the differences were perceived.

The results show that mechanical pruning led to wines with lower alcohol content, color intensity, total anthocyanin content as well as less tannin power and inferior tannin content. This type of pruning technique also led to lesser accumulation of minerals and lower levels of some heavy metals. In the sensory analysis, the wines from mechanically pruned vines were less appreciated by the tasters receiving worse scores, including lower global appreciation marks. The organic amendment that led to lower overall results in the studied parameters was sewage sludge, it reduced alcoholic content and color intensity as well as total anthocyanin content while grime and the control plots led to better results.

Keywords: Pruning technique, organic amendments, wine

Resumo Alargado

O objetivo do presente trabalho é avaliar a influência da técnica de poda escolhida e da aplicação de corretivos orgânicos na qualidade de vinhos da casta Syrah. Para tal foram realizados ensaios em três vinhas de regiões vitivinícolas diferentes – Quinta do Côro na região vitivinícola do Tejo, Quinta do Gradil pertencente à região de Lisboa e Herdade de Rio Frio em Setúbal. A avaliação do efeito da poda foi feita com base na comparação entre a técnica tradicional – poda manual (MAN) – e poda mecânica simulada, em sebe (MEC). Este ensaio da poda foi feito na Quinta do Côro e na Quinta do Gradil, isto porque a vinha da Herdade de Rio Frio ainda é demasiado recente para a poda mecânica ser aplicada. Os corretivos orgânicos aplicados foram as lamas de ETAR (ETAR), estrume de bovino (ESTR), resíduos sólidos urbanos compostados (RSUC) e pó de carvão (BIOC). As parcelas de controlo (TEST), para efeitos de comparação de resultados não sofreram adição de qualquer tipo de corretivo orgânico.

A influência dos tratamentos aplicados na qualidade do vinho foi avaliada através de análises físico-químicas onde se determinou o teor alcoométrico volúmico, o pH, a acidez total, a acidez volátil e o teor de sulfuroso livre e total. Neste último caso, quando necessário, foram efetuadas correções. A determinação dos parâmetros da cor dos vinhos e da sua composição fenólica incluíram determinações como a intensidade da cor e sua tonalidade, teor de antocianinas totais e ionizadas, teor de pigmentos totais e polimerizados, fenóis totais, fenóis flavonoides e não flavonoides, e poder tanante. Para além da concentração de azoto total, foi feita uma análise mineral onde se determinaram os teores de potássio, cálcio, ferro e cobre. A análise dos metais pesados e elementos de terras raras incluiu a quantificação de um total de 35 elementos, entre os quais o arsénio, o tálio, manganês e chumbo. Para determinar se as diferenças registadas nos ensaios laboratoriais se traduziram na forma como os vinhos são caracterizados pelos provadores, foi feita uma análise sensorial com um painel constituído por 11 provadores profissionais. Os resultados foram obtidos através de processos de amostragem, nomeadamente os obtidos à vindima, com amostras representativas dos três blocos existentes em cada vinha. Cada bloco tem linhas onde se efetuou os dois tipos de poda e se aplicaram os corretivos orgânicos. Após a vindima, os resultados obtidos são uma média de 3 repetições dos ensaios laboratoriais, exceto nos metais pesados onde se efetuaram duas repetições e na análise físico-química onde apenas se efetuou uma medição. Os resultados foram analisados estatisticamente através do método ANOVA com testes de Tuckey.

Os resultados mostram que a poda manual foi a técnica de poda que resultou em maiores valores nos parâmetros da análise físico-química – os vinhos provenientes de videiras podadas manualmente apresentam maior teor alcoométrico volúmico, maior pH e valores de acidez volátil também mais elevados. No entanto, os maiores valores de acidez total registaram-se quando os vinhos provêm de videiras onde se aplicou a poda mecânica simulada. O efeito dos corretivos orgânicos na qualidade dos vinhos não registou nenhuma tendência em particular,

exceto para o teor alcoométrico volúmico na Quinta do Côro e Quinta do Gradil onde as lamas de ETAR aplicadas originaram vinhos menos alcoólicos. Este corretivo orgânico também originou vinhos com menores valores de acidez volátil na Quinta do Côro e na Herdade de Rio Frio.

A intensidade cromática dos vinhos resultantes das videiras podadas manualmente foi significativamente mais elevada que nos vinhos derivados da poda mecânica. Esta técnica de poda também originou vinhos com maior teor de fenóis totais, maior concentração de antocianinas e maior poder tanante. Pelo contrário, o grau de ionização das antocianinas aumentou em vinhos provenientes de videiras onde se aplicou a poda mecânica simulada. No geral, o corretivo orgânico que deu origem a vinhos com maiores resultados em parâmetros cromáticos foi o pó de carvão, a par dos vinhos provenientes das parcelas de controlo. Esta tendência verificou-se no teor de antocianinas ionizadas, pigmentos polimerizados, índice de polimerização, intensidade da cor, fenóis totais e poder tanante quer na Quinta do Côro quer na Quinta do Gradil.

O teor de azoto total foi mais elevado em vinhos provenientes de videiras podadas manualmente em ambas as Quintas onde o efeito da poda foi avaliado. Após análise dos resultados da determinação do teor de minerais nos vinhos é possível afirmar que o potássio, o ferro e o cobre estão presentes em maiores concentrações em vinhos derivados de videiras podadas manualmente. Pelo contrário, o teor de cálcio é superior quando a técnica de poda escolhida foi a poda mecânica simulada. No que diz respeito aos corretivos orgânicos, mais uma vez as lamas de ETAR influenciaram de forma significativa os teores de cálcio sendo que quando este tratamento foi aplicado os vinhos resultantes apresentam concentrações superiores. O teor de metais pesados, e outros elementos entre os quais as terras raras, foi influenciado pela técnica de poda escolhida e pelos corretivos orgânicos aplicados. No entanto, os resultados mostram tendências diferentes de acordo com o elemento em questão, o que demonstra a necessidade de mais estudos para determinar a influência quer da poda mecânica quer da aplicação de corretivos orgânicos na concentração destes elementos nos vinhos. Apesar deste facto, é possível afirmar que a poda mecânica simulada provocou o aumento do teor de alguns elementos nos vinhos, tais como arsénio, vanádio e estrôncio. No que diz respeito aos corretivos orgânicos, no geral as lamas de ETAR e o estrume de bovino causaram o aumento do teor destes elementos nos vinhos.

A análise sensorial mostrou que apesar de os resultados analíticos demonstrarem inúmeras diferenças significativas estatisticamente, elas não se podem traduzir na prática uma vez que os provadores apenas encontraram diferenças em alguns dos atributos avaliados. Na Quinta do Côro os atributos aos quais os provadores atribuíram valores significativamente diferentes dos outros quando a variável em estudo foi a técnica de poda foi a adstringência e o equilíbrio do vinho na boca. Em ambos os atributos, os vinhos provenientes de videiras podadas manualmente receberam pontuações mais elevadas. Na Quinta do Gradil a poda influenciou o corpo dos vinhos, o seu equilíbrio na boca e a apreciação global sendo que mais uma vez a poda manual originou vinhos com pontuações superiores. As parcelas de controlo originaram vinhos com pontuações mais elevadas no equilíbrio no nariz e na boca, no corpo e apreciação global. Na Herdade de Rio Frio não existiram tendências tão marcadas, os corretivos orgânicos influenciaram de forma diferente as pontuações dadas pelos provadores a cada atributo.

Após análise dos resultados é possível afirmar que uma vez que os efeitos dos tratamentos aplicados foram estudados para três *terroirs* diferentes, as conclusões apenas se aplicam a esses casos. Quer na Quinta do Côro quer na Quinta do Gradil, os vinhos resultantes da poda manual registaram valores mais elevados para os parâmetros em estudo. No que diz respeito aos corretivos orgânicos, a sua influência na qualidade do vinho não é tão clara e depende da vinha em estudo.

A decisão em relação ao tipo de poda a aplicar deve ser tomada tendo por base uma análise fundamentada de custos e benefícios, sendo que por vezes pode ser preferível adotar ambas as técnicas consoante o tipo de vinho a fazer. Nas regiões vitivinícolas consideradas neste estudo, uma estratégia economicamente sustentável pode passar por dividir a vinha em blocos de acordo com a qualidade e rendimento pretendidos. Desta forma, cada bloco pode ser tratado de forma diferente para se obterem diferentes tipos de vinho reduzindo os custos de produção.

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List of abbreviations

MEC	Simulated Mechanical Pruning
MAN	Manual Pruning
TEST	Control plot
ETAR	Sewage Sludge
ESTR	Cattle manure
RSUC	Municipal Solid Waste Compost
BIOC	Grime
MPCT	Minimal Pruning of Cordon Trained vines

1. Introduction

Agriculture is one of the main activities ever since the first sedentary populations settled. However, with the evolution of mankind and the exponential increase of population numbers the natural resources have become our most precious treasure. These resources are limited and over the years have not been carefully managed, that is why sustainability is one of the most discussed topics all over the world.

Viticulture is one of the most important agricultural activities of the world and wine is one of the most exported products worldwide. Activities in this sector are highly dependent on natural resources – solar energy, water, climate, soils – that must be preserved through sustainable practices in order not to compromise the ability of future generations to meet their own needs, as found in Long Island Sustainable Winegrowers Organization website (http://www.lisustainablewine.org/).

Sustainable viticulture is defined by the OIV (2008) as a global initiative to produce grapes with quality and without risks for consumers' health, integrating the different aspects of sustainability – economic viability, environmental concern and social equity.

Social equity is the capability of wine companies to understand the desires and needs of the nearby populations, considering the effects of their actions on the community allowing people to be responsible for their well-being in the future. Economic viability is what allows a vineyard and winery to have sustainable practices implemented, besides the fact that the economic activity of a company helps the economy of the community where it is inserted. The last aspect of sustainability is environmental concern, meaning the protection of the environment through the adoption of the "best practices" – the ones that allow a vineyard to produce the highest quality fruit possible while being able to keep the ecosystem's balance (OIV 2008).

In Portugal, viticulture is one of the most important activities in the agricultural sector. It originates seasonal employment and generates large volumes of exports. However, the appearance of New World country wine producers increased the competition in international markets. These countries produce in higher quantities and with lower costs, so in order to compete in a more equilibrated level with them it is necessary to reduce the production costs, without compromising the quality of the wines and the health of the vineyards. The reduction of the costs can be achieved by different practices – introducing mechanization, reduce the amounts of chemical amendments and herbicides. At the same time, it's necessary to increase the productivity of the vineyards and the price of the wine. This increase in productivity is not going to be enough due to the small size of the Portuguese vineyards, other strategies must be used in order to differentiate our wines from the others, for example explore the appreciation of the consumers for companies that show extra environmental concerns (organic and sustainable vineyards).

There are two vineyard practices that allocate more costs – pruning and harvesting. Pruning allows the grapegrowers to control the yield obtained in the next years' production but it is a time and money consuming operation, according to Gatti et al (2011) it can require 60 to 120 worker hr/ha depending on vine vigor, trellis type and design, equipment and skilled labor, which is difficult to find in many viticultural areas around the world. One way to reduce the labor costs with this operation is introducing a new technique, the mechanical pruning. The advantages of the mechanization are the reduction of the need of skilled manual labor, that sometimes it's difficult to find, improvement of work conditions, reduction of the time spent to prune a vineyard.

Another way to control yield is through fertilizers' application. A well-nourished vine will produce more grapes and with better quality. Along with this low production worry, the environmental concern lead to the use of organic amendments in vineyards, these provide not only the mineral nutrients required by the vine, but are also a source of organic matter to the soil and prevent soil erosion (Leita et al 1999). Organic matter is important to the soils' characteristics as it improves soil structural stability and water infiltration rate (allowing the water to reach the vine roots) (Goulet at al 2004). In some cases, by using organic amendments there is no need of application of a mineral fertilizer and separately apply organic matter, reducing the number of labor hours. Another advantage of using this type of fertilizer is the fact that it can be used in organic and biodynamic viticulture.

The present work is inserted in a ProDer project named Fertilpoda, measure 4.1 for "Cooperation for Innovation", and the objective is to compare the effect of simulated mechanical pruning (MEC) (as opposed to manual pruning) and organic amendments addition in wine quality. The experimental vineyards are located in three different regions of Portugal – Quinta do Gradil (Lisboa), Quinta do Côro (Tejo) and Herdade de Rio Frio (Setúbal). The organic amendments used were Cow Manure (ESTR), Sewage Sludge (ETAR), Municipal Solid Waste Compost (RSUC) and Grime (BIOC). The wines' quality was evaluated by the analysis of physicochemical parameters, analysis of wine color, phenolics composition, total nitrogen determination, mineral and heavy metal content and sensory analysis.

2. Bibliographic Review

2.1. Grapevine Pruning

2.1.1. Manual Pruning

Winter pruning is a cultural intervention that consists in the suppression of the canes, total or partial, in order to obtain a certain level of buds, usually it removes up to 95% of the preceding year wood (Magalhães 2008, Intrieri et al. 2011). When performed manually it allows retaining a specific number of nodes, the ones with the most potential to produce, in an optimal distribution (Zabadal et al. 2002). By removing most of the vegetative growth of the preceding year, this operation is designed to maintain vine shape and size, control bud and shoot number and, hence, bunch number and size (Intrieri et al. 2011). When the number of buds is too low the cane vigor increases and the yield decreases because the vegetative growth and the reproductive growth compete for available resources (Keller et al. 2004). When the number of buds is excessive the fertility is lower, the canes and bunches are lighter and the viability of the plants can be affected. Therefore, it is of extreme importance to achieve a balance between shoot and fruit growth in order to obtain a sufficient photosynthetic leaf area enough to ripen the berries to the desired level and to reload reserves in the permanent structure of the vine (Keller et al. 2004). In addition to the yield, pruning influences the quality of the harvest – excessive yield can cause maturation problems and creates the appropriate microclimate for the installation of fungal diseases (Magalhães 2008).

Traditionally, winter pruning was done manually. This operation is time-consuming and labor-intensive, it is the second biggest cost factor in vineyard operations being only inferior to harvest (Morris 2008). According to Intrieri et al. (2011) this process may require from 70 to 100 hr/ha of human labor, depending on training system. Archer and Van Schalkwyk (2007) referred that the labor costs of winter pruning can be responsible for over 60% of total annual costs of wine grape production in South Africa. In order to reduce this high cost, other techniques as mechanical pruning can be implemented. Morris (2008) concluded that machine farming of vines reduced costs from 44% up to 61% as compared to hand farming.

The mechanical techniques usually applied are hedge pruning or minimal pruning, non-selective techniques. Although effort has been made to develop robotic mechanical pruning, a selective technique close to manual pruning, mechanical pruning nowadays select areas of the vine to prune instead of specific canes to leave specific nodes. This means that mechanical pruning is often less precise and creates a less orderly vine structure than manual pruning (Zabadal et al. 2002). Another aspect that may condition the adoption of mechanical pruning is the inclination of the soil in which the vineyard is planted on, many areas in Portugal have high slopes that do not allow machine work.

2.1.2. Mechanical Pruning

2.1.2.1. Hedge Pruning

According to Gatti et al. (2011) the high labor demand caused by hand pruning can be reduced by 50 to 90% if mechanical pruning is adopted, the time savings depend on the extend of hand clean-up that follows the mechanical pruning. The suitability of a grapevine to mechanical pruning is function of numerous factors such as the vine capacity for yield self-regulation.

Mechanical pruning can be done fully or partially. Partial mechanical pruning consists in prune the vines using a pneumatic system that consists in a set of shears with a compressor mounted on a tractor, which has some inconvenient aspects as the need of a driver for the tractor (extra cost) and it implies that the workers prune the vines at the same speed. In alternative, this system can be adapted through the use of a static compressor at the top of the rows. Instead of the use of the pneumatic system, electric shears can be used. With the objective of improving the efficiency of manual pruning, a mechanical pre-pruning can be done in order to reduce the size of the canes and, consequently, making it easier to remove (Magalhães 2008). Total mechanical pruning consists in hedging the vines on top and on the sides, originating a box shaped grapevine. This type of pruning has some disadvantages, once the canes are not suppressed there is too much accumulation of old wood in the interior of the box shaped canopy, increasing its density which in turn can lead to a deficient sunlight exposure and high temperature, increasing the probability of fungal development.

In a study of the effects of mechanical hedge pruning on growth, yield and quality of Cabernet Sauvignon grapevines, Lopes et al. (2000) stated that at harvest both the control must (derived from manually pruned vines) and the one from mechanical pruned plants had similar levels of sugars, titratable acidity and skin anthocyanins content. However, in one of the three years of this study, the total phenols presented significant differences - manual pruning led to higher values. In this study, the authors concluded that mechanical pruning is a technique that can be used in this vineyard without great loss of quality in wines.

Vine growers are concerned that over cropping due to high node numbers retained on mechanical pruned vines might lead to reduction of grape quality and eventual vine decline (Keller et al. 2004). Mechanical hedging can have different levels of severity but according to Intrieri et al. (2011) the results of various studies have been satisfactory in terms of crop yield and quality. In Old World countries, there is an assumption that a higher node number left by hedge pruning, in comparison to hand pruning, would result in a higher yield which in turn can lead to a decrease in grape quality (Poni 2004). According to the same author, increasing node number per vine can lead to an increase in total effective leaf area which can counteract the yield increase, allowing to maintain similar quality. Similarly, the higher node number can reduce the vigor of the canes which generates more favorable conditions for sugar accumulation after veraison. Thus, mechanical pruning is a viable tool for identifying the yield threshold beyond which quality declines. In contrast, Gatti et al. (2011) reported that field trials on mechanical pruning have shown that some grape varieties are difficult to adapt to mechanical pruning, they easily tend to over crop leading to a loss in grape quality. Ideally, from an economic perspective, growers should try to maximize both the quantity and quality of the harvest at sustainable levels at the lowest possible cost (Keller et al. 2004).

2.1.2.2. Minimal Pruning

Minimal pruning of cordon trained vines (MPCT) was developed in Australia, in irrigated vineyards in warm climatic regions (Poni et al. 2000) and it consists in mechanical skirting of the vines to a certain level above the soil (Morris 2008). According to Morris (2008) MPCT increases yield and produces numerous small clusters with delayed fruit maturity, especially in the first year. In the second year, the yield decreases in response to the high crop load from the previous year. After three years, yield stabilizes and the composition of the grapes is similar to the other pruning methods.

In a practical point of view, this technique is promising for wine production because it is suitable for mechanical harvest, maximizes production and can be used as a tool for controlling excessive vigor (Clingeleffer 1988). Besides, it has a low severity only removing 10% or less of year-old wood allowing to decrease pruning costs by drastically curtailing labor demand to 5 to 10 hr/ha. MPCT trials all over the world have led to different conclusions, in general it is sensitive to environmental conditions and management regimes and it proves to be better adapted to warm climates and early to midseadon ripening varieties – environmental and cultural conditions (Intrieri et al. 2011).

Poni et al (2000) concluded that yields have increased by 20 to 40% compared to hand pruned vines, while ripening has been delayed by about one week with a similar grape composition being attained. MPCT has also been studied in cooler areas of Australia and the results were an increase in vine vigor and a shorter ripening season. In these climatic conditions, vineyards experienced overcroping and delayed or incomplete ripening which can be corrected by using other techniques to limit crop size (for example crop thinning). Nevertheless, minimally pruned vines normally have 3 to 10 times higher shoot number, smaller and less compact clusters with fewer and smaller berries and generally higher yields with slightly lower soluble solids concentrations (Poni et al. 2000). Clingeleffer and Krake (1992) stated that MPCT reduces berry size by up to 30% increasing the skin to pulp ratio which contributes to enhance wine quality through effects on color and flavor components.

2.1.3. Organic Amendments

According to Morlat and Chaussod (2008) natural organic restitutions in vineyards are low hence the soils tend to have low levels of organic matter. Besides, many vineyards have been maintained several years without floor vegetation and often without organic manure additions, which contributed to the decrease in the organic matter content of agricultural soils because organic carbon inputs are below the outputs. This reduces soil fertility and increases erosion (Leita et al. 1999).

Soil organic matter is important in agriculture for many reasons – it increases soil stability, pore size, aeration and water-holding capacity, it serves as a reservoir and source of soil nutrients providing sites for cation exchange and chelates, it assists in buffering soil pH and since generally it has dark color it helps increasing the rate of warming by the sun (Bugg and Van Horn 1988).

In a study of the effects of the addition of organic amendments in a vineyard in Loire Valley, Morlat and Chaussod (2008) stated that organic amendments affect specially two soil properties – bulk density and water content at field capacity. In this study, all organic treatments (pruned vine-wood, cattle manure and spent mushroom compost) significantly lowered bulk density, compared to the control (without amendments), the decrease was 6%. Soil moisture at field capacity increased because the water holding capacity of organic matter. The mineral content of the soil have also suffered some changes, with the application of these treatments the soil N and K concentrations increased. When the organic amendments were applied in high rates, the N content exceeded the plant requirements, increasing the risk of N leaching.

Cattle manure is the oldest organic amendment ever used. However, with the increase in global population the levels of waste have suffered an alerting increase and one way to give it a sustainable use is to utilize these wastes as soil amendments (Leita et al. 1999). Several wastes can be used as organic amendments – sewage sludge, municipal soil waste compost and biochar, for example.

2.1.3.1 Sewage Sludge (ETAR)

Sewage sludge is an organic residue that results from municipal wastewater treatment after aerobic or anaerobic digestion, and due to its high nutritional value they constitute a valuable amendment. The high level of organic matter improves some physical e chemical properties of the soil, resulting in a better plant growth. One aspect to consider before the application of this amendment is the fact that sewage sludge may contain high amounts of potentially toxic elements, which means that sewage sludge should be analyzed before being applied (Mendonza et al. 2006). According to Silveira et al. (2003) domestic sewage sludge tend to have lower heavy metal content than industrial ones. Heavy metals most commonly found in this type of organic amendment are lead (Pb), nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn).

Mendonza et al. (2006) pointed another aspect to consider in the use of sludges in agriculture, it is the distance between the place where sludges are produced and the agricultural area where it could potentially be applied. If the treatment station is far from the agricultural land where this amendment is to be used, the transport represents a higher cost.

In a study of the response to potassium fertilization of soil with long term application of sewage sludge, Miah et al. (1999) stated that the fertility of the soil increased over a long period of time. However, despite the fact that usually sludge contains considerable amounts of nitrogen (N), phosphorus (P) and potassium (K), this investigation showed that K deficiency became evident after 17 years of sewage sludge application.

As described before, this type of amendment alters soil properties which, in turn, are one of the main factors affecting the organoleptic characteristics of wine. Consequently, application of sewage sludge in a vineyard will probably affect the volatile organic composition of wine. Korboulewsky et al. (2004) found differences in the concentrations of volatile organic composition of wine in the first harvest after the application of the treatment. In this study, wines from plots with the highest rate of application of sewage sludge (90 t compost fresh wt/ ha) had the lowest volatile organic composts concentration with very low concentrations of fruity aroma compounds, which suggest a lower organoleptic quality. These wines had the highest animal aroma, which depresses olfactory quality and led to lower overall wine quality (lowest global mark in sensory analysis). In viticultural terms, sewage sludge in this study increased vine vigor presumably because of high input of mineral nitrogen. As a result, the maturation of grapes was delayed once nitrogen favors vegetative growth.

2.1.3.2 Cattle Manure (ESTR)

Cattle manure is the waste product resultant from the feces of bovines, their bleeding material, wasted feed and water.

This type of amendment is used since the beginning of agriculture and it can be used in every type of agricultural production – conventional, organic, and biodynamic. Clark et al. (1998) studied the soil chemical properties during the transition from conventional to organic and low-input farming practices. Inputs of carbon (C), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were higher in the organic and low-input systems as a result of manure applications and cover crop incorporations.

In another study, Ramos and López-Acevedo (2004) concluded that the use of this organic amendment indeed increased the organic matter content in the soil, although it caused an increase in the electric conductivity values up to limits that could affect vine yield. In addition, cattle manure also lead to a rise in zinc (Zn) levels which in some points of the studied area doubled to its original values after the first application. One aspect to take into account before the use of organic amendments is the rate of application, some of the Mediterranean farmers where the mechanization of fields is leading to poor soils, are applying the same amounts of cattle manure to disturbed and non-disturbed soils leading to an increase in Zn concentration between 60 and 100%.

Morlat (2008) conducted a trial over 28 years which consisted in studying the effect of long-term additions of organic amendments in a Loire Valley vineyard. One of the organic amendments used in this experiment was cattle manure, which over the years increased soil salinity – soluble salts tend to reduce soil quality and productivity once it decreases vineyards' photosynthetic capacity originating a decline in growth rate and crop yield. Since 1999 to 2004 cattle manure in a rate of 20 ton/ha/year provided the highest mineral nitrogen content that caused an increase in vigor, which in turn caused the delay in sugar accumulation in the berries decreasing total soluble solids, although titratable acidity wasn't altered. Despite the lower sugar content, wines made from plots amended whit cattle manure had a higher fermentation rate due to the high nitrogen level. These wines had the highest ethanol content and total K along with highest pH. According to the results presented by Morlat (2008) there was a decrease in color intensity shown by the results of sensory analysis and color measurements that is caused by a lower anthocyanin concentration. This suggests that these wines probably had lower aging potential. In terms of organoleptic analysis, these wines had lower astringency, intensity and after taste, higher herbaceous and animal odor. Morlat (2008) concluded his study by saying that if the winegrowers' goal is to produce high quality grapes to produce top-quality wines the application of these type of organic amendments, specially cattle manure, must be avoided and vine nutrition should be managed with a flexible approach always considering the environmental conditions in which the vineyard grows.

2.1.3.3 Municipal Solid Waste Compost (RSUC)

The quantity of municipal solid waste produced is increasing in parallel with the increasing size of population. The amount of sanitary landfills to dispose this biodegradable wastes is limited and the incineration of this waste produces significant quantities of greenhouse gases (GHG) and toxic solid residues. One way to utilize municipal soil waste is by composting which consists in accelerating the decomposition and stabilization of the biodegradable components for a sustainable recycling, producing compost that can be used as organic fertilizer (Moldes et el. 2007). RSUC is frequently promoted as an inexpensive and simple solution for a variety of agronomic, environmental and socio-economic problems (Fagnano et al. 2011).

RSUC diminishes the volume of waste, the high temperatures of the composting process kill pathogens that may be present, decreases germination of weeds in agricultural fields and destroys malodorous compounds. This material, which can be used in organic viticulture, is also gaining popularity due to its positive effect on soil properties (Hargreaves et al. 2008). Weber et al. (2007) and Weber et al. (2014) proved that RSUC application in the soil increases its porosity, improves water penetration, air circulation and water retention in soil. It also improves the stability of soil aggregates.

These benefits may be seen in agricultural land but this compost should only be applied after it has been analyzed and shown to be safe (Moldes et al. 2007). Weber et al. (2007) indicated that the addition of municipal solid waste compost increased heavy metal concentration in soil, in several cases, especially in sandy soils, because they usually form relatively insoluble species. In the other hand, compost application leads to soil enrichment of organic matter. This organic matter in mature composts indicates a high ability to bind the cations of heavy metals (ion exchange between the solid and solution phase of the soil or by the formation of coordinative complexes). This means that the environmental hazard related with the soil enrichment in metals depends on the concentration of mobile and plant-available forms instead of the total amount that the compost introduces in the soil. Consequently, the beneficial aspects should be assessed together with the potentially detrimental ones.

Hargreaves et al. (2008) reported that large amounts of RSUC are used to meet nitrogen requirements of the cultures and to add organic matter to the soil. The problem with this is that in some cases metals and excess nutrients can move through the soil contaminating the groundwater. In addition, this compost can have high salt concentrations that can inhibit plant growth and negatively affect soil structure. Another effect registered by these authors is that the use of this material may increase soil pH proportionally to the application rate, which can be an advantage when the soil has high acidity levels. The increase in pH can possibly be due to the mineralization of carbon which produces OH^- ions and introduces basic cations in the soil like K^+, Ca^{2+} and Mg^{2+}.

2.1.3.4 Grime (BIOC)

Biochar is the product that results from the thermal decomposition (pyrolysis) of organic material in a low oxygen medium under temperatures that can reach 700 °C. The difference between biochar and charcoal is only the intention of use once biochar is produced with the objective of being applied to the soil in order to improve soil productivity, carbon accumulation and filtration of percolating soil water (Lehman and Joseph 2015), it helps retain water and increases saturated hydraulic conductivity in the soils' top layers (Uzoma et al. 2011).

Baronti et al. (2014) studied the impact of biochar application on plant relations in Vitis vinifera (L.). They affirmed that the reduction of soil organic matter in agricultural soils due to production intensification and the use of chemical fertilizers has damaged the water holding capacity of soils, which can have negative effects on plants in terms of capability to adapt to climate change. The same authors stated that the predicted increase in extreme weather events probably will have negative effects on Mediterranean viticulture which will result in an increase in the areas affected by water deficit resulting in limitations to growth, irregular ripening and diminished berry quality.

In terms of soil quality, the application of biochar leads to significant changes in soil quality. Chan et al. (2007) studied the influence of the application of biochar on the soil quality by applying three rates of this amendment with and without additional nitrogen application. These authors registered an increase in soil pH and organic carbon content as well as exchangeable cations. This material also increases soils' field capacity.

Ameloot et al. (2005) investigated the effects of biochar on nitrogen mineralization and biological soil properties. These authors noted that soil nitrogen mineralization rates are affected by biochar, as it is a source of this element for plants. Lehman et al. (2006) concluded that biochar and its application to soil improve soil fertility and crop production.

The application of biochar is a sustainable agricultural practice which allows using local and renewable materials. In addition, this organic amendment represents an environmental friendly way to indirectly help in the mitigation of climate change by diminishing the greenhouse gases from landfills, decreasing industrial energy use due to recycling, producing bioenergy and decreasing the need of long-distance transport of waste (Lehman and Joseph 2015). By applying biochar into the soil farmers increase the consumption of atmospheric carbon dioxide in terrestrial ecosystems which decrease environmental pollution, since biochar has a relatively stable nature (Chan et al. 2007).

2.2. Objectives

The aim of the present work is to evaluate the viability of the applied techniques in future viticulture. This work was developed in the scope of the Fertilpoda: effect of different organic amendments on soil quality, vines growth, grape production and wine quality of mechanically pruned vineyards.

For that two techniques were applied – in one hand a comparison between manual and mechanical pruning was made. This comparison enlightens about the adaptability of the mechanical pruning in vineyards and its effects on Syrah wine quality.

In the other hand four different organic amendments were tested in contrast with the control – sewage sludge, cattle manure, municipal solid waste compost and biochar. The purpose of using these type of amendments is to assess its effects on grape and wine quality in order to apply them in vineyards, independent of which production regime is used (organic, biodynamic, conventional). In the process of choosing which organic amendment to use in a vineyard, it's necessary to consider that each vineyard may respond with some differences once it is planted in a different *terroir* which highly influences the characteristics of the resultant wine.

3. Materials and Methods

3.1. Experimental Design

This experiment was done in vineyards of the three companies mentioned before, with Syrah grapevines, and this is the 3rd year of data collecting and analysis. In Quinta do Côro the vineyard was planted with the 99R rootstock with 2,5m between rows and 1m between the vines in a row. In Quinta do Gradil the SO4 rootstock was utilized and the space between the rows is 2,6m and 1m between plants.

In Quinta do Côro and Quinta do Gradil the vineyards were divided into three blocks, each block had a portion of the vines pruned by hand and a portion pruned in way to simulate mechanical pruning. The canopy of the manually pruned vines is organized according to vertical shoot positioning, with bilateral Royat cordon in Quinta do Côro and unilateral in Quinta do Gradil. The mechanically pruned vines have free vegetation (sprawl).

Within each type of pruning technique, the vineyard was divided into five parcels - four with organic amendments and one without any type of amendment (Test). In Herdade de Rio Frio, since the vineyard is only 3 years old, the vines were all pruned by hand so only the organic amendment treatment was carried out. The next table synthetizes this information (Table 1).

Table 1 – Experimental Design

Company	Blocks	Pruning technique	Organic Amendment
Quinta do Gradil and Quinta do Côro	1, 2, 3	Manual pruning	TEST
			ETAR
			ESTR
			RSUC
			BIOC
	1, 2, 3	Mechanical pruning	TEST
			ETAR
			ESTR
			RSUC
			BIOC
Herdade de Rio Frio	1, 2, 3	Manual pruning	TEST
			ETAR
			ESTR
			RSUC
			BIOC

TEST- control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

In what pruning concerns, two types of pruning techniques were compared. The first one, the traditional way, is the manual pruning that consists in spur prune the vines leaving generally 12 buds per vine (six spurs, each with two buds) and the second one is the mechanical pruning in which the hedge pruning was done by a pruning machine leaving approximately 34 buds per vine.

The organic amendments were applied in each block, which means that for each one of them three repetitions were made. In order to apply the correct amount of amendment, the calculations for these applications were based on the supply of 5.000 kg/ha/year of organic matter to the soil. Thus, the quantities of each treatment were different and are described in table 2, along with a short characterization of these materials. In this study instead of using biochar, grime was used.

Table 2 – Quantities and characterization of the organic amendments applied

Organic Amendment	Rate of application (kg/ha/year)	Humidity (%)	Organic matter content (% dry weight)	Nitrogen content (g/kg dry weight)	pH
TEST	-	-	-	-	-
ETAR	31 300	78,0	72,7	65,0	9,6
ESTR	23 000	68,9	67,5	24,0	8,2
RSUC	15 500	27,3	40,9	20,1	9,2
BIOC	8 600	23,8	76,3	14,3	9,0

TEST- control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

3.2. Winemaking

Before the harvest, the grapes from the vineyards involved in this project were controlled, in order to access their quality and maturation stage. The parameters controlled in this phase were weight of a hundred berries (g), °Brix, Potential Alcohol Content (%), pH and Total Acidity (g tartaric acid/L).

When the grapes were at the ideal stage of maturation the manual harvest was performed and the grapes were transported to the experimental winery at Instituto Superior de Agronomia (Lisbon), where the fermentation took place. After the transport, a sample of grapes from each treatment was taken and analyzed – weight of a hundred berries, volume of must from those berries (mL), °Brix, Potential Alcohol Content, pH, Total Acidity, Total Anthocyanins (mg/L) and Total Phenols (a.u.).

Then, the grapes were de-stemmed, crushed and sulfur dioxide was added (50 mg/L). The crushed grapes were placed into 50 L stainless-steel tanks and inoculated with the yeast Zymasil Bayanus. In the day after these operations, a sample of must from each vineyard and treatment was taken to analyze °Brix, Potential Alcohol Content, pH, Total Acidity, Density and Temperature (°C).

The alcoholic fermentation lasted between 7 and 9 days at the average temperature of 24 °C, every day density and temperature were measured. The maceration was made by cap punching three times per day, assuring a major extraction of the color and astringency compounds into the wine. When the fermentation ended, the wine was transferred to another tank and the solids (skins and seeds) were pressed in a small vertical press.

When alcoholic fermentation ended, more samples were analyzed to determine Density, Temperature, pH, Total Acidity, Volatile Acidity, Total and Free Sulfur Dioxide (mg/L), Alcohol Content and Reducing Sugars (g/L).

The malolactic fermentation developed after the alcoholic fermentation, spontaneously, and it’s progression was controlled using the Paper Cromatography Method. In February, this process was ended for all the wines. In order to remove the residues that settled, the wines were racked and then a new analysis took place to control Total and Free Sulfur Dioxide, Volatile Acidity and pH. If the Sulfur Dioxide was too low, the corrections were made and then the wines were bottled.

After the bottling process, the wine’s chromatic characteristics, mineral and heavy metals content and sensory analysis were performed.

3.3. Physicochemical and Chromatic Characteristics Analysis

Physicochemical analysis has a major importance in enology for many reasons – it allows controlling the wine’s quality, the development of spoilage phenomena, improves the winemaking process, helps the blending technique and it’s a tool for certification of wines. It also allows the winemaker to see if their wines respect the legal limits of certain substances (Zoecklein et al. 1999).

In this case, it allowed the understanding on one hand of the influence of the pruning technique and in the other hand the influence of the organic amendments in wine composition and quality. These analyzes were made in the Enology Laboratory of Instituto Superior de Agronomia and the heavy metals specifically in Instituto Nacional de Investigação Agrária e Veterinária in a small town near Lisbon called Dois Portos.

All parameters were analyzed according to OIV recommended methods, except for Anthocyanins (total and ionized), Tannin Power, Color due to copigmentation, Total and Polymeric Pigments, Total Phenols and Non-Flavonoid Phenols - the procedures were the ones developed in published scientific articles.

a) °Brix

The measure of °Brix is performed in grapes and must only, during the maturation control and harvest period, it translates the amount of soluble solids per 100 g of must. Although there are many soluble solids, such as pigments and acids, the sugars are the biggest fraction therefore in general this parameter is considered as a measurement of sugar levels.

This determination is made through the use of a refractometer.

b) Potential Alcohol Content (%)

This parameter is determined through the measurement of density and specific gravity at 20 °C on the sample by areometry (OIV 2015).

c) pH

The wine's pH was measured according to the OIV method – determine the difference of potential between two electrodes immersed in the wine (OIV 2015).

d) Total Acidity (g tartaric acid/L)

This parameter should be controlled because it must be higher than 3,5 g/ L (IVV 2015).

Total acidity was determined by titration with bromothymol blue as indicator and compared with an end-color standard (OIV 2015).

e) Volatile Acidity (g acetic acid/L)

The volatile acidity provides information on the sanitary state of the grapes, of the winery and it allows controlling the conservation of a wine. As it is a quality factor, it has legal limits that must be respected – 1,2 g acetic acid/ L in red wines (IVV 2015). Normally, this parameter is expressed in acetic acid because of its importance but the volatile acidity includes all the steam-distillable acids present in wine such as lactic acid, formic, butyric and propionic acids. (Zoecklein et al. 1999).

This determination starts with the removal of carbon dioxide from the sample. Then, volatile acids are separated from the wine by steam distillation and titrated using sodium hydroxide (OIV 2015).

f) Sulfur Dioxide (mg/ L)

In wines, this compound can be in two forms – free and combined. The free sulfur dioxide is the form that has the beneficial properties – antiseptic, antioxidant and it inhibits enzyme activity.

Its addition must be carefully made because it has a legal limit of ≤ 150 mg/ L for red wines (IVV 2015).

The free sulfur dioxide is determined by direct titration with iodine while the combined one is subsequently determined by iodometric titration after alkaline hydrolysis. The total sulfur dioxide is the sum of free and combined sulfur dioxide (OIV 2015).

g) Alcohol content (% vol.)

The method used was the ebulliometry. The principle of this methodology is based upon the fact that the boiling point of a wine sample is lower than the one from pure water and higher than ethanol's. The difference between these temperatures is related to percent ethanol (Zoecklein et al. 1999).

h) Reducing Substances (g inverted sugar/ L)

This determination allows confirming if the alcoholic fermentation is over – if the wine has a concentration of these substances lower than 2 g/ L technologically it's considered dry.

The reducing substances are determined in two steps. In the first one - clarification – the reducing substance other than sugars are eliminated and in the second stage the sample is heated until the boiling point is achieved in order to accelerate their reduction in the presence of an alkaline solution of a copper salt, and then a titration of the excessive copper allows determining this parameter (OIV 2015).

i) Malolactic Fermentation

The malolactic fermentation is a catabolic pathway that enzymatically oxidizes malic acid.

The method used to monitor this process was paper chromatography developed by Ribéreau-Gayon (1953). The ascending chromatography on paper uses acetic-butanol as solvent with bromophenol blue and the concentration of malic acid is estimated by comparison with a standard scale prepared in the same conditions.

j) Wine color and phenolic composition

The wine color and phenolic composition of a wine give an idea of the wine's age, as well as what winemaking techniques were applied.

The wine's color is one of the first characteristics evaluated during sensorial analysis and influences the consumers' perception of the wine's quality.

A spectrophotometric method was applied to determine color intensity and shade, in order to obtain the absorbency values necessary to calculate these parameters – radiation measurements of wavelengths 420, 520 and 620 nm (OIV 2015).

The color intensity, I, is given by the following expression:

$$\mathrm{I}\ (\mathrm{a.u.}) = \mathrm{A}_{420} + \mathrm{A}_{520} + \mathrm{A}_{620}$$

The shade, N, is given by the following expression:

$$\mathrm{N} = \frac{A_{420}}{A_{520}}$$

j.i) Total phenols (u. a.)

The methodology used consisted in the measurement of the absorbency at wavelength 280 nm (A_{280}) of the diluted wine sample (Somers and Evans 1977).

The total phenols of the wine are given by the following expression:

$$\text{Total phenols} = \text{A}_{280} * \text{dilution factor}$$

j.ii) Nonflavonoid phenols (u.a.)

The total phenols of the wine can be divided in flavonoid phenols (catechins, epicatechins, flavonols, anthocyanins and condensed tanins) and nonflavonoid phenols such as phenolic acids (benzoic and cinamic) and their derivatives, stilbenes and other volatile phenols.

The used method is based on the absorbency measurement at 280 nm wavelength of the sample before and after the precipitation of the flavonoids through a reaction with formaldehyde under certain conditions (low pH, room temperature) (Kramling and Singleton 1969).

j.iii) Anthocyanins (mg/ L)

Anthocyanins are red pigments, responsible for the red wine color, present in the skin of the grapes and also in the flesh of the "*teinturier*" grape varieties. These molecules can be in the aglycone form (anthocyanidin) or in a more stable glycoside form (anthocyanin). Their structure is the flavylium cation that includes two benzene rings bonded by an unsaturated cationic oxygenated heterocycle (Ribéreau-Gayon et al. 2006).

Some anthocyanins are present in wine in colorless forms, the ones responsible for the color are the ionized anthocyanins. The total anthocyanin content and ionized anthocyanin content were determined using the methodology developed by Somers and Evans (1977), by spectrophotometry in a cuvette with a 10 mm path length, and the expressions to the calculations are:

$$\text{Total anthocyanins (mg/L)} = 20 * (\text{A}_{520}^{\text{HCl}} - \frac{5}{3}\text{A}_{520}^{\text{SO}_2})$$

$$\text{Ionized anthocyanins (mg/L)} = 20 * (\text{A}_{520} - \text{A}_{520}^{\text{SO}_2})$$

$$\text{Degree of ionised anthocyanins (\%)} = \frac{\text{A}_{520} - \text{A}_{520}^{\text{SO}_2}}{\text{A}_{520}^{\text{HCl}} - (\frac{5}{3}\text{A}_{520}^{\text{SO}_2})}$$

j.iv) Total Pigments (u.a.)

The total pigments are the sum of a wide variety of molecules such as phenolic compounds (such as flavonoids), anthocyanins and associations between them.

The total pigments are calculated through the methodology described by Boulton (1999) based on the following expression:

$$\text{Total pigments (u.a.)} = \text{A}_{520}^{\text{HCl}} \times 101$$

j.v) Polymeric Pigments (u.a.)

The polymeric molecules comprehend associations of anthocyanins and tannins, polymeric tannins such as procyanidins. These substances contribute to the color, body and astringency of wine. The polymeric pigments are essentially polymerized anthocyanins and polymers of anthocyanins and condensed tannins. In young wines, the red color is also intensified by those anthocyanin polymers. This determination is carried out by the methodology described by Boulton (1999) and the result is calculated through the expressions:

$$\text{Polymeric Pigments (u.a.)} = \mathrm{A}_{520}^{\mathrm{SO_2}} \times 10$$

$$\text{Polymerization Index (\%)} = \frac{A_{520}^{SO_2}}{A_{520}^{HCl}}$$

j.vi) Color due to copigmentation (u.a.)

The copigmentation is a molecular association between pigments (anthocyanins) and other organic molecules (copigmentation cofactors) in solution and it causes the intensification in the color of the wine. The copigmentation can happen between anthocyanins (self-association) or between anthocyanins and other cofactors. This phenomena is usual in young red wines (Boulton 2001).

The method used was the one described by Boulton et al. (1999) and the expression used to calculate the results are as follows:

$$\text{Color due to copigmentation (u.a.)} = A_{520}^{a} - A_{520}^{b}$$

Where:

$\mathrm{A}_{520}^{\mathrm{a}}$: Absorbency of the wine sample with acetaldehyde after 45 min waiting.

A_{520}^{b}: Absorbency of the wine sample and acetaldehyde mixture diluted in a hidroalcoholic solution adjusted to the wine's pH after 45 min waiting.

j.vii) Tannin Power (NTU/ mL)

Polyphenols are a group of molecules responsible for the astringency of the wine, especially the polymerized tannins. The astringency is the result of the interaction between tannins and salivary protein molecules by cross linking resulting in the formation of aggregates that precipitate reducing the palate lubrication by decreasing the viscosity of saliva. These aggregates cause the friction of tissues in the mouth originating the sensation of dryness or roughness (Bajec and Pickering 2008).

This parameter was determined by the method developed by Freitas and Mateus (2001), which measures the turbidity caused by the aggregates of tannins and proteins by nephelometry (nephelometer Hach 2100N), after adding BSA (bovine serum albumin) to cause the precipitation. The expression used to calculate the results is:

$$\text{Tannin Power } (NTU/mL) = \frac{d - d_0}{0{,}08}$$

Where:

d: Turbidity of the diluted wine sample (wine and hidroalcoholic solution adjusted to the pH 3,2).

d_0: Turbidity of the diluted wine sample added with BSA after 45 min waiting.

3.4. Total Nitrogen Content

The nitrogen composts of wine can influence fermentation, clarification and potential microbial instability (Zoecklein et al. 1999).

The total nitrogen includes various organic forms (such as amino acids, proteins, pyrazines) and one inorganic form (ammonia salts). Other forms of nitrogen include urea, ethyl carbamate and bioamines (Ribéreau-Gayon et al. 2006).

The nitrogen content of a must and wine is influenced by many factors for example the fertilization of the vineyard, the type of soil, grape variety and harvest maturity (Zoecklein et al. 1999). Since the treatments applied to fertilize the vineyards had different nitrogen concentration this determination helps understand their influence in wine's quality.

The principles of the OIV method used consists in wet ash the wine sample using sulfuric acid in the presence of a catalyst and then titrimetrically determine the ammonia liberated with sodium hydroxide (OIV 2015).

3.5. Mineral Analysis and Heavy Metals

The potassium and calcium concentrations were also determined because of its interference with the stabilization of wines. One of the most common problems is the potassium bitartrate and calcium tartrate precipitation which are natural phenomena in wine evolution that cause no threat to human health but can influence the consumer's perception on wine quality (Ribéreau-Gayon et al. 2006).

These determinations were made directly on diluted wine samples by atomic absorption spectrophotometry after the addition of an ionization suppression agent (cesium chloride for potassium).

Iron and copper are heavy metals present in wines in small concentrations but they are responsible for some instabilities - ferric casse and copper casse. The second instability is more common in white wines. The iron content of the wine is an important determination because

ferric ion may react with phenols in red wines originating a soluble complex that causes an increase in color intensity, significant in young wines (Ribéreau-Gayon et al. 2006).
The methodology used for these determinations is atomic absorption spectrophotometry, in the iron case it's necessary to dilute the wine sample and remove it's alcohol by reducing the sample's volume to half of its original size (OIV 2015).

The heavy metals present in wines include a wide range of elements such as Pb, Mn, Zn, Ni, among others. They are naturally present in the environment at low concentrations, but in case of contamination they become toxic at higher concentrations (Ribéreau-Gayon et al. 2006). Some elements, besides their toxicity, have legal limits that must be respected, so in order to quantify the presence of heavy metals in the wines the ICP-MS (inductively coupled plasma-mass spectrometry) was applied and the semi-quantitative method was used (Catarino et al. 2006).

3.6. Sensorial Analysis

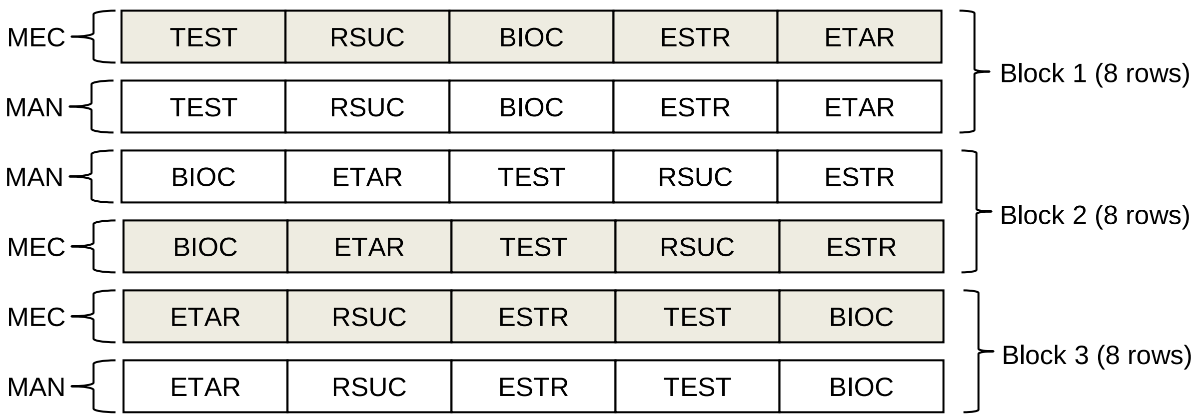
The sensorial analysis allows identifying and appreciating a wine's characteristics through the sensorial organs of the human body. This analysis can be helpful in the understanding of the consumer's tendencies, and in this case it made possible to realize if the pruning technique and organic amendment used in a certain sample had any influence in the organoleptic characteristics of the wine.
The analysis was done in a standardized environment, the samples were randomly coded with three different numbers and tasted by 11 trained panelists, professionals of the area.
The tasting sheet was suggested by the Laboratory Ferreira da Lapa (Enology Department) of Instituto Superior de Agronomia, and had two scales according to which the sample was scored. The attributes to punctuate were color (red or violet), aroma (fruity, floral, herbal, jam, intensity and equilibrium which includes the interaction between aroma and taste) and taste (body, bitter, astringency, persistency and equilibrium) and finally the global appreciation. The tasting sheet used is presented in the annex nº 3.

3.7. Statistical Analysis

The statistical analysis allows understanding if the values obtained before are significantly different in order to recognize if the pruning techniques, organic amendments and interactions between them affected wine quality.
Before the vinification, the results were obtained through an average of samples from the three blocks from each vineyard (Figure 1), analyzed separately. For each block the analytical

procedures were repeated three times. At the vinification, the grapes from the three blocks from each vineyard were mixed and fermented together which means that the results are an average of three repetitions in the laboratorial determinations, except for the ones before bottling (only one measurement was carried out) and the heavy metals that result from two repetitions.

This analysis was made using the software Statistica 6.0 and all the treatments were analyzed by a variance test - ANOVA with the Tuckey test, consisting in a split-plot with 3 repetitions (in most cases), in which the main factor is the pruning technique and the secondary factor is the organic amendment. The p value was used to determine statistic differences between each treatment and the symbol * shows the significance of that difference – * is equal and below 0.05, ** is below 0.001, *** is below 0.0001. When no significant differences were found the letters “ns” were used.



MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Figure 1 – Experimental Design

4. Results and Discussion

This section of the work presents the results of all the analyzes performed since the harvest. In order to facilitate the data presentation and discussion the results were grouped by chronological order – first the grape samples collected at harvest and grape must analysis then the physicochemical analysis, followed by the chromatic characteristics, mineral and heavy metal analysis and in the end the sensorial analysis of the wines.

4.1. Grape and Must Analysis

At harvest samples were taken and analyzed. The harvest at Quinta do Côro was on 8th September 2014. In the three vineyards the grapes were hand harvested and the samples taken from each block were analyzed, the results are presented in the next tables (Table 3 - 5). The absolute values of the analyzed parameters have some differences and only in total acidity mechanical pruning had the highest values, in general. The organic amendments did not influence these parameters in a significant way with one exception – ETAR globally decreased probable alcohol content.

Table 3 – Grape analysis of Quinta do Côro Block 1

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN TEST	167.0	25.7	15.1	3.52	5.10	1512.3	59.1
MAN ETAR	176.4	23.4	13.8	3.48	5.40	1132.3	46.8
MAN ESTR	187.9	24.0	14.1	3.51	5.10	1335.4	54.1
MAN RSUC	176.6	23.6	13.9	3.49	5.30	1201.5	47.2
MAN BIOC	179.8	23.6	13.9	3.46	5.00	1190.8	45.9
MEC TEST	149.5	23.7	13.9	3.41	5.00	1353.9	54.2
MEC ETAR	147.4	20.3	11.9	3.34	5.40	1043.1	41.3
MEC ESTR	147.1	21.5	12.6	3.36	5.40	1318.5	52.3
MEC RSUC	166.9	22.3	13.1	3.41	5.30	1109.2	43.3
MEC BIOC	147.6	24.0	14.1	3.40	5.10	1230.8	49.7

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 4 – Grape analysis of Quinta do Côro Block 2

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN TEST	168.5	24.4	14.4	3.47	4.80	1646.2	62.4
MAN ETAR	174.5	23.8	14.0	3.66	4.80	1418.5	53.2
MAN ESTR	183.2	23.5	13.8	3.46	4.80	1483.1	56.9
MAN RSUC	187.1	23.7	13.9	3.71	5.30	1864.6	72.6
MAN BIOC	156.0	25.2	14.8	3.65	5.00	2375.4	95.5
MEC TEST	165.3	21.6	12.7	3.36	5.10	1155.4	44.8
MEC ETAR	131.3	20.3	11.9	3.54	5.90	893.9	33.3
MEC ESTR	164.8	19.3	11.4	3.34	5.70	884.6	34.4
MEC RSUC	168.3	19.9	11.7	3.50	5.90	878.5	34.9
MEC BIOC	126.4	22.5	13.2	3.43	5.60	1529.2	58.2

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 5 – Grape analysis of Quinta do Côro Block 3

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN TEST	182.4	22.6	13.3	3.49	5.00	111.7	40.7
MAN ETAR	187.8	22.4	13.2	3.52	5.00	1350.8	52.2
MAN ESTR	170.8	24.5	14.4	3.54	4.70	1295.4	48.2
MAN RSUC	164.1	24.1	14.2	3.68	4.50	1409.2	54.0
MAN BIOC	175.1	23.3	13.7	3.46	5.30	1160.0	44.4
MEC TEST	150.99	22.6	13.3	3.41	4.80	1267.7	47.3
MEC ETAR	162.1	19.1	11.2	3.41	5.00	790.8	30.8
MEC ESTR	163.8	20.6	12.1	3.40	6.20	932.3	36.4
MEC RSUC	144.7	20.6	12.1	3.50	5.30	1150.8	44.2
MEC BIOC	164.9	21.9	12.9	3.40	5.30	969.2	37.0

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

In Quinta do Gradil the harvest was on 15th September 2014, the results from the berries' analysis at this stage are presented below for the three blocks (Tables 6 - 8). When considering the absolute values, manual pruning led to heavier berries with higher sugar levels, which translated in higher potential alcohol content.

Table 6 – Grape analysis of Quinta do Gradil Block 1

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN TEST	244.3	21.8	12.8	3.51	6.00	1261.5	51.8
MAN ETAR	250.4	19.8	11.6	3.47	6.00	1035.4	26.5
MAN ESTR	241.3	20.4	12.0	3.44	6.00	840.0	29.8
MAN RSUC	234.5	21.7	12.8	3.43	6.90	1090.8	36.8
MAN BIOC	221.2	21.3	12.5	3.42	6.20	1053.9	39.0
MEC TEST	164.8	20.2	11.9	3.32	6.00	1112.3	43.6
MEC ETAR	202.4	18.7	11.0	3.35	6.00	1126.2	30.7
MEC ESTR	191.9	19.7	11.6	3.33	6.00	790.8	32.4
MEC RSUC	167.4	19.8	11.6	3.38	6.00	918.5	34.5
MEC BIOC	154.1	19.4	11.4	3.37	5.60	1264.6	39.2

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 7 – Grape analysis of Quinta do Gradil Block 2

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN TEST	194.5	21.7	12.8	3.45	5.30	835.4	29.0
MAN ETAR	241.1	19.1	11.2	3.35	6.90	644.6	22.3
MAN ESTR	248.5	20.0	11.8	3.52	5.60	475.4	16.2
MAN RSUC	243.5	20.7	12.2	3.58	6.00	447.7	17.3
MAN BIOC	223.5	19.9	11.7	3.53	6.50	535.4	19.4
MEC TEST	184.1	21.5	12.6	3.40	5.90	324.6	12.5
MEC ETAR	219.1	19.1	11.2	3.29	6.60	616.9	23.4
MEC ESTR	205.1	20.2	11.9	3.39	6.00	1069.2	41.3
MEC RSUC	195.2	19.6	11.5	3.57	5.60	967.7	35.1
MEC BIOC	191.6	19.9	11.7	3.36	6.80	849.2	32.0

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 8 – Grape analysis of Quinta do Gradil Block 3

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN TEST	226.0	20.6	12.1	3.46	6.00	1358.5	49.6
MAN ETAR	246.3	20.4	12.0	3.41	6.50	383.1	13.3
MAN ESTR	237.9	20.7	12.2	3.47	6.30	156.9	5.0
MAN RSUC	242.3	19.2	11.3	3.36	7.10	783.1	30.5
MAN BIOC	224.5	20.0	11.8	3.40	6.60	364.6	12.3
MEC TEST	189.2	19.2	11.3	3.35	6.20	392.3	13.3
MEC ETAR	224.6	19.3	11.4	3.37	6.50	292.3	11.5
MEC ESTR	193.3	20.4	12.0	3.39	5.90	680.0	27.2
MEC RSUC	199.8	19.1	11.2	3.31	6.30	392.3	14.9
MEC BIOC	186.8	20.3	11.9	3.37	6.60	1127.7	46.6

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

In Herdade do Rio Frio the hand harvest was on 22nd September 2014. The values presented in the following table (Table 9) are from the berries' sample taken at that operation. In this case, contrary to the other two vineyards, the organic amendment that resulted in higher values was ETAR.

Table 9 – Grape analysis of Herdade do Rio Frio

Treatment	Weight of 100 berries (g)	°Brix	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
TEST	177.0	18.8	11.1	3.69	4.40	529.23	25.0
ETAR	172.1	19.4	11.4	3.72	4.40	716.92	35.3
ESTR	177.3	18.9	11.1	3.52	4.10	641.54	31.1
RSUC	188.0	19.8	11.6	3.68	4.20	595.38	29.3
BIOC	167.9	19.6	11.5	3.69	3.90	384.62	18.8

TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Statistical Analysis of Harvest Result's

Table 10 - Statistical Analysis of Quinta do Côro Grape Samples

Treatment	Weight of 100 berries (g)	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN	175.3	14.0	3.54	4.98	1365.8	55.5
MEC	154.2	12.6	3.41	5.37	1101.5	42.8
Sig.	***	***	***	**	ns	**
TEST	163.4	13.8a	3.44	4.95	1174.5	51.4
ETAR	162.9	12.7b	3.49	5.23	1104.9	42.9
ESTR	169.3	13.1ab	3.44	5.30	1208.2	47.1
RSUC	170.6	13.2ab	3.55	5.23	1269.0	49.4
BIOC	157.2	13.8a	3.47	5.18	1409.2	55.1
Sig.	ns	*	ns	ns	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 11 - Statistical Analysis of Quinta do Gradil Grape Samples

Treatment	Weight of 100 berries (g)	Potential Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Total Anthocyanin (mg/L)	Total Phenols (a.u.)
MAN	233.8	12.1	3.45	6.24	751.1	26.6
MEC	191.5	11.6	3.37	6.11	795.0	29.2
Sig.	ns	ns	*	ns	ns	ns
TEST	199.9c	12.3a	3.42	5.88	880.8	33.3
ETAR	231.4a	11.4b	3.37	6.40	683.1	21.3
ESTR	218.5ab	11.9ab	3.42	5.95	668.7	25.3
RSUC	213.3bc	11.8ab	3.44	6.30	766.7	28.2
BIOC	200.2c	11.8ab	3.41	6.35	865.9	31.4
Sig.	***	*	ns	ns	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

In both cases, the berry weight was higher in grapes from manually pruned vines compared to the ones from mechanically pruned plants (Table 10 and 11), the same tendency was registered in last year a analysis (Kaushal 2014). This difference might be due to the smaller number of buds retained by the manual pruning (12 buds/vine) as opposed to mechanical pruning (34buds/vine), similar results were obtained by Gatti et al (2011). Wessner and Kurtural (2013) concluded the same in a study of the interaction between pruning systems and canopy management practices with yield and fruit composition of Syrah. These authors concluded that manual (spur) pruning reduced yield and number of clusters harvested but berry and cluster weights were increased when compared to other pruning techniques that retained more buds, as the mechanical pruning. The organic amendment that led to a significant increase in berry weight in Quinta do Gradil was ETAR, this can be explained by its higher level of nitrogen that according to Magalhães (2008) can increase productivity once it enhances bud burst and also berry and cluster size.

The potential alcohol content was higher when manual pruning was applied, the same result was found by Zabadal et al. (2002) in a study which aimed to evaluate the strategies for pruning and crop control of Concord grapevines. The lower values of grapes from vines mechanically pruned can be explained by the increased canopy density which leads to reduced sunlight exposure of berries as the number of leaves proximate to fruits increase, their size is reduced and the vine assimilation is lower at ripening. In what organic amendments are concerned, the plots where BIOC was applied and the control ones have the higher absolute values of potential alcohol content in Quinta do Côro and Quinta do Gradil, while ETAR led to lower values. The same results were found in last harvest data (Kaushal, 2014).

When mechanical pruning was applied in the vines, the grapes had lower pH in both vineyards while results for total acidity are not conclusive although it is possible to say that Quinta do Gradil had higher total acidity values than Quinta do Côro, which can be explained by the lower temperatures in that area. There was no organic amendment that significantly influenced either of these parameters. The lower berry pH values in mechanically pruned vines can be explained by the higher shade levels in bunch zone (Archer and Shalkwyk 2007).

Total anthocyanin and total phenols content were not significantly affected either by pruning technique or organic amendment applied. Intrieri et al. (2011) in a study of the semi-minimal-pruned hedge technique found that total anthocyanin content was higher in these grapevines as compared to the control ones, similar to what happened in Quinta do Gradil and to the data recorded by Wessner and Kurtural (2013), however this is contradictory to what happened in Quinta do Côro where manual pruning led to grapes with higher total anthocyanin content.

After analyzing the berries from each block, they were mixed and fermented together. The must analysis is presented in the next tables (Table 12 – 14). In general, manual pruning led to higher values in probable alcohol content (which is determined through °Brix) and pH but lower results in total acidity. The organic amendment responsible for the inferior values of probable alcohol content was ETAR, except in Herdade de Rio Frio. BIOC led to higher pH values in Quinta do Gradil and Herdade de Rio Frio and ETAR increased total acidity results in Quinta do Côro and Quinta do Gradil.

Table 12 - Must Analysis of Quinta do Côro

Treatment	°Brix	Probable Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)
MAN TEST	23.9	14.1	3.46	5.00
MAN ETAR	22.9	13.5	3.46	5.90
MAN ESTR	23.6	13.9	3.44	5.40
MAN RSUC	23.4	13.8	3.52	5.40
MAN BIOC	24.1	14.2	3.46	5.10
MEC TEST	22.3	13.1	3.35	5.30
MEC ETAR	18.6	10.9	3.36	5.70
MEC ESTR	20.1	11.8	3.34	5.40
MEC RSUC	20.6	12.1	3.37	5.40
MEC BIOC	22.9	13.5	3.36	5.40

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 13 - Must Analysis of Quinta do Gradil

Treatment	°Brix	Probable Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)
MAN TEST	22.1	13.0	3.56	5.70
MAN ETAR	21.1	12.4	3.53	6.20
MAN ESTR	21.8	12.8	3.58	5.90
MAN RSUC	21.9	12.9	3.59	6.00
MAN BIOC	21.3	12.5	3.58	6.20
MEC TEST	21.4	12.6	3.48	5.70
MEC ETAR	19.9	11.7	3.50	6.50
MEC ESTR	20.4	12.0	3.52	6.00
MEC RSUC	19.8	11.6	3.51	6.00
MEC BIOC	20.6	12.1	3.54	5.90

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 14– Must Analysis of Herdade de Rio Frio

Treatment	°Brix	Probable Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)
TEST	19.3	11.4	3.47	4.50
ETAR	20.8	12.2	3.45	4.80
ESTR	20.9	12.3	3.51	5.30
RSUC	19.4	11.4	3.47	4.80
BIOC	20.2	11.9	3.56	4.20

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Statistical Analysis of Must Result's

Table 15 – Statistical Analysis of Quinta do Côro Must Results

Treatment	Probable Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)
MAN	13.9	3.47	5.34
MEC	12.3	3.36	5.43
Sig.	*	***	ns
TEST	13.6	3.41	5.10 b
ETAR	12.2	3.41	5.78 a
ESTR	12.9	3.39	5.40 ab
RSUC	12.9	3.45	5.40 ab
BIOC	13.8	3.41	5.25 ab
Sig.	ns	ns	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 16 - Statistical Analysis of Quinta do Gradil Must Results

Treatment	Probable Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)
MAN	12.7	3.57	5.97
MEC	12.0	3.51	6.00
Sig.	**	**	ns
TEST	12.8	3.52	5.70
ETAR	12.1	3.52	6.30
ESTR	12.4	3.55	5.93
RSUC	12.3	3.55	6.00
BIOC	12.3	3.56	6.00
Sig.	ns	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Statistical analysis show significant differences between pruning techniques in probable alcohol content and pH in both cases (Tables 15 and 16). Manual pruning led to higher probable alcohol content and pH values, the same results were achieved by Kaushal (2014), in last years' analysis. The same tendency was observed in the grapes collected at harvest for both wineries. Quinta do Gradil has the more elevated pH values while Quinta do Côro has the lowest. The probable alcohol content in Quinta do Côro is the highest and in Herdade de Rio Frio the lowest values were registered, a similar trend to what was observed in harvest samples' analysis.

Total acidity was higher when mechanical pruning was applied in both cases, the samples at harvest show an opposite result in Quinta do Gradil. Herdade de Rio Frio registered lower total acidity values and Quinta do Gradil has the higher values, once the vineyards are in a cooler area.

Assimilable nitrogen content was determined for Quinta do Gradil and Herdade de Rio Frio must (Annex 1). The values are all in a close range except for the must from plots treated with ETAR, which registered the higher value. Mendes (2014) in a study of the possibility of valorization of sewage sludge as an organic amendment for agriculture and Miah et al. (1999) in a study of the effects of long-term application of this type of product stated that ETAR has considerable amounts of nitrogen, among other plant essential nutrients. However, all values are below the technological level considered by Bell and Henschke (2005) of 140 mg N/L – a lower concentration increases the risk of a slow or stuck fermentation.

4.2. Physicochemical Analysis of the Wines

After the alcoholic fermentation, sulfur dioxide was not added to the wines in order to allow the development of lactic acid bacteria (without the need of inoculation). The first wines to complete the malolactic fermentation were the ones from Herdade de Rio Frio (ended in October), then the wines from Quinta do Gradil (November) and finally the ones from Quinta do Côro (January and February). There are some factors that combined influence the development of these microorganisms – pH, temperature, sulfur dioxide and ethanol. The determining factor in this case was the alcohol content, the wines with lower ethanol concentration ended malolactic fermentation before the others.

After this reaction, the physicochemical characteristics of the wines were analyzed before the bottling process, in order to monitor the alcohol content, pH, total acidity, volatile acidity and sulfur dioxide levels (the last one was corrected when necessary). Unlike last years' results (Kaushal 2014), the alcohol content of the wines is lower – no more than 14,7% v/v and manual pruning led to higher alcohol, as before. Volatile acidity and pH are higher when manual pruning was applied but total acidity registered higher values in wines from mechanical pruned vines. The results are presented in the following tables (Table 17 – 19), in Quinta do Côro no organic amendment significantly influenced the analyzed parameters while in Quinta do Gradil pH was the only parameter that registered differences between pruning techniques and organic amendments application.

Table 17 – Physicochemical Analysis of Quinta do Côro Wines

Treatment	Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Volatile Acidity (g acetic acid/L)	Free Sulfur Dioxide (mg/L)	Total Sulfur Dioxide (mg/L)
MAN TEST	14.7	3.54	6.15	0.41	45	75
MAN ETAR	14.0	3.42	6.30	0.29	29	68
MAN ESTR	14.3	3.52	6.60	0.32	43	78
MAN RSUC	14.3	3.56	6.30	0.31	50	88
MAN BIOC	15.2	3.44	6.75	0.39	13	43
MEC TEST	14.2	3.30	7.20	0.26	42	85
MEC ETAR	11.0	3.18	7.05	0.27	34	68
MEC ESTR	12.2	3.26	6.90	0.28	28	65
MEC RSUC	13.2	3.30	7.20	0.28	43	75
MEC BIOC	14.0	3.25	6.90	0.23	39	75

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 18 - Physicochemical Analysis of Quinta do Gradil Wines

Treatment	Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Volatile Acidity (g acetic acid/L)	Free Sulfur Dioxide (mg/L)	Total Sulfur Dioxide (mg/L)
MAN TEST	13.2	3.92	5.9	0.46	50	78
MAN ETAR	12.2	3.88	6.2	0.48	48	80
MAN ESTR	12.8	3.90	6.0	0.42	49	73
MAN RSUC	13.4	3.88	6.2	0.51	45	60
MAN BIOC	12.8	3.81	6.2	0.42	46	68
MEC TEST	13.1	3.70	6.0	0.49	48	78
MEC ETAR	11.6	3.79	6.0	0.42	47	80
MEC ESTR	12.2	3.72	6.0	0.36	53	95
MEC RSUC	11.8	3.64	6.2	0.49	41	60
MEC BIOC	13.3	3.75	6.5	0.50	39	70

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 19 - Physicochemical Analysis of Herdade de Rio Frio Wines

Treatment	Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Volatile Acidity (g acetic acid/L)	Free Sulfur Dioxide (mg/L)	Total Sulfur Dioxide (mg/L)
TEST	11.4	3.73	5.3	0.49	32	90
ETAR	12.2	3.74	5.4	0.46	34	100
ESTR	12.4	4.02	5.4	0.54	32	78
RSUC	11.4	3.81	5.3	0.48	35	75
BIOC	12.0	4.00	5.0	0.49	36	70

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Statistical Analysis of Physicochemical Parameters' Results

Table 20 - Statistical Analysis of Quinta do Côro Physicochemical Results

Treatment	Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Volatile Acidity (g acetic acid/L)
MAN	14.5	3.50	6.42	0.34
MEC	12.9	3.26	7.05	0.26
Sig.	*	***	**	*
TEST	14.5	3.42	6.68	0.34
ETAR	12.5	3.30	6.68	0.28
ESTR	13.3	3.39	6.75	0.30
RSUC	13.8	3.43	6.75	0.30
BIOC	14.6	3.35	6.83	0.31
Sig.	ns	ns	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 21 – Statistical Analysis of Quinta do Gradil Physicochemical Results

Treatment	Alcohol Content (%)	pH	Total Acidity (g tartaric acid/L)	Volatile Acidity (g acetic acid/L)
MAN	12.9	3.88	6.06	0.46
MEC	12.4	3.72	6.12	0.45
Sig.	ns	**	ns	ns
TEST	13.2	3.81	5.93	0.47
ETAR	11.9	3.84	6.08	0.45
ESTR	12.5	3.81	6.00	0.39
RSUC	12.6	3.76	6.15	0.50
BIOC	13.1	3.78	6.30	0.46
Sig.	ns	ns	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

In Quinta do Côro and Quinta do Gradil no significant difference was found in alcohol content of the wines from plots treated with different organic amendments (Tables 20 and 21), however comparing the absolute values it's possible to affirm that ETAR led to wines with less alcoholic content. This can be explained by the fact that ETAR has considerable levels of nitrogen which increases vine vigor from the plots treated with this organic amendment (Morlat and Symoneaux 2008), which diminished the clusters exposition to sunlight especially when mechanical pruning was applied. Schreiner et al. (2013) studied the impact of nitrogen, potassium and phosphorus supply on vine nutrient status, growth physiology and yield in Pinot Noir grapevines. These authors concluded that nitrogen is the most important nutrient to manage in vineyards once it has a great impact on vine vegetative and reproductive growth. Excessive nitrogen supply diminishes berry quality due in part to increase shading of clusters which can cause maturity problems. In Quinta do Gradil the type of pruning did not influence this parameter but in Quinta do Côro wines from manual pruning registered higher alcohol content. Similarly to what happened with must, the wines from Herdade de Rio Frio have the lower alcohol content.

Manual pruning led to wines with higher pH values, the same results were achieved by Kaushal (2014) in last year's analysis, this can be explained by the higher potassium concentration in wines from plots where this technique was applied. Once more organic amendments did not have any influence with statistical relevance. The wines from Quinta do Côro have the lowest pH values while the ones from Herdade de Rio Frio have the highest ones. Quinta do Gradil and Herdade de Rio Frio have the higher pH values that can be explained by the fact that potassium concentration in these wines is higher than in Quinta do Côro (Table 28). Davies et al (2006) stated that although potassium is an essential nutrient for grapevines, it's accumulation in berries at harvest may reduce wine quality particularly when it comes to red wines. This reduction in wine quality is due to the fact that potassium combines itself with tartaric acid resulting in potassium bitartrate, a stability problem that occurs during winemaking and storage. Besides this precipitation, pH increase leads to negative impacts on wine color and flavor and microbiological stability.

In what volatile acidity is concerned no significant difference was found and the tendency varied according to each winery – in Quinta do Côro the plots without any amendment led to higher volatile acidity values, in Quinta do Gradil was RSUC and in Herdade de Rio Frio was ESTR. According to Bell and Henschke (2005) high must yeast assimilable nitrogen leads to an increase in biomass and higher maximum heat output due to a greater fermentation rate and increases the formation of acetic acid and volatile acidity. In our experiment in Herdade de Rio Frio, the organic amendment that increased nitrogen content did not led to the higher volatile acidity values, even after malolactic fermentation that always increases acetic acid concentration.

Total acidity registered similar values in both wineries, the higher total acidity belongs to wines from plots pruned mechanically, the same tendency as last year (Kaushal 2014). Herdade de Rio Frio has the wines with less total acidity while Quinta do Côro wines' have the higher content.

These parameters were analyzed before the bottling of the wines, free sulfur dioxide was corrected when necessary to values in the order of 35 mg/L and only after that the bottling was done. Hence, the wines were protected against oxidation phenomena and microbial spoilage.

4.3. Analysis of the wines' color and phenolic composition

After the bottling of the wines, the chromatic characteristics were determined and the results are presented in the following tables (Tables 22 – 27). The analysis, unlike last year's, show statistically different results however in a practical point of view sometimes that difference is not translated. In general terms, the manual pruning contributed to higher color intensity and shade, total anthocyanin content, polymerization index and higher tannin power. In general, the treatments with biochar and control plots present the higher values. In Quinta do Côro the only analysis that wasn´t significantly different according to the pruning technique and organic amendment applied is nonflavonoid phenols. In Quinta do Gradil polymerization index was not influenced by the pruning technique and nonflavonoid phenols were not influenced by organic amendments. In Herdade de Rio Frio the application of organic amendments did not change the levels of nonflavonoid phenols and tannin power of the wines.

Table 22 – Color and phenolic composition of the wine samples from Quinta do Côro (Part I)

Treatment	Tot. Anthoc. (mg/L)	Ion. Anthoc. (mg/L)	Ionization degree of anthoc. (%)	Total Pigments (a.u.)	Polym. Pigments (a.u.)	Polymerization Index(%)	Color Intensity (a.u.)
MAN TEST	490.08 (25.67)	62.67 (4.40)	12.81 (1.08)	29.59 (1.22)	3.05 (0.08)	10.33 (0.60)	11.173 (0.185)
MAN ETAR	468.50 (12.10)	78.20 (2.03)	16.69 (0.05)	28.35 (0.57)	2.95 (0.02)	10.42 (0.27)	11.933 (0.110)
MAN ESTR	467.28 (16.95)	63.80 (3.14)	13.65 (0.18)	28.92 (0.87)	3.33 (0.04)	11.53 (0.32)	11.883 (0.276)
MAN RSUC	473.12 (15.19)	56.47 (1.17)	11.95 (0.64)	29.22 (0.82)	3.34 (0.05)	11.43 (0.23)	11.390 (0.108)
MAN BIOC	390.56 (127.18)	78.00 (2.16)	22.02 (9.44)	25.86 (6.41)	3.80 (0.04)	15.39 (4.30)	13.663 (0.125)
MEC TEST	511.16 (19.54)	143.73 (5.00)	28.12 (0.18)	31.11 (0.96)	3.33 (0.02)	10.71 (0.37)	17.380 (0.321)
MEC ETAR	286.41 (5.57)	102.33 (4.01)	35.76 (2.05)	17.2 (0.31)	1.73 (0.03)	10.06 (0.15)	10.800 (0.269)
MEC ESTR	371.05 (9.07)	88.73 (3.24)	23.93 (1.27)	22.12 (0.44)	2.14 (0.01)	9.68 (0.23)	10.933 (0.255)
MEC RSUC	418.29 (5.29)	92.47 (2.19)	22.11 (0.49)	25.05 (0.27)	2.48 (0.01)	9.90 (0.11)	11.830 (0.122)
MEC BIOC	484.92 (10.93)	132.67 (1.86)	27.37 (0.78)	31.01 (0.52)	4.06 (0.03)	13.09 (0.28)	18.027 (0.084)

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 23 - Color and phenolic composition of the wine samples from Quinta do Côro (Part II)

Treatment	Shade	Color due to copigm. (a.u.)	Color due to copigm (%)	Total Phenols (a.u.)	Nonflavonoid phenols (a.u.)	Flavonoid Phenols (a.u.)	Tannin Power (NTU/mL)
MAN TEST	0.583 (0.013)	2.24 (0.22)	24.33 (1.91)	51.27 (0.25)	5.20 (0.17)	46.07 (0.29)	193.13 (15.26)
MAN ETAR	0.546 (0.005)	2.95 (0.10)	30.20 (0.77)	49.77 (0.55)	5.70 (0.10)	44.07 (0.47)	201.29 (5.41)
MAN ESTR	0.591 (0.007)	2.61 (0.28)	26.71 (3.07)	52.37 (0.12)	4.90 (0.10)	47.47 (0.15)	188.83 (13.35)
MAN RSUC	0.611 (0.002)	1.87 (0.19)	20.04 (1.28)	55.87 (0.15)	5.60 (0.10)	50.23 (0.12)	206.54 (9.82)
MAN BIOC	0.554 (0.003)	2.42 (0.20)	22.84 (1.74)	55.00 (0.26)	5.87 (0.67)	49.13 (0.90)	206.42 (9.41)
MEC TEST	0.475 (0.006)	3.63 (0.26)	29.78 (1.70)	55.87 (1.43)	5.80 (0.20)	50.07 (1.23)	170.79 (9.17)
MEC ETAR	0.437 (0.005)	2.56 (0.07)	35.87 (1.08)	34.83 (0.59)	4.93 (0.95)	29.90 (1.18)	74.67 (6.62)
MEC ESTR	0.492 (0.004)	2.53 (0.21)	30.31 (1.57)	43.73 (0.93)	5.40 (0.00)	38.33 (0.93)	129.42 (14.65)
MEC RSUC	0.493 (0.006)	2.59 (0.63)	27.82 (5.15)	46.43 (0.83)	5.30 (0.44)	41.13 (1.20)	130.54 (2.65)
MEC BIOC	0.496 (0.002)	3.12 (1.14)	24.73 (7.57)	56.17 (0.75)	5.70 (0.17)	50.47 (0.60)	156.50 (18.28)

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 24 - Color and phenolic composition of the wine samples from Quinta do Gradil (Part I)

Treatment	Tot. Anthoc. (mg/L)	Ion. Anthoc. (mg/L)	Ionization degree of anthoc. (%)	Total Pigments (a.u.)	Polym. Pigments (a.u.)	Polymerization Index(%)	Color Intensity (a.u.)
MAN TEST	657.32 (19.06)	49.13 (5.80)	7.48 (0.94)	36.43 (0.94)	2.14 (0.04)	5.87 (0.21)	8.823 (0.323)
MAN ETAR	508.70 (12.60)	31.93 (1.30)	6.28 (0.41)	28.21 (0.65)	1.67 (0.02)	5.91 (0.11)	6.393 (0.098)
MAN ESTR	662.48 (54.05)	44.00 (0.35)	6.67 (0.60)	36.63 (2.68)	2.10 (0.02)	5.76 (0.46)	8.323 (0.049)
MAN RSUC	643.43 (38.24)	46.53 (3.60)	7.23 (0.39)	35.28 (1.93)	1.87 (0.02)	5.30 (0.26)	7.953 (0.268)
MAN BIOC	614.01 (45.60)	45.93 (1.53)	7.51 (0.66)	34.27 (2.26)	2.14 (0.02)	6.27 (0.44)	8.410 (0.135)
MEC TEST	547.69 (21.71)	48.33 (3.30)	8.82 (0.38)	30.43 (1.08)	1.83 (0.01)	6.02 (0.23)	7.880 (0.246)
MEC ETAR	426.86 (9.68)	23.80 (0.53)	5.58 (0.22)	23.40 (0.48)	1.23 (0.01)	5.27 (0.12)	4.673 (0.061)
MEC ESTR	515.05 (21.90)	33.47 (1.89)	6.50 (0.32)	28.32 (1.11)	1.54 (0.01)	5.43 (0.19)	6.060 (0.121)
MEC RSUC	460.41 (17.97)	41.93 (2.47)	9.10 (0.18)	25.62 (0.88)	1.56 (0.01)	6.09 (0.25)	6.700 (0.173)
MEC BIOC	530.84 (13.52)	52.53 (1.55)	9.90 (0.25)	29.49 (0.71)	1.77 (0.02)	6.00 (0.08)	8.047 (0.121)

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – bioc

Table 25 - Color and phenolic composition of the wine samples from Quinta do Gradil (Part II)

Treatment	Shade	Color due to copigm. (a.u.)	Color due to copigm (%)	Total Phenols (a.u.)	Nonflavonoid phenols (a.u.)	Flavonoid Phenols (a.u.)	Tannin Power (NTU/mL)
MAN TEST	0.672 (0.030)	1.96 (0.10)	24.79 (1.48)	50.33 (0.71)	5.20 (0.10)	45.13 (0.78)	154.88 (7.17)
MAN ETAR	0.710 (0.009)	1.29 (0.12)	21.46 (1.84)	42.40 (0.75)	5.03 (0.23)	37.37 (0.76)	154.17 (11.74)
MAN ESTR	0.680 (0.002)	1.66 (0.10)	21.95 (1.21)	50.10 (0.56)	5.17 (0.35)	44.93 (0.74)	159.13 (16.06)
MAN RSUC	0.657 (0.015)	1.90 (0.04)	25.25 (0.39)	49.37 (0.61)	5.00 (0.10)	44.37 (0.71)	177.00 (7.74)
MAN BIOC	0.655 (0.006)	1.91 (0.03)	24.22 (0.57)	48.70 (1.30)	5.07 (0.21)	43.63 (1.50)	110.46 (6.19)
MEC TEST	0.623 (0.01)	1.88 (0.08)	22.92 (0.97)	45.93 (0.90)	5.83 (0.21)	40.10 (0.70)	117.00 (27.23)
MEC ETAR	0.693 (0.010)	0.73 (0.07)	12.67 (1.22)	36.03 (0.49)	6.67 (1.24)	29.37 (1.57)	133.38 (21.19)
MEC ESTR	0.661 (0.013)	1.59 (0.00)	21.29 (0.07)	44.17 (0.38)	6.17 (0.32)	38.00 (0.35)	92.21 (17.32)
MEC RSUC	0.612 (0.015)	1.55 (0.06)	21.15 (0.83)	40.20 (0.46)	6.23 (0.25)	33.97 (0.64)	107.58 (24.30)
MEC BIOC	0.604 (0.007)	1.32 (0.04)	16.56 (0.48)	46.53 (1.27)	5.57 (0.21)	40.97 (1.06)	83.79 (21.00)

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 26 - Color and phenolic composition of the wine samples from Herdade do Rio Frio (Part I)

Treatment	Tot. Anthoc. (mg/L)	Ion. Anthoc. (mg/L)	Ionization degree of anthocyanin (%)	Total Pigments (a.u.)	Polym. Pigments (a.u.)	Polymerization Index(%)	Color Intensity (a.u.)
TEST	289.68 (19.66)	20.13 (1.63)	6.96 (0.49)	16.36 (0.97)	1.13 (0.02)	6.90 (0.46)	4.063 (0.145)
ETAR	297.60 (6.43)	20.07 (2.53)	6.73 (0.73)	17.04 (0.32)	1.29 (0.01)	7.59 (0.14)	4.507 (0.203)
ESTR	379.40 (17.69)	22.67 (0.81)	5.99 (0.41)	21.11 (0.88)	1.28 (0.01)	6.09 (0.26)	4.823 (0.075)
RSUC	326.62 (11.13)	19.73 (1.21)	6.04 (0.26)	18.28 (0.56)	1.17 (0.01)	6.40 (0.18)	4.110 (0.092)
BIOC	334.34 (2.70)	15.47 (0.61)	4.63 (0.22)	18.55 (0.15)	1.10 (0.02)	5.93 (0.08)	3.743 (0.095)

TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Table 27 - Color and phenolic composition of the wine samples from Herdade do Rio Frio (Part II)

Treatment	Shade	Color due to copigm. (a.u.)	Color due to copigm (%)	Total Phenols (a.u.)	Nonflavonoid phenols (a.u.)	Flavonoid Phenols (a.u.)	Tannin Power (NTU/mL)
TEST	0.681 (0.002)	1.56 (0.04)	36.71 (0.73)	30.83 (0.15)	4.27 (0.06)	26.57 (0.15)	127.88 (2.61)
ETAR	0.735 (0.024)	1.63 (0.06)	35.98 (1.46)	35.30 (0.20)	4.77 (0.15)	30.53 (0.21)	143.96 (7.89)
ESTR	0.745 (0.007)	1.16 (0.04)	28.02 (0.79)	36.40 (0.10)	4.77 (0.29)	31.63 (0.38)	164.63 (4.01)
RSUC	0.680 (0.008)	1.47 (0.01)	33.46 (0.22)	32.37 (0.21)	4.43 (0.21)	27.93 (0.21)	128.33 (2.62)
BIOC	0.756 (0.015)	1.12 (0.01)	29.29 (0.33)	33.50 (0.36)	4.67 (0.15)	28.83 (0.38)	138.38 (34.45)

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC - biochar

Statistical Analysis of Chromatic Characteristics' Results

Table 28 - Statistical Analysis of color and phenolic composition results of Quinta do Côro wines (Part I)

Treatment	Tot. Anthoc. (mg/L)	Ion. Anthoc. (mg/L)	Ionization degree of anthoc. (%)	Total Pigments (a.u.)	Polym. Pigments (a.u.)	Polymerization Index(%)	Color Intensity (a.u.)
MAN	457.91	67.83	15.42	28.39	3.30	11.82	13.794
MEC	414.36	111.99	27.46	25.28	2.75	10.69	12.009
Sig.	*	***	***	***	***	*	***
TEST	500.62a	103.20a	20.47bc	30.35a	3.19b	10.52b	14.277a
ETAR	377.45b	90.27b	26.22a	22.78c	2.34e	10.24b	11.367b
ESTR	419.16b	76.27c	18.79c	25.52bc	2.74d	10.61b	11.408b
RSUC	445.71ab	74.47c	17.03c	27.14ab	2.91c	10.67b	11.610b
BIOC	437.74ab	105.33a	24.69ab	28.43ab	3.93a	14.24a	15.845a
Sig.	*	***	*	*	***	**	***
MAN TEST	490.08ab	62.67f	12.81d	29.59d	3.05a	10.33	11.173e
MAN ETAR	468.50ab	78.20e	16.69bcd	28.35d	2.95a	10.42	11.933d
MAN ESTR	467.28ab	63.80f	13.65cd	28.92c	3.33a	11.53	11.883d
MAN RSUC	473.12ab	56.47f	11.95d	29.22c	3.34a	11.43	11.390de
MAN BIOC	390.56abc	78.00e	22.02bc	25.86b	3.8ab	15.39	13.663c
MEC TEST	511.16a	143.73a	28.12ab	31.11c	3.33a	10.71	17.380b
MEC ETAR	286.41c	102.33c	35.76a	17.20g	1.73c	10.06	10.800e
MEC ESTR	371.05bc	88.73d	23.93b	22.12f	2.14bc	9.68	10.933e
MEC RSUC	418.29ab	92.47d	22.11bc	25.05e	2.48ab	9.90	11.830d
MEC BIOC	484.92ab	132.67b	27.37ab	31.01a	4.06a	13.09	18.027a
Sig.	*	*	*	***	*	ns	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 29 - Statistical Analysis of color and phenolic composition results of Quinta do Côro wines (Part II)

Treatment	Shade	Color due to copigm. (a.u.)	Color due to copigm (%)	Total Phenols (a.u.)	Nonflavonoid phenols (a.u.)	Tannin Power (NTU/mL)
MAN	0.577	2.42	24.83	52.90	5.45	199.24
MEC	0.478	2.88	29.70	47.40	5.43	132.38
Sig.	***	*	***	***	ns	***
TEST	0.529c	2.93	27.05b	53.60 b	5.50	181.96a
ETAR	0.491d	2.75	33.03a	42.30 e	5.32	137.98c
ESTR	0.541b	2.57	28.51ab	48.10 d	5.15	159.13b
RSUC	0.552a	2.23	23.93b	51.20 c	5.45	168.54ab
BIOC	0.525c	2.77	23.79b	55.60 a	5.78	181.46a
Sig.	*	ns	*	***	ns	*
MAN TEST	0.583c	2.24b	24.33	51.27 b	5.20	193.13ab
MAN ETAR	0.546c	2.95ab	30.20	49.77 b	5.70	201.29ab
MAN ESTR	0.591b	2.61ab	26.71	52.37 b	4.90	188.83abc
MAN RSUC	0.611a	1.87b	20.04	55.87 a	5.60	206.54a
MAN BIOC	0.554c	2.42ab	22.84	55.00 a	5.87	170.79b
MEC TEST	0.475e	3.63a	29.78	55.87 a	5.80	74.67e
MEC ETAR	0.437f	2.56ab	35.87	34.83 e	4.93	74.67e
MEC ESTR	0.492de	2.53ab	30.31	43.73 d	5.40	129.42d
MEC RSUC	0.493d	2.59ab	27.82	46.43 c	5.30	130.54d
MEC BIOC	0.496d	3.12ab	24.73	56.17 a	5.70	156.50cd
Sig.	*	*	ns	**	ns	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 30 - Statistical Analysis of color and phenolic composition results of Quinta do Gradil wines (Part I)

Treatment	Tot. Anthoc. (mg/L)	Ion. Anthoc. (mg/L)	Ionization degree of anthoc. (%)	Total Pigments (a.u.)	Polym. Pigments (a.u.)	Polymerization Index(%)	Color Intensity (a.u.)
MAN	617.19	43.51	7.04	34.16	1.98	5.82	7.981
MEC	496.17	40.01	7.98	27.45	1.58	5.76	6.672
Sig.	***	**	***	***	***	ns	***
TEST	602.51a	48.73ab	8.15a	33.43a	1.98a	5.94ab	8.352a
ETAR	467.78c	27.87d	5.93b	25.81c	1.45d	5.59b	5.533c
ESTR	588.76ab	38.73c	6.59b	32.47ab	1.82b	5.60b	7.192b
RSUC	551.92b	44.23b	8.17a	30.45b	1.71c	5.70ab	7.327b
BIOC	572.42ab	49.23a	8.70a	31.88ab	1.96a	6.14a	8.228a
Sig.	*	*	***	*	***	*	***
MAN TEST	657.32a	49.13ab	7.48bc	36.43a	2.14a	5.87abc	8.823a
MAN ETAR	508.70bcde	31.93c	6.28cd	28.21cd	1.67d	5.91abc	6.393d
MAN ESTR	662.48a	44.00b	6.67cd	36.63a	2.10a	5.76abc	8.323abc
MAN RSUC	643.43a	46.53ab	7.23c	35.28a	1.87b	5.30c	7.953bc
MAN BIOC	614.01ab	45.93ab	7.51bc	34.27ab	2.14a	6.27a	8.410ab
MEC TEST	547.69bc	48.33ab	8.82ab	30.43bc	1.83b	6.02abc	7.880c
MEC ETAR	426.86e	23.80d	5.58d	23.40e	123e	5.27c	4.673e
MEC ESTR	515.05c	33.47c	6.50cd	28.31cd	1.54d	5.43b	6.060d
MEC RSUC	460.41de	41.93b	9.10a	25.62de	1.56d	6.09ab	6.700d
MEC BIOC	530.84bc	52.53a	9.90a	29.49cd	1.77c	6.00abc	8.047b
Sig.	*	*	*	*	*	*	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 31 - Statistical Analysis of color and phenolic composition results of Quinta do Gradil wines (Part II)

Treatment	Shade	Color due to copigm. (a.u.)	Color due to copigm (%)	Total Phenols (a.u.)	Nonflavonoid phenols (a.u.)	Tannin Power (NTU/mL)
MAN	0.675	1.74	23.54	48.2	5.09	151.13
MEC	0.638	1.41	18.92	42.6	6.09	106.79
Sig.	***	***	***	***	***	***
TEST	0.648bc	1.92a	23.85a	48.1a	5.52	135.94a
ETAR	0.702a	1.01c	17.07c	39.2c	5.85	143.77a
ESTR	0.670b	1.62b	21.62b	47.1a	5.67	125.67ab
RSUC	0.634c	1.72b	23.20a	44.8b	5.62	142.29a
BIOC	0.629c	1.61b	20.39b	47.6a	5.32	97.13b
Sig.	**	**	*	***	ns	**
MAN TEST	0.672	1.96a	24.79ab	50.3a	5.20	154.88
MAN ETAR	0.710	1.29c	21.46c	42.4ef	5.03	154.17
MAN ESTR	0.680	1.66b	21.95bc	50.1a	5.17	159.13
MAN RSUC	0.657	1.90a	25.25a	49.4a	5.00	177.00
MAN BIOC	0.655	1.91a	24.22abc	48.7ab	5.07	110.46
MEC TEST	0.623	1.88a	22.92abcd	45.9cd	5.83	117.00
MEC ETAR	0.693	0.73d	12.67f	36.0g	6.67	133.38
MEC ESTR	0.661	1.59b	21.29c	44.2de	6.17	92.21
MEC RSUC	0.612	1.55b	21.15d	40.2f	6.23	107.58
MEC BIOC	0.604	1.31c	16.56e	46.5bc	5.57	83.79
Sig.	ns	*	*	*	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 32 - Statistical Analysis of color and phenolic composition results of Herdade do Rio Frio wines (Part I)

Treatment	Tot. Anthoc. (mg/L)	Ion. Anthoc. (mg/L)	Ionization degree of anthoc. (%)	Total Pigments (u.a.)	Polym. Pigments (a.u.)	Polymerization Index(%)	Color Intensity (a.u.)
TEST	289.68d	20.13a	6.96a	16.36c	1.13c	6.90ab	4.063bc
ETAR	297.60cd	20.07a	6.73a	17.04bc	1.29a	7.59a	4.507a
ESTR	379.40a	22.67a	5.99a	21.11a	1.28a	6.09c	4.823a
RSUC	326.62bc	19.73a	6.04a	18.28b	1.18b	6.40bc	4.110b
BIOC	334.34b	15.47b	4.63b	18.55b	1.10c	5.93c	3.743c
Sig.	*	*	*	***	***	*	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 33 - Statistical Analysis of color and phenolic composition results of Herdade do Rio Frio wines (Part II)

Treatment	Shade	Color due to copigm. (a.u.)	Color due to copigm (%)	Total Phenols (a.u.)	Nonflavonoid phenols (a.u.)	Tannin Power (NTU/mL)
TEST	0.681b	1.56ab	36.71a	30.83e	4.27	127.88
ETAR	0.735a	1.63a	35.98a	35.30b	4.77	143.96
ESTR	0.745a	1.16c	28.02c	36.40a	4.77	164.63
RSUC	0.680b	1.47b	33.46b	32.37d	4.43	128.33
BIOC	0.756a	1.12c	29.29c	33.50c	4.67	138.38
Sig.	**	**	*	**	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Statistical analysis show that the manual pruning technique has some influence in color intensity, in what organic amendments are concerned the biochar treatment and the control plots present significantly higher values than the others, except in Herdade de Rio Frio. Quinta do Côro has wines with higher color intensity (Tables 28 and 29) and Herdade de Rio Frio registered lower values, in certain cases with half the color intensity (Tables 32 and 33). The treatment that caused less color intensity is ETAR due to its effect in vegetative growth, and in Quinta do Gradil it increased pH causing a reduction in ionized anthocyanins, which are the ones responsible for wine color (Tables 30 and 31). As the pH of a red wine decreases its red color intensifies because there is an increase in the proportion of anthocyanins in the red form (flavylium cation) (Ribéreau-Gayon et al. 2006).

Shade is also higher when manual pruning was applied, Quinta do Gradil and Herdade de Rio Frio have higher shade levels than Quinta do Côro. In what organic amendments are concerned, RSUC registered higher shade values in Quinta do Côro, however in Quinta do Gradil ETAR was the one achieving higher results. In Herdade de Rio Frio the results were statistically more equilibrated – TEST and RSUC treatments resulted in lower shade.

The total anthocyanin content is higher when the manual pruning technique was applied because it reduced the canopy density, when compared to mechanical pruning, and that allowed more light in the bunch zone to stimulate the anthocyanin production. According to Herderich et al. (2006) in a study of wine grape tannin and color specifications, moderately and highly exposed bunches had a higher ratio of skin tannins to anthocyanins than shaded bunches. This might explain why wines made from berries that developed and ripened under moderate to high sunlight exposure presented higher pigmented polymers. The same tendency was registered by Kaushal (2014) in last year analysis and Gatti et al (2011) in their study of long-term effects of mechanical pruning on Barbera grapevines also registered a slightly lower anthocyanin concentration resultant from mechanically pruned vines. Quinta do Gradil wines have a higher total anthocyanin concentration and Herdade de Rio Frio ones have the lowest concentration. The control plot originated wines with higher total anthocyanin concentration except in Herdade de Rio Frio where ESTR was the amendment that led to higher levels. Morlat et Symoneaux (2008) determined that organic amendments that provide a high level of available nitrogen to the vines caused a decrease in anthocyanin and tannin content of berries which is in agreement to the results found in this experiment – ETAR was responsible for the lower anthocyanin concentration both in Quinta do Côro and Quinta do Gradil.

Ionized anthocyanins concentration in Quinta do Côro was higher when mechanical pruning was applied, while in Quinta do Gradil the higher values were registered when the vines were manually pruned. BIOC was the organic amendment that led to a higher ionized anthocyanin concentration except in Herdade de Rio Frio where all the values are very close to each other except for BIOC, which led to the lower value. The ionization degree of anthocyanins is higher when mechanical pruning was applied both in Quinta do Côro and Quinta do Gradil, the results belong to the same range of values as last years' (Kaushal 2014). In the organic amendments, no special tendency was found, in each vineyard a different treatment led to the higher result unlike what happened in the results presented by Kaushal (2014) in which the wines from control plots presented higher ionization degree of anthocyanins.

Total pigments are higher in wines derived from manual pruning for both wineries, although the values are higher in Quinta do Gradil. Organic amendments influenced this parameter in different ways – in Quinta do Côro BIOC led to higher total pigments, in Quinta do Gradil TEST was the one resulting in higher values and in Herdade de Rio Frio ESTR was the one leading to higher total pigments.

Polymerized pigments are present in higher concentrations when manual pruning was applied and Quinta do Côro wines present the higher values. BIOC was the organic amendment that caused an increase in this parameter results' except in Herdade de Rio Frio where ESTR and ETAR were the treatments that induced higher polymerized pigments concentration in wines. Polimerization index has superior values in both wineries when manual pruning was the chosen technique. Once more Quinta do Gradil and Quinta do Côro wines' have higher polymerization index when BIOC was applied and in Herdade de Rio Frio ETAR was the treatment that led to higher results. Polymerization index is higher in Quinta do Côro than in Quinta do Gradil and Herdade de Rio Frio possibly because of the higher amount of tannins which increases the reactivity between these molecules and anthocyanins leading to a higher polymerization index.

The color due to copigmentation phenomena has higher values in Quinta do Côro and lower ones in Herdade de Rio Frio, in general terms the plots without any type of organic amendment addition led to higher values of this parameter. This difference found between vineyards can be explained by the pH of the wines – pH values close to 3,5 favor copigmentation phenomena – which is why the wines from Herdade de Rio Frio with higher pH values (between 3,8 and 4) have lower color due to copigmentation (Ribéreau-Gayon et al. 2006).

Total phenols are present in higher concentrations in wines from Quinta do Côro and the lower ones were from Herdade de Rio Frio. Manual pruning led to greater values in both cases, and the organic amendment that caused a decrease in this parameter was ETAR, except in Herdade de Rio Frio were control plots led to less total phenols. In what nonflavonoid phenols are concerned no tendency was found between vineyards, all the wines presented similar values independently of the pruning technique and organic amendment applied.

Tannin power presented higher values for wines from manually pruned vines. Organic amendments influenced this parameters in different ways, according to each vineyard. In Quinta do Côro control plots led to wines with higher tannin power while in Quinta do Gradil ETAR had that effect and in Herdade de Rio Frio was ESTR.

4.4. Total Nitrogen Content

Total nitrogen content was mainly influenced by the pruning technique, in both vineyards manual pruning originated wines with a higher concentration of this element. Organic amendments did not significantly influenced total nitrogen amount, however if the absolute values are considered it is possible to affirm that ETAR led to an accumulation of nitrogen in the wines.

Table 34 – Nitrogen content of the wines from Quinta do Côro, Quinta do Gradil and Herdade de Rio Frio

	Quinta do Côro	Quinta do Gradil	Herdade de Rio Frio
Treatment	Total Nitrogen (mg/L)	Total Nitrogen (mg/L)	Total Nitrogen (mg/L)
MAN TEST	232.3	265.8	101.1
MAN ETAR	354.7	356.5	196.3
MAN ESTR	215.9	337.9	127.9
MAN RSUC	256.9	331.9	132.3
MAN BIOC	183.3	265.1	149.4
MEC TEST	89.7	137.6	
MEC ETAR	154.0	260.2	
MEC ESTR	137.1	200.0	
MEC RSUC	188.1	202.3	
MEC BIOC	90.8	167.3	

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Statistical Analysis of Wines' Nitrogen Content Results

Table 35 – Statistical analysis of wines' nitrogen content

	Quinta do Côro	Quinta do Gradil
Treatment	Total Nitrogen (mg/L)	Total Nitrogen (mg/L)
MAN	248.6	311.4
MEC	131.9	193.5
Sig.	*	**
TEST	161.0	201.7
ETAR	254.3	308.4
ESTR	176.5	268.9
RSUC	222.5	267.1
BIOC	137.1	216.2
Sig.	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Total Nitrogen content is higher when manual pruning was applied and when the plot was treated with sewage sludge (ETAR) in the three vineyards (Tables 35-35). This means that the nitrogen added by this treatment is easily mineralized becoming available to the vines, besides it's high levels of nitrogen, calcium and phosphorus ETAR has the advantage of being rich in organic matter (Hue 1988, Miah et al. 1999). Bell and Henschke (2005) studied the implications of nitrogen nutrition and alerted for the fact that residual nitrogen in wine under some circumstances can lead to microbiological instability and consequent loss of wine quality. Quinta do Gradil wines registered the major nitrogen contents while Herdade de Rio Frio have the minimum concentrations.

4.5. Mineral Analysis of the Wines

Mineral analysis of the wines from the three vineyards of this experiment showed that manual pruning tends to result in higher concentrations of potassium, iron and copper (Tables 36-38). Only calcium content increased with mechanical pruning, despite the difference is not statistically significant. Pruning technique did not influenced calcium and iron concentration and the application of organic amendments did not lead to significantly different results. By the values obtained it is possible to affirm that no contamination occurred during vinification and wine storage.

Table 36 - Mineral Analysis of the wine samples from Quinta do Côro

Treatment	Potassium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Copper (mg/L)
MAN TEST	972	15	2.2	0.03
MAN ETAR	910	15	2.6	0.05
MAN ESTR	1048	14	2.6	0.08
MAN RSUC	1065	13	2.6	0.06
MAN BIOC	974	13	2.2	0.03
MEC TEST	850	15	2.5	0.01
MEC ETAR	717	20	2.1	0.02
MEC ESTR	853	18	2.0	0.03
MEC RSUC	773	17	1.9	0.03
MEC BIOC	863	14	2.3	0.01

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 37 - Mineral Analysis of the wine samples from Quinta do Gradil

Treatment	Potassium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Copper (mg/L)
MAN TEST	1283	11	2.1	0.07
MAN ETAR	1292	16	2.3	0.02
MAN ESTR	1281	13	2.3	0.06
MAN RSUC	1265	13	2.2	0.05
MAN BIOC	1246	13	2.1	0.06
MEC TEST	1092	11	1.3	<0.0005
MEC ETAR	1186	17	1.7	0.004
MEC ESTR	1149	14	1.7	0.09
MEC RSUC	1045	14	1.7	0.04
MEC BIOC	1291	14	1.9	0.05

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 38 - Mineral Analysis of the wine samples from Herdade de Rio Frio

Treatment	Potassium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Copper (mg/L)
MAN TEST	1099	18	1.6	<0.0005
MAN ETAR	1091	17	1.9	<0.0005
MAN ESTR	1575	15	1.8	<0.0005
MAN RSUC	1225	17	1.7	<0.0005
MAN BIOC	1465	16	1.5	<0.0005

MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Statistical Analysis of Mineral Content Results

Table 39 - Statistical Analysis of mineral determination of the wine samples from Quinta do Côro

Treatment	Potassium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Copper (mg/L)
MAN	994	14	2.4	0.05
MEC	811	17	2.1	0.02
Sig.	**	ns	ns	*
TEST	911	15	2.3	0.02
ETAR	814	18	2.3	0.03
ESTR	950	16	2.3	0.05
RSUC	919	15	2.2	0.05
BIOC	918	13	2.3	0.02
Sig.	ns	ns	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 40 - Statistical Analysis of mineral determination of the wine samples from Quinta do Gradil

Treatment	Potassium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Copper (mg/L)
MAN	1273	13	2.2	0.05
MEC	1153	14	1.6	0.04
Sig.	*	ns	***	ns
TEST	1188	11b	1.7	0.04
ETAR	1239	17a	2.0	0.01
ESTR	1215	14ab	2.0	0.08
RSUC	1155	14ab	2.0	0.05
BIOC	1268	13ab	2.0	0.05
Sig.	ns	**	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Potassium concentration is higher when manual pruning was made, it was higher in Quinta do Côro (Table 39) and Herdade de Rio Frio when the vineyards were treated with cattle manure (ESTR), the same results were presented by Morlat et Symoneaux (2008) and Kaushal (2014). Calcium content was superior for plots amended with ETAR and when mechanical pruning was applied. These two minerals can combine themselves with tartaric acid originating potassium bitartrate and calcium tartrate – instabilities that can apear during winemaking and wine conservation.

The amount of iron and copper present in these wines is higher when manual pruning was chosen (Table 39 and 40). The values are close to each other independently of the vineyard and are within the range of usual values (Catarino et al. 2008). Iron participates in oxidation and copigmentation phenomena, when it is present in concentrations above 10 mg/L can lead to iron casse. Copper is a metal with a maximum level permitted by the OIV – 1 mg/L but generally it is present in wines in concentrations between 0,1 and 0,2 mg/L (Catarino et al. 2008). Comparing these values to the ones obtained in the analysis of the wines from the three wineries it is possible to say that there is no risk of iron or copper casse.

4.6. Heavy Metals and Other Element Analysis of the Wines

Heavy metals and rare earth elements are considered contaminants in wines, when above certain concentrations. Mineral composition of wine not only reflects its origin, once the elements are absorbed by the vine roots system, but also the possible contaminations that can take place from vinification to the wine storage (Catarino et al 2008). Since organic amendments addition can result in the accumulation of some of these elements in the soil, some of which have to obey to legal limits, the determination of approximately 35 of these elements was carried out.

In general terms, the results obtained show some variability between pruning technique and the elements concentration – in the majority of the elements manual pruning led to higher concentrations especially in Quinta do Côro. Contrarily in Quinta do Gradil it was mechanical pruning that conducted to the higher levels. In what organic amendments are concerned in Quinta do Côro ETAR and ESTR were the ones leading to higher concentrations of these elements (Tables 41 and 42), in Quinta do Gradil the treatments with this effect were the control plots, ETAR and BIOC (Tables 43 and 44) and, finally, in Herdade de Rio Frio BIOC and ETAR resulted in wines with more contaminants’ concentration (Tables 45 and 46). The Limits of Detection (LOD) and Limits of Quantification (LOQ) for the applied methodology are in Annex 2.

Table 41 – Metals and Other Elements Analysis of Quinta do Côro Wines (Part I – manual pruning)

Element	MAN TEST	MAN ETAR	MAN ESTR	MAN RSUC	MAN BIOC
Li (µg/L)	2.92 (0.01)	2.74 (0.02)	3.0 (0.1)	2.65 (0.08)	2.61 (0.02)
Be (µg/L)	0.060 (0.004)	0.066 (0.001)	0.05 (0.02)	0.07 (0.02)	0.05 (0.01)
Na (mg/L)	4.09 (0.02)	5.458 (0.009)	4.12 (0.02)	4.9 (0.2)	4.01 (0.04)
Mg (mg/L)	94 (1)	90 (2)	91.5 (0.6)	92 (1)	91.7 (0.8)
Al (mg/L)	0.0557 (0.0004)	0.057 (0.003)	0.0561 (0.0004)	0.069 (0.002)	0.0707 (0.0003)
V (µg/L)	0.28 (0.02)	0.29 (0.03)	0.3 (0.1)	0.27 (0.02)	0,40 (0.02)
Mn (mg/L)	0.59 (0.01)	0.63 (0.01)	0.630 (0.004)	0.57 (0.01)	0.591 (0.002)
Co (µg/L)	2.682 (0.002)	2.45 (0.03)	2.94 (0.03)	2.35 (0.02)	2.60 (0.03)
Ni (µg/L)	15.1 (0.2)	17.4 (0.7)	15.0 (0.3)	14 (1)	15.3 (0.1)
Zn (mg/L)	0.58 (0.01)	0.813 (0.005)	0.67 (0.01)	0.58 (0.01)	0.604 (0.002)
Ga (µg/L)	7.1 (0.2)	4.96 (0.02)	6.33 (0.01)	7.4 (0.2)	7.0 (0.2)
As (µg/L)	0.242 (0.003)	0.32 (0.01)	0.261 (0.005)	0.25 (0.01)	0.24 (0.02)
Se (µg/L)	0.35 (0.02)	0.4 (0.3)	0.4 (0.1)	0.2 (0.1)	0.3 (0.2)
Rb (mg/L)	6.1 (0.1)	5.56 (0.04)	6.04 (0.03)	6.48 (0.05)	6.3 (0.1)
Sr (µg/L)	485 (10)	530 (4)	565 (19)	501 (2)	520 (9)
Y (ng/L)	24 (2)	21 (1)	31 (14)	25 (1)	21 (3)
Cd (µg/L)	0.237 (0.005)	0.22 (0.02)	0.20 (0.07)	0.19 (0.06)	29.8 (0.5)
Cs (µg/L)	66.8 (0.8)	123 (1)	55.14 (0.07)	63.1 (0.7)	72.7 (0.7)
Ba (µg/L)	233 (3)	267 (4)	300.4 (0.6)	255 (2)	244 (3)
Ce (ng/L)	24.0 (0.3)	31 (4)	43 (22)	27 (11)	29.8 (0.5)
Pr (ng/L)	3*	4 (1)	19#*	2.69 (0.07)	2*
Nd (ng/L)	7*	7.6 (0.7)	13 (10)	8*	6*
Sm (ng/L)	< LOQ	< LOQ	14#*	< LOQ	< LOQ
Eu (ng/L)	20.8 (0.9)	18.9 (0.7)	33 (13)	20.9 (0.7)	20 (1)
Gd (ng/L)	< LOQ	< LOQ	20#*	< LOQ	< LOQ
Tb (ng/L)	< LOQ	< LOQ	15#*	< LOQ	< LOQ
Dy (ng/L)	< LOQ	< LOQ	18#*	< LOQ	< LOQ
Ho (ng/L)	< LOQ	< LOQ	18#*	< LOQ	< LOQ
Er (ng/L)	< LOQ	< LOQ	11#*	< LOQ	< LOQ
Tm (ng/L)	< LOQ	< LOQ	16#*	< LOQ	< LOQ
Yb (ng/L)	< LOQ	< LOQ	13#*	< LOQ	< LOQ
Lu (ng/L)	< LOQ	< LOQ	13#*	< LOQ	< LOQ
Tl (µg/L)	4.7 (0.1)	5.3 (0.1)	5.08 (0.07)	4.46 (0.05)	5.44 (0.09)
Pb (µg/L)	8.0 (0.2)	7.8 (0.2)	7.001 (0.003)	4.46 (0.05)	6.8 (0.1)

After recalibration of the apparatus; * Only one value of the two repetitions is bigger than the LOQ; LOQ = limit of quantification (Annex 2); MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 42 – Metals and Other Elements Analysis of Quinta do Côro Wines (Part II – mechanical pruning)

Element	MEC TEST	MEC ETAR	MEC ESTR	MEC RSUC	MEC BIOC
Li (µg/L)	3.01 (0.06)	3.1 (0.2)	4.43 (0.07)	2.75 (0.05)	3.60 (0.02)
Be (µg/L)	0.07 (0.02)	0.07 (0.03)	0.05 (0.01)	0.08 (0.02)	0.05 (0.02)
Na (mg/L)	4.131 (0.002)	3.7 (0.3)	3.40 (0.07)	3.96 (0.05)	3.540 (0.002)
Mg (mg/L)	103.1 (0.5)	85 (8)	91 (2)	95.6 (0.2)	97.7 (0.4)
Al (mg/L)	0.0531 (0.0003)	0.049 (0.004)	0.060 (0.002)	0.130 (0.004)	0.0554 (0.0004)
V (µg/L)	0.33 (0.01)	0.44 (0.05)	0.29 (0.01)	0.28 (0.02)	0.25 (0.02)
Mn (mg/L)	0.915 (0.001)	0.60 (0.05)	0.64 (0.01)	0.60 (0.01)	0.661 (0.004)
Co (µg/L)	2.366 (0.003)	2.3 (0.1)	2.38 (0.05)	2.568 (0.001)	2.10 (0.01)
Ni (µg/L)	14.2 (0.4)	19 (1)	16.9 (0.4)	14.8 (0.1)	15.4 (0.2)
Zn (mg/L)	0.418 (0.004)	0.37 (0.03)	0.38 (0.01)	0.4555 (0.0002)	0.31 (0.01)
Ga (µg/L)	5.8 (0.1)	3.2 (0.3)	4.71 (0.03)	5.72 (0.04)	5.6 (0.2)
As (µg/L)	0.339 (0.002)	0.28 (0.04)	0.257 (0.005)	0.27 (0.02)	0.248 (0.002)
Se (µg/L)	0.42 (0.03)	0.5 (0.1)	0.25 (0.03)	0.3 (0.1)	0.4 (0.1)
Rb (mg/L)	5.10 (0.09)	4.6 (0.4)	5.1 (0.1)	4.583 (0.002)	4.80 (0.02)
Sr (µg/L)	620.9 (0.4)	500 (39)	609 (15)	517 (5)	583 (6)
Y (ng/L)	16 (3)	7.7 (0.4)	15.6 (0.1)	16.2 (0.7)	14.3 (0.9)
Cd (µg/L)	0.23 (0.07)	0.24 (0.04)	0.15 (0.01)	0.21 (0.01)	0.201 (0.003)
Cs (µg/L)	59.4 (0.2)	96 (8)	43 (1)	54.1 (0.7)	205.21 (0.03)
Ba (µg/L)	293 (6)	253 (21)	351 (6)	264 (1)	290 (2)
Ce (ng/L)	34 (3)	21 (2)	24.6 (0.9)	22 (2)	23 (6)
Pr (ng/L)	2.9 (0.6)	< LOQ	< LOQ	< LOQ	< LOQ
Nd (ng/L)	7 (3)	< LOQ	< LOQ	< LOQ	7*
Sm (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Eu (ng/L)	23.7 (0.9)	19 (6)	26.6 (0.5)	23 (2)	22.3 (0.2)
Gd (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Tb (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Dy (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ho (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Er (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Tm (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Yb (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Lu (ng/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Tl (µg/L)	4.21 (0.04)	5.1 (0.4)	3.97 (0.03)	4.1 (0.1)	4.00 (0.01)
Pb (µg/L)	6.3 (0.3)	8.1 (0.6)	7.5 (0.2)	7.93 (0.02)	6.35 (0.04)

After recalibration of the apparatus; * Only one value of the two repetitions is bigger than the LOQ; LOQ = limit of quantification (Annex 2); MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 43 – Metals and Other Elements Analysis of Quinta do Gradil Wines (Part I – manual pruning)

Element	MAN TEST	MAN ETAR	MAN ESTR	MAN RSUC	MAN BIOC
Li (µg/L)	9.293 (0.003)	7.6 (0.4)	6.7 (0.2)	8.2 (0.2)	7.863 (0.003)
Be (µg/L)	0.740 (0.006)	0.73 (0.03)	0.6 (0.1)	0.50 (0.02)	0.86 (0.04)
Na (mg/L)	10.05 (0.04)	7.04 (0.09)	9.6 (0.1)	9.42 (0.07)	9.3 (0.3)
Mg (mg/L)	92.1 (0.9)	96 (3)	101 (3)	96 (1)	95 (3)
Al (mg/L)	0.09 (0.01)	0.083 (0.003)	0.075 (0.001)	0.098 (0.002)	0.085 (0.003)
Sc (µg/L)	1.1 (0.2)	2.6 (0.3)	1.2 (0.5)	1.1 (0.1)	0.9 (0.3)
V (µg/L)	0.16 (0.06)	0.31 (0.04)	0.2 (0.3)	0.049*	0.058*
Mn (mg/L)	4.43 (0.06)	10.1 (0.2)	4.42 (0.09)	9.459 (0.004)	8.4 (0.1)
Co (µg/L)	4.3 (0.1)	5.0 (0.3)	3.95 (0.09)	4.65 (0.01)	4.9 (0.2)
Ni (µg/L)	30.4 (0.8)	33.3 (0.3)	25 (1)	26.83 (0.02)	32 (1)
Zn (mg/L)	0.73 (0.03)	0.53 (0.01)	0.48 (0.01)	0.482 (0.003)	0.53 (0.03)
Ga (µg/L)	8.4 (0.8)	7.3 (0.2)	7.6 (0.2)	7.93 (0.09)	7.8 (0.6)
As (µg/L)	1.02 (0.03)	1.0 (0.1)	1.36 (0.05)	0.91 (0.03)	0.89 (0.06)
Se (µg/L)	1.4 (0.3)	0.75 (0.03)	1.3 (0.1)	0.9 (0.3)	0.8 (0.2)
Rb (mg/L)	1.89 (0.01)	2.31 (0.05)	1.62 (0.01)	2.37 (0.02)	1.86 (0.05)
Sr (µg/L)	547 (6)	500 (12)	543 (2)	535 (1)	563 (13)
Y (ng/L)	10.6 (0.9)	11.5 (0.9)	18 (6)	9.8 (0.1)	8.1 (0.8)
Cd (µg/L)	0.407 (0.002)	0.56 (0.03)	0.3 (0.1)	0.42 (0.05)	0.43 (0.01)
Cs (µg/L)	4.00 (0.04)	2.88 (0.06)	2.32 (0.05)	3.60 (0.06)	3.4 (0.2)
Ba (µg/L)	377 (2)	339 (3)	395 (9)	352 (14)	363 (7)
Ce (ng/L)	24.4 (0.6)	27.0 (0.7)	22 (14)	22 (3)	18 (2)
Pr (ng/L)	2*	<LOQ	10[#]*	<LOQ	<LOQ
Nd (ng/L)	<LOQ	5[#]	8 (3)	7*	<LOQ
Sm (ng/L)	<LOQ	<LOQ	8[#]*	<LOQ	<LOQ
Eu (ng/L)	12 (2)	10.9 (0.6)	20 (6)	13.5 (0.9)	14.1 (0.3)
Gd (ng/L)	<LOQ	<LOQ	10[#]*	<LOQ	<LOQ
Tb (ng/L)	<LOQ	<LOQ	9[#]*	<LOQ	<LOQ
Dy (ng/L)	<LOQ	<LOQ	7[#]*	<LOQ	<LOQ
Ho (ng/L)	<LOQ	<LOQ	7[#]*	<LOQ	<LOQ
Er (ng/L)	<LOQ	<LOQ	6[#]*	<LOQ	<LOQ
Tm (ng/L)	<LOQ	<LOQ	6[#]*	<LOQ	<LOQ
Yb (ng/L)	<LOQ	<LOQ	6[#]*	<LOQ	<LOQ
Lu (ng/L)	<LOQ	<LOQ	5[#]*	<LOQ	<LOQ
Tl (µg/L)	0.31 (0.01)	0.20 (0.01)	0.20 (0.03)	0.212 (0.002)	0.20 (0.01)
Pb (µg/L)	14.3 (0.3)	12.3 (0.4)	12.9 (0.6)	14.75 (0.07)	13.32 (0.03)

[#] After recalibration of the apparatus; * Only one value of the two repetitions is bigger than the LOQ; LOQ = limit of quantification (Annex 2); MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 44 – Metals and Other Elements Analysis of Quinta do Gradil Wines (Part II – mechanical pruning)

Element	MEC TEST	MEC ETAR	MEC ESTR	MEC RSUC	MEC BIOC
Li (µg/L)	7.1 (0.1)	6.2 (0.2)	6.32 (0.02)	5.8 (0.3)	7.4 (0.1)
Be (µg/L)	0.54 (0.09)	0.6 (0.1)	0.44 (0.06)	0.34 (0.02)	0.6 (0.1)
Na (mg/L)	8.82 (0.02)	6.8 (0.2)	8.01 (0.02)	7.9 (0.2)	8.49 (0.002)
Mg (mg/L)	99 (1)	89 (3)	93.9 (0.6)	93 (3)	97.7 (0.4)
Al (mg/L)	0.069 (0.001)	0.069 (0.002)	0.0801 (0.0003)	0.10 (0.05)	0.0682 (0.0003)
Sc (µg/L)	3.0 (1.0)	1.20 (0.03)	1.1 (0.4)	1.7 (0.7)	2.5 (0.4)
V (µg/L)	0.27 (0.07)	0.41 (0.01)	0.47 (0.04)	0.33 (0.01)	0.29 (0.06)
Mn (mg/L)	5.32 (0.08)	5.5 (0.1)	3.43 (0.06)	3.9 (0.1)	9.9 (0.1)
Co (µg/L)	3.66 (0.03)	3.7 (0.2)	4.299 (0.004)	3.98 (0.09)	4.36 (0.06)
Ni (µg/L)	25.4 (0.8)	35 (3)	27.69 (0.05)	26.5 (0.9)	32.2 (0.8)
Zn (mg/L)	0.36 (0.01)	0.38 (0.01)	0.345 (0.004)	0.36 (0.01)	0.3 (4.6)
Ga (µg/L)	7.8 (0.3)	6.8 (0.2)	7.2 (0.1)	6.74 (0.09)	7.6 (0.1)
As (µg/L)	1.23 (0.09)	1.11 (0.01)	1.1 (0.1)	1.46 (0.07)	0.96 (0.09)
Se (µg/L)	0.7 (0.2)	0.5 (0.4)	0.61 (0.01)	0.6 (0.1)	0.79 (0.03)
Rb (mg/L)	1.72 (0.05)	1.92 (0.05)	1.43 (0.01)	1.48 (0.03)	1.62 (0.02)
Sr (µg/L)	570 (4)	533 (15)	553 (5)	519 (11)	565 (11)
Y (ng/L)	4 (1)	12 (3)	10.0 (2.0)	5.6 (0.3)	6.3 (0.2)
Cd (µg/L)	0.31 (0.02)	0.39 (0.04)	0.24 (0.04)	0.37 (0.03)	0.50 (0.01)
Cs (µg/L)	6.28 (0.01)	8.5 (0.2)	5.97 (0.01)	166 (5)	5.85 (0.09)
Ba (µg/L)	346 (2)	346 (13)	415 (3)	339 (6)	397 (10)
Ce (ng/L)	24.07 (0.02)	31 (1)	28.9 (3.0)	21.8 (0.8)	26.0 (0.8)
Pr (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Nd (ng/L)	<LOQ	6*	6*	<LOQ	<LOQ
Sm (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Eu (ng/L)	11.8 (0.2)	13 (3)	13 (1)	11 (2)	14 (1)
Gd (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tb (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Dy (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ho (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Er (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tm (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Yb (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lu (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tl (µg/L)	0.44 (0.02)	0.19 (0.01)	0.18 (0.02)	0.16 (0.01)	0.18 (0.01)
Pb (µg/L)	8.2 (0.1)	11.1 (0.7)	6.7 (0.1)	9.3 (0.2)	8.46 (0.02)

* Only one value of the two repetitions is bigger than the LOQ; LOQ = limit of quantification (Annex 2); MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 45 – Metals and Other Elements Analysis of Rio Frio Wines

Element	MAN TEST	MAN ETAR	MAN ESTR	MAN RSUC	MAN BIOC
Li (µg/L)	4.44 (0.06)	6.9 (0.1)	3.902 (0.004)	5.165 (0.003)	12.7 (0.2)
Be (µg/L)	0.139 (0.004)	0.152 (0.002)	0.2836 (0.0003)	0.13 (0.01)	0.46 (0.01)
Na (mg/L)	9.1 (0.2)	15.97 (0.2)	11.49 (0.05)	9.3 (0.1)	9.6 (0.2)
Mg (mg/L)	88 (1)	101 (2)	107 (1)	83.9 (0.2)	106.7 (0.7)
Al (mg/L)	0.219 (0.002)	0.30 (0.01)	0.261 (0.003)	0.20 (0.01)	0.179 (0.003)
V (µg/L)	0.41 (0.02)	0.48 (0.01)	0.31 (0.02)	0.32 (0.03)	0.294 (0.001)
Mn (mg/L)	0.85 (0.01)	1.24 (0.01)	0.95 (0.01)	0.922 (0.004)	1.23 (0.02)
Co (µg/L)	2.85 (0.03)	3.5 (0.1)	3.20 (0.01)	2.98 (0.01)	6.4 (0.1)
Ni (µg/L)	14.5 (0.9)	18.3 (0.7)	13.3 (0.3)	13.9 (0.3)	20.6 (0.7)
Zn (mg/L)	1.21 (0.04)	1.32 (0.02)	1.078 (0.004)	0.972 (0.004)	1.37 (0.05)
Ga (µg/L)	5.27 (0.07)	5.19 (0.01)	5.9 (0.1)	4.7 (0.1)	5.2 (0.2)
As (µg/L)	0.92 (0.02)	1.096 (0.001)	0.85 (0.01)	0.86 (0.03)	0.841 (0.001)
Se (µg/L)	0.45 (0.04)	0.772 (0.001)	0.74 (0.08)	0.6 (0.1)	0.6 (0.2)
Rb (mg/L)	1.746 (0.002)	2.02 (0.03)	2.35 (0.01)	1.97 (0.02)	2.16 (0.02)
Sr (µg/L)	324.31 (0.01)	396 (5)	415 (2)	349 (2)	397 (2)
Y (ng/L)	14 (2)	18 (2)	51 (1)	14 (1)	53 (2)
Cd (µg/L)	0.14 (2.00)	0.22 (0.01)	0.09 (0.04)	0.101 (0.003)	0.19 (0.05)
Cs (µg/L)	3.78 (0.04)	3.14 (0.01)	2.94 (0.02)	2.95 (0.01)	6.51 (0.02)
Ba (µg/L)	172.3 (0.3)	153 (1)	182.5 (0.8)	221 (7)	185.7 (0.1)
La (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ce (ng/L)	39.9 (0.8)	43 (8)	61 (2)	45 (4)	78 (4)
Pr (ng/L)	3*	3.3 (0.6)	5.1 (0.4)	<LOQ	8.0 (0.3)
Nd (ng/L)	<LOQ	7*	20 (3)	6*	12 (4)
Sm (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Eu (ng/L)	20.1 (0.7)	16.9 (0.9)	21 (5)	23 (2)	22.4 (0.6)
Gd (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	4.9 (0.4)
Tb (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Dy (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ho (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Er (ng/L)	<LOQ	<LOQ	6 (2)	<LOQ	5*
Tm (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Yb (ng/L)	<LOQ	<LOQ	4*	<LOQ	3*
Lu (ng/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tl (µg/L)	0.320 (0.003)	0.327 (0.004)	0.610 (0.003)	0.4819 (0.0005)	0.62 (0.04)
Pb (µg/L)	20.2 (0.1)	17.4 (0.2)	15.4 (0.1)	15.05 (0.2)	16.7 (0.4)

* Only one value of the two repetitions is bigger than the LOQ; LOQ = limit of quantification (Annex 2); MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Statistical Analysis of Heavy Metal and Other Elements Content Results

Table 46 - Statistical Analysis of the results from Metals and Other Elements Analysis of Quinta do Côro Wines (Part I)

	Li (µg/L)	Be (µg/L)	Na (mg/L)	Mg (mg/L)	Al (mg/L)	V (µg/L)	Mn (mg/L)	Co (µg/L)
MAN	2.78	0.06	4.51	91.90	0.0616	0.307	0.60	2.61
MEC	3.38	0.06	3.74	94.58	0.0694	0.319	0.68	2.34
Sig.	***	ns	***	ns	***	ns	***	***
TEST	2.96b	0.07	4.11b	98.63a	0.0544cd	0.305	0.75a	2.52b
ETAR	2.94b	0.07	4.57a	87.82c	0.0528d	0.369	0.62bc	2.37c
ESTR	3.71a	0.05	3.76c	91.37bc	0.0580c	0.286	0.63b	2.66a
RSUC	2.70c	0.07	4.43a	93.68abc	0.0992a	0.279	0.58c	2.46bc
BIOC	3.10b	0.05	3.77c	94.70ab	0.0630b	0.326	0.63b	2.35c
Sig.	*	ns	*	*	*	ns	*	*
MAN TEST	2.92cde	0.06	4.09cd	94.18abc	0.0557cd	0.279ab	0.59cde	2.68b
MAN ETAR	2.74de	0.07	5.46a	90.35bc	0.0569c	0.294ab	0.63bc	2.45cde
MAN ESTR	2.98cd	0.05	4.12c	91.54bc	0.0561cd	0.285ab	0.63bd	2.94a
MAN RSUC	2.65e	0.07	4.90b	91.77bc	0.0685b	0.274ab	0.57de	2.35e
MAN BIOC	2.61e	0.05	4.01cd	91.66bc	0.0707b	0.402ab	0.59cde	2.60bc
MEC TEST	3.01cd	0.07	4.13c	103.08a	0.0531cd	0.331ab	0.91a	2.37e
MEC ETAR	3.13c	0.07	3.68def	85.28c	0.0486d	0.444a	0.60bd	2.28ef
MEC ESTR	4.43a	0.05	3.40f	91.21bc	0.0599c	0.286ab	0.64bc	2.38de
MEC RSUC	2.75de	0.08	3.96cde	95.60abc	0.1300a	0.283ab	0.60bd	2.57bc
MEC BIOC	3.60b	0.05	3.54ef	97.74ab	0.0554cd	0.250b	0.66b	2.10f
Sig.	*	ns	*	*	*	*	*	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 47 - Statistical Analysis of the results from Metals and Other Elements Analysis of Quinta do Côro Wines (Part II)

	Ni (µg/L)	Zn (mg/L)	Ga (µg/L)	As (µg/L)	Se (µg/L)	Rb (mg/L)	Sr (µg/L)	Y (ng/L)
MAN	15.4	0.65	6.56	0.26	0.32	6.09	520.2	244.9
MEC	16.0	0.39	5.02	0.28	0.39	4.83	565.9	140.8
Sig.	ns	***	***	ns	ns	***	***	***
TEST	14.7	0.50c	6.49a	0.29ab	0.38	5.62a	552.9ab	81.5
ETAR	18.0	0.59a	4.09c	0.30a	0.45	5.09b	515.4cd	57.9
ESTR	15.9	0.53b	5.52b	0.26bc	0.32	5.55a	587.1a	93.6
RSUC	14.6	0.52bc	6.54a	0.26bc	0.28	5.53a	508.7d	82.4
BIOC	15.4	0.46d	6.31a	0.24c	0.34	5.53a	551.1bc	70.3
Sig.	**	*	***	*	ns	**	*	ns
MAN TEST	15.1	0.58c	7.14	0.24c	0.35	6.14	485.0d	48.5
MAN ETAR	17.4	0.81a	4.96	0.32ab	0.37	5.56	530.4bcd	42.4
MAN ESTR	15.0	0.67b	6.33	0.26bc	0.38	6.04	565.3ac	62.4
MAN RSUC	14.5	0.58c	7.36	0.25c	0.24	6.48	500.6d	49.9
MAN BIOC	15.3	0.60c	7.03	0.24c	0.25	6.25	519.6cd	41.7
MEC TEST	14.2	0.42de	5.83	0.34a	0.42	5.10	620.9a	33.0
MEC ETAR	18.6	0.37f	3.23	0.28bc	0.54	4.61	500.3d	15.5
MEC ESTR	16.9	0.38ef	4.71	0.26c	0.25	5.06	609.0a	31.3
MEC RSUC	14.8	0.46d	5.72	0.27bc	0.33	4.58	516.8cd	32.5
MEC BIOC	15.4	0.31g	5.59	0.25c	0.43	4.80	582.6ab	28.6
Sig.	ns	*	ns	*	ns	ns	*	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 48 - Statistical Analysis of the results from Metals and Other Elements Analysis of Quinta do Côro Wines (Part III)

	Cd (µg/L)	Cs (µg/L)	Ba (µg/L)	Ce (ng/L)	Eu (ng/L)	Tl (µg/L)	Pb (µg/L)
MAN	0.21	76.06	259	31	22.9	5.01	6.81
MEC	0.20	91.68	290	25	22.9	4.28	7.23
Sig.	ns	***	***	ns	ns	***	**
TEST	0.23	63.07c	263b	29	22.3	4.48bc	7.14bc
ETAR	0.23	109.50b	260b	26	19.0	5.21a	7.91a
ESTR	0.18	49.20d	326a	34	30.0	4.53bc	7.25b
RSUC	0.20	58.59d	260b	25	22.3	4.29c	6.20cd
BIOC	0.19	138.98a	267b	26	21.4	4.72b	6.60d
Sig.	ns	**	***	ns	ns	*	*
MAN TEST	0.24	66.76de	233f	24	20.8	4.74bc	7.99a
MAN ETAR	0.22	122.56b	267cde	31	18.9	5.35a	7.76abc
MAN ESTR	0.20	55.14f	300b	43	33.3	5.08ab	7.00bcd
MAN RSUC	0.19	63.10df	255ef	27	20.9	4.46cd	4.46e
MAN BIOC	0.18	72.74d	244ef	30	20.4	5.44a	6.85cd
MEC TEST	0.23	59.38ef	293bc	34	23.7	4.21cd	6.29d
MEC ETAR	0.24	96.44c	253ef	21	19.1	5.07ab	8.05a
MEC ESTR	0.15	43.26g	351a	25	26.6	3.97d	7.51abc
MEC RSUC	0.21	54.09f	265cde	22	22.7	4.13d	7.93ab
MEC BIOC	0.20	205.21a	290bd	23	22.3	4.00d	6.35d
Sig.	ns	*	*	ns	ns	*	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 49 - Statistical Analysis of the results from Metals and Other Elements Analysis of Quinta do Gradil Wines (Part I)

	Li (µg/L)	Be (µg/L)	Na (mg/L)	Mg (mg/L)	Al (mg/L)	Sc (µg/L)	V (µg/L)	Mn (mg/L)	Co (µg/L)
MAN	7.91	0.68	9.08	96.27	0.0854	1.4	0.15	7.36	4.56
MEC	6.56	0.51	8.00	94.53	0.0773	2.0	0.35	5.60	3.99
Sig.	***	***	***	ns	ns	*	**	***	***
TEST	8.17a	0.64ab	9.43a	95.46	0.0769	2.2	0.21	4.88d	3.98c
ETAR	6.90c	0.69ab	6.90c	92.74	0.0758	1.9	0.36	7.77b	4.32ab
ESTR	6.50c	0.51bc	8.78b	97.64	0.0777	1.2	0.35	3.93e	4.12bc
RSUC	6.96c	0.42c	8.66b	94.66	0.0995	1.4	0.18	6.66c	4.32b
BIOC	7.64b	0.72a	8.92b	96.50	0.0768	1.7	0.16	9.19a	4.64a
Sig.	*	*	**	ns	ns	ns	ns	***	*
MAN TEST	9.29a	0.74	10.05a	92.07bc	0.0851	1.1b	0.16	4.43e	4.29bc
MAN ETAR	7.55bc	0.73	7.04g	96.39abc	0.0829	2.6ab	0.31	10.08a	4.99a
MAN ESTR	6.69deg	0.59	9.55ab	101.38a	0.0752	1.2ab	0.24	4.42e	3.95ce
MAN RSUC	8.15b	0.50	9.42b	96.26abc	0.0982	1.1ab	0.02	9.46b	4.65ab
MAN BIOC	7.86b	0.86	9.34bc	95.27abc	0.0854	0.9b	0.03	8.43c	4.92a
MEC TEST	7.05cde	0.54	8.82cd	98.85ab	0.0686	3.3a	0.27	5.32d	3.65e
MEC ETAR	6.25fg	0.65	6.76g	89.08c	0.0686	1.2ab	0.41	5.47d	3.66e
MEC ESTR	6.32ef	0.44	8.01ef	93.91abc	0.0801	1.1b	0.47	3.43f	4.30bc
MEC RSUC	5.76f	0.34	7.90f	93.06abc	0.1009	1.7ab	0.33	3.85f	3.98ce
MEC BIOC	7.41bd	0.57	8.49de	97.73ab	0.0682	2.5ab	0.29	9.94a	4.36bc
Sig.	*	ns	*	*	ns	*	ns	*	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 50 - Statistical Analysis of the results from Metals and Other Elements Analysis of Quinta do Gradil Wines (Part II)

	Ni (µg/L)	Zn (mg/L)	Ga (µg/L)	As (µg/L)	Se (µg/L)	Rb (mg/L)	Sr (µg/L)	Y (ng/L)
MAN	29.48	0.55	7.81	1.04	1.02	2.01	537.67	11.7
MEC	29.28	0.36	7.22	1.18	0.64	1.63	547.99	7.4
Sig.	ns	***	**	**	**	***	*	**
TEST	27.91b	0.54a	8.09a	1.12ac	1.05	1.81c	558.29a	7.1b
ETAR	33.96a	0.46b	7.05b	1.07bcd	0.62	2.12a	516.48c	11.6ab
ESTR	26.36b	0.41c	7.39ab	1.25a	0.96	1.52d	548.33ab	14.2a
RSUC	26.66b	0.42c	7.34ab	1.18ab	0.74	1.92b	527.33bc	7.7b
BIOC	32.01a	0.43bc	7.70ab	0.92d	0.78	1.74c	563.70a	7.2b
Sig.	**	*	**	*	ns	**	**	*
MAN TEST	30.39abcd	0.73a	8.40	1.02cd	1.40	1.89b	546.60abd	10.6
MAN ETAR	33.34a	0.53b	7.33	1.03cd	0.75	2.31a	500.18c	11.5
MAN ESTR	25.03e	0.48b	7.62	1.36ab	1.31	1.62d	543.29abd	18.4
MAN RSUC	26.83ce	0.48b	7.93	0.91d	0.86	2.37a	535.39abcd	9.8
MAN BIOC	31.80abc	0.53b	7.78	0.89d	0.78	1.86bc	562.88ab	8.1
MEC TEST	25.43de	0.36c	7.78	1.23ac	0.70	1.72cd	569.99a	3.5
MEC ETAR	34.58a	0.38c	6.77	1.11bcd	0.49	1.92b	532.78bc	11.6
MEC ESTR	27.69be	0.34c	7.17	1.13bcd	0.61	1.43e	553.37abd	10.0
MEC RSUC	26.48de	0.36c	6.74	1.46a	0.63	1.48e	519.27cd	5.6
MEC BIOC	32.21ab	0.34c	7.62	0.96cd	0.79	1.62d	564.51ab	6.3
Sig.	*	***	ns	*	ns	*	*	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 51 - Statistical Analysis of the results from Metals and Other Elements Analysis of Quinta do Gradil Wines (Part III)

	Cd (µg/L)	Cs (µg/L)	Ba (µg/L)	Ce (ng/L)	Eu (ng/L)	Tl (µg/L)	Pb (µg/L)
MAN	0.42	3.25	365.12	22.70	14.3	0.22	13.50
MEC	0.36	38.49	368.33	26.40	12.4	0.23	8.74
Sig.	*	***	ns	ns	ns	ns	***
TEST	0.36bc	5.14b	361.28bc	24.22	12.1	0.37a	11.22ab
ETAR	0.48a	5.71b	342.66c	29.09	11.8	0.19b	11.70a
ESTR	0.26c	4.15b	404.96a	25.67	16.6	0.19b	9.80c
RSUC	0.40ab	84.70a	345.09c	22.03	12.1	0.19b	12.00a
BIOC	0.47a	4.65b	379.64b	21.74	14.0	0.19b	10.89b
Sig.	*	***	**	ns	Ns	***	*
MAN TEST	0.41abcd	4.00b	376.95bc	24.38	12.5	0.31b	14.25ab
MAN ETAR	0.56a	2.88b	339.08d	27.00	11.0	0.20c	12.29cd
MAN ESTR	0.28cd	2.32b	395.41ab	22.40	20.4	0.20c	12.92c
MAN RSUC	0.42abc	3.60b	351.65cd	22.24	13.5	0.21c	14.75a
MAN BIOC	0.43abc	3.45b	362.52cd	17.50	14.1	0.20c	13.32bc
MEC TEST	0.31cd	6.28b	345.62cd	24.07	11.8	0.44a	8.19e
MEC ETAR	0.39bcd	8.54b	346.24cd	31.19	12.6	0.19c	11.12d
MEC ESTR	0.24d	5.97b	414.51a	28.94	12.7	0.18c	6.69f
MEC RSUC	0.37bcd	165.79a	338.53d	21.83	10.8	0.16c	9.25e
MEC BIOC	0.50ab	5.85b	396.77ab	25.98	13.9	0.18c	8.46e
Sig.	*	***	*	ns	ns	**	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 52 - Statistical Analysis of the results from Metals and Other Elements Analysis of Herdade de Rio Frio Wines (Part I)

	Li (µg/L)	Be (µg/L)	Na (mg/L)	Mg (mg/L)	Al (mg/L)	V (µg/L)	Mn (mg/L)	Co (µg/L)
TEST	4.44d	0.14c	9.13c	87.78c	0.219c	0.41a	0.85c	2.85d
ETAR	6.92b	0.15c	15.97a	100.78b	0.301a	0.48a	1.24a	3.49b
ESTR	3.90e	0.28b	11.49b	107.11a	0.261b	0.31b	0.95b	3.20bc
RSUC	5.17c	0.13c	9.30c	83.85c	0.201cd	0.32b	0.92b	2.98cd
BIOC	12.73a	0.46a	9.55c	106.77a	0.179d	0.29b	1.23a	6.41a
Sig.	*	***	***	*	**	*	**	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 53 - Statistical Analysis of the results from Metals and Other Elements Analysis of Herdade de Rio Frio Wines (Part II)

	Ni (µg/L)	Zn (mg/L)	Ga (µg/L)	As (µg/L)	Se (µg/L)	Rb (mg/L)	Sr (µg/L)	Y (ng/L)
TEST	14.5b	1.21b	5.27b	0.92b	0.45	1.75d	324.3d	14b
ETAR	18.3a	1.32ab	5.19b	1.10a	0.77	2.02c	395.6b	18b
ESTR	13.3b	1.08c	5.89a	0.85b	0.74	2.35a	415.0a	51a
RSUC	13.9b	0.97c	4.65c	0.86b	0.60	1.97c	348.8c	14b
BIOC	20.6a	1.37a	5.22b	0.84b	0.60	2.16b	396.7b	53a
Sig.	**	*	*	**	ns	**	**	***

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

Table 54 - Statistical Analysis of the results from Metals and Other Elements Analysis of Herdade de Rio Frio Wines (Part III)

	Cd (µg/L)	Cs (µg/L)	Ba (µg/L)	Ce (ng/L)	Eu (ng/L)	Tl (µg/L)	Pb (µg/L)
TEST	0.14ab	3.78b	172.3c	39.9c	20.1	0.320c	20.2a
ETAR	0.22a	3.14c	152.8d	43.0c	16.9	0.327c	17.4b
ESTR	0.09b	2.94d	182.5bc	61.0ab	21.4	0.610a	15.4c
RSUC	0.10b	2.95d	220.9a	44.7bc	23.4	0.482b	15.1c
BIOC	0.19ab	6.51a	185.7b	78.0a	22.4	0.617a	16.7b
Sig.	*	**	*	*	ns	**	**

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

In general terms, the pruning technique did not influence Mg, Ni, Ce and Eu concentrations in both vineyards were two types of techniques were applied. In what organic amendments are concerned, ETAR was the treatment that conducted to higher heavy metal content in the wine samples. The same results were obtained by Mendoza et al (2006) in a study of metal availability and uptake by plants amended with this type of product, however more years are necessary to study the long-term effects of the ETAR application and use as an organic amendment.

Some heavy metals have legal limits to obey – Zn, As, Cd and Pb – as they can have impact on consumer's health and present a risk for environment (OIV 2015). In the three wineries, the wine samples have lower concentrations than these limits which is a positive aspect in what consumers' health in concerned (Tables 46-54). After statistical analysis is possible to affirm that there is a tendency for wines from manually pruned vines to have higher contents of Zn and Cd, while As levels were more elevated when mechanical pruned was used. In Pb amount Quinta do Côro had higher values for mechanical pruning while in Quinta do Gradil manual pruning led to higher concentrations. In organic amendments, there´s a tendency for ETAR to increase the amount of As and Cd although the results are sometimes different for each element which suggests that different mechanisms are involved in metal bioavailability as suggested by Leita et al (1999). Besides, these differences mean that these results are specific for that soil characteristics and soil's nutrient composition.

Antolín et al (2005) conducted an experiment in which they analyzed the effect of the application of sewage sludge in heavy metal concentration, among other parameters, in a Mediterranean soil. These authors observed that this type of organic amendment increased soil extractable Cd, Mn, Pb and Zn. The same results were obtained in this experiment, especially for Cd.

Due to its nature, it was expected that RSUC influenced the amount of these elements in wine once it is a heterogeneous mixture of municipal wastes that usually contains metals (Woodbury 1992). However, the results from our experiment show that this organic amendment is the one that led to less increase in the heavy metals content.

According to Catarino et al (2008) Mn concentration in wine usually is below 3 mg/L, however in Quinta do Gradil all the values are higher for all treatments applied. This shows a possible soil contamination by the previous use of amendments rich in this element, or the use of phytosanitary products rich in manganese salts.

In Quinta do Côro, Tl concentration varies between 4 and 5 µg/L which suggests a possible contamination according to Catarino et al (2008), the authors state that Tl concentration in non-contaminated wines is usually lower than 1 µg/L although contaminations originated in the soil and previous amendments used can increase this content to 2 to 8 µg/L.

4.7. Sensory Analysis of the Wines

Sensory analysis of the wines showed that generally when manual pruning was applied the wines received higher scores (Tables 39 and 41). Organic amendments also influenced the score given by the tasters, the wines from plots without any treatment had higher scores in 8 of the 15 attributes (Tables 40, 42 and 43). The wines from Quinta do Côro received the best marks while the ones from Herdade de Rio Frio had the lowest.

Table 55 - Effect of Pruning Technique on Sensory Analysis of Quinta do Côro Wines

General Attribute	Tasting Attribute	Pruning Technique (average values)		Sig.
		MAN	MEC	
Color	Red	3.73	3.59	ns
	Violet	2.75	2.64	ns
Aroma	Fruity	3.28	3.27	ns
	Floral	2.30	2.17	ns
	Vegetal	2.26	2.07	ns
	Jam	2.47	2.18	ns
	Intensity	3.44	3.25	ns
	Balance (nose)	3.36	3.11	ns
Taste	Body	2.79	2.81	ns
	Bitterness	1.83	1.90	ns
	Astringency	3.71	3.21	**
	Acidity	2.52	2.67	ns
	Persistency	3.42	3.19	ns
	Balance (mouth)	3.41	3.05	*
Global Appreciation		3.31	2.98	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

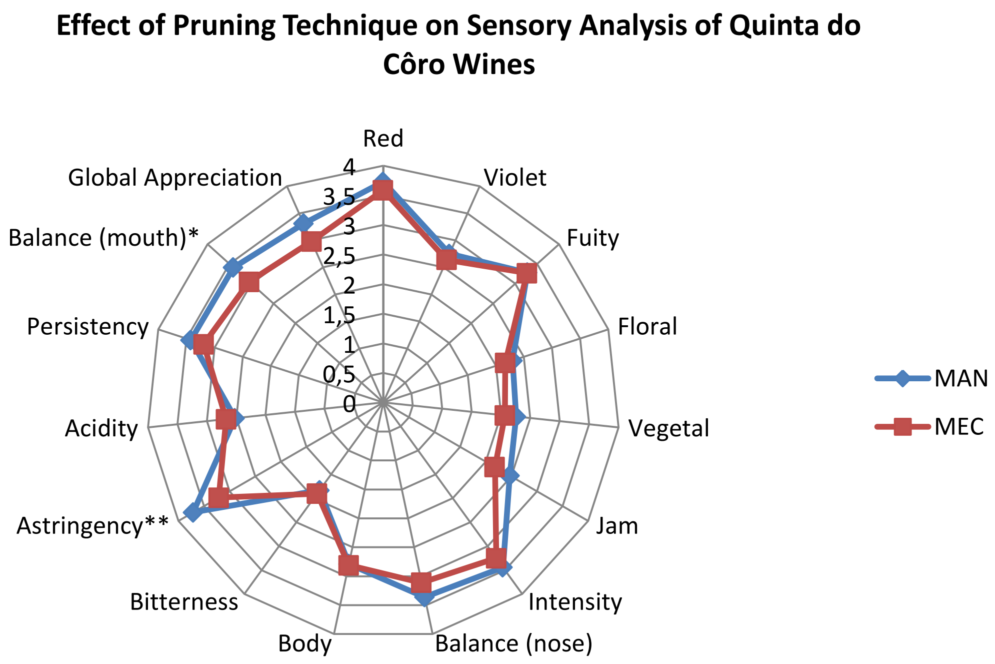


Figure 2 – Effect of Pruning Technique on Sensory Analysis of Quinta do Côro Wines

Table 56 - Effect of Organic Amendments on Sensory Analysis of Quinta do Côro Wines

General Attribute	Tasting Attribute	Organic Amendment (average values)					*Sig.*	*Int.*
		TEST	ETAR	ESTR	RSUC	BIOC		
Color	Red	3.70	3.38	3.58	3.80	3.85	ns	ns
	Violet	3.03	2.28	2.58	2.50	3.10	ns	ns
Aroma	Fruity	3.40	3.25	3.15	3.18	3.40	ns	ns
	Floral	2.30	2.40	2.28	2.00	2.20	ns	ns
	Vegetal	1.98	2.15	2.15	2.43	2.13	ns	ns
	Jam	2.63	2.30	2.18	2.05	2.48	ns	ns
	Intensity	3.45	3.35	3.35	3.20	3.38	ns	ns
	Balance (nose)	3.50	3.23	3.10	3.08	3.28	ns	ns
Taste	Body	2.93	2.58	2.88	2.75	2.88	ns	ns
	Bitterness	1.88	1.80	1.95	1.85	1.85	ns	ns
	Astringency	3.65	3.23	3.53	3.30	3.60	ns	ns
	Acidity	2.60	2.85	2.58	2.48	2.48	ns	ns
	Persistency	3.50	3.05	3.43	3.13	3.43	ns	ns
	Balance (mouth)	3.53	2.98	3.13	3.15	3.38	ns	ns
Global Appreciation		3.40	2.98	2.98	3.08	3.30	ns	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

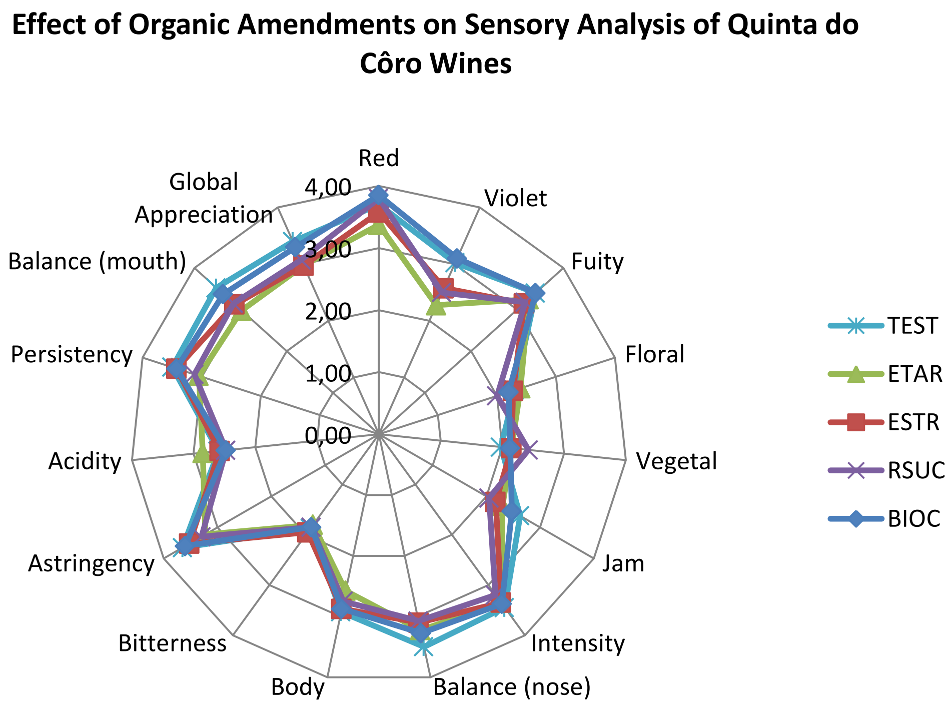


Figure 3 – Effect of Organic Amendments on Sensory Analysis of Quinta do Côro Wines

Table 57 - Effect of Pruning Technique on Sensory Analysis of Quinta do Gradil Wines

General Attribute	Tasting Attribute	Pruning Technique (average values)		*Sig.*
		MAN	MEC	
Color	Red	3.56	3.20	ns
	Violet	2.58	2.36	ns
Aroma	Fruity	3.23	3.01	ns
	Floral	2.06	1.91	ns
	Vegetal	1.82	1.93	ns
	Jam	2.35	1.98	ns
	Intensity	3.20	3.10	ns
	Balance (nose)	3.05	2.85	ns
Taste	Body	3.12	2.85	*
	Bitterness	2.05	1.82	ns
	Astringency	2.65	2.45	ns
	Acidity	2.80	2.73	ns
	Persistency	3.07	3.02	ns
	Balance (mouth)	2.89	2.60	*
Global Appreciation		3.11	2.83	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

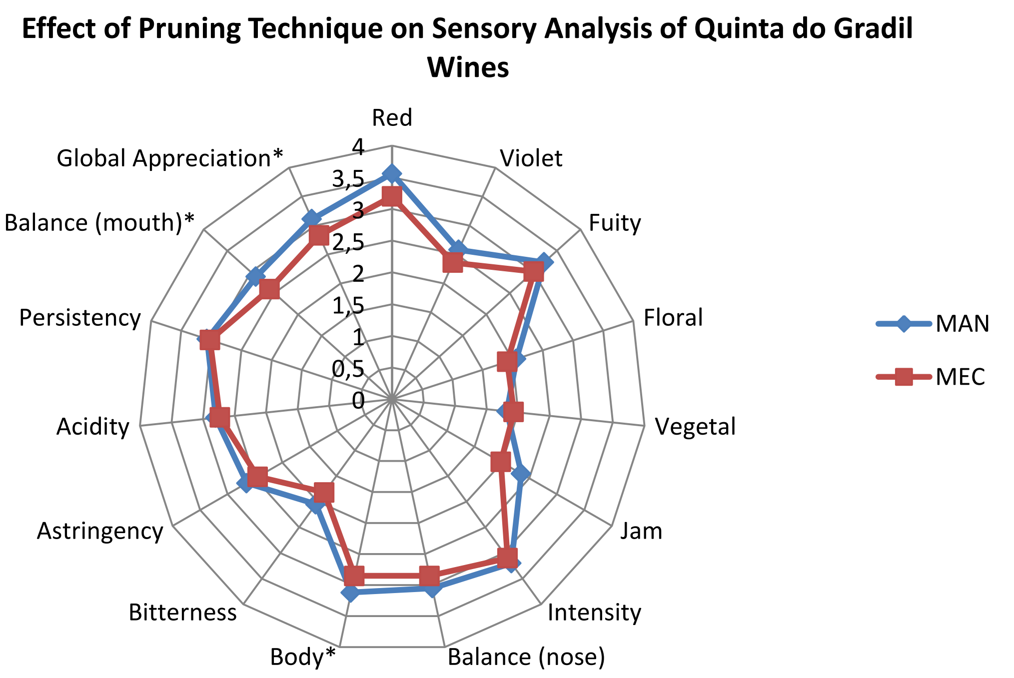


Figure 4 - Effect of Pruning Technique on Sensory Analysis of Quinta do Gradil Wines

Table 58 - Effect of Organic Amendments on Sensory Analysis of Quinta do Gradil Wines

General Attribute	Tasting Attribute	Organic Amendment (average values)					*Sig.*	*Int.*
		TEST	ETAR	ESTR	RSUC	BIOC		
Color	Red	3.50	2.85	3.35	3.58	3.63	ns	ns
	Violet	2.75	2.20	2.40	2.45	2.55	ns	ns
Aroma	Fruity	3.13	2.85	3.43	3.18	3.03	ns	ns
	Floral	2.05	1.90	1.95	2.00	2.03	ns	ns
	Vegetal	1.90	2.15	1.80	1.80	1.73	ns	ns
	Jam	2.33	2.00	2.25	2.10	2.15	ns	ns
	Intensity	3.40	2.85	3.20	3.15	3.15	ns	ns
	Balance (nose)	3.20 a	2.63 b	3.00 ab	3.1 ab	2.83 ab	*	ns
Taste	Body	3.38 a	2.55 b	2.88 ab	3.03 ab	3.10 a	*	ns
	Bitterness	1.73	2.13	1.93	1.88	2.03	ns	ns
	Astringency	2.68	2.43	2.43	2.63	2.60	ns	ns
	Acidity	2.68	2.88	2.75	2.70	2.83	ns	ns
	Persistency	3.33	2.70	3.05	3.00	3.15	ns	ns
	Balance (mouth)	3.03 a	2.33 b	2.83 ab	2.75 ab	2.80 ab	*	ns
Global Appreciation		3.33 a	2.60 b	2.98 ab	3.00 ab	2.95 ab	*	ns

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

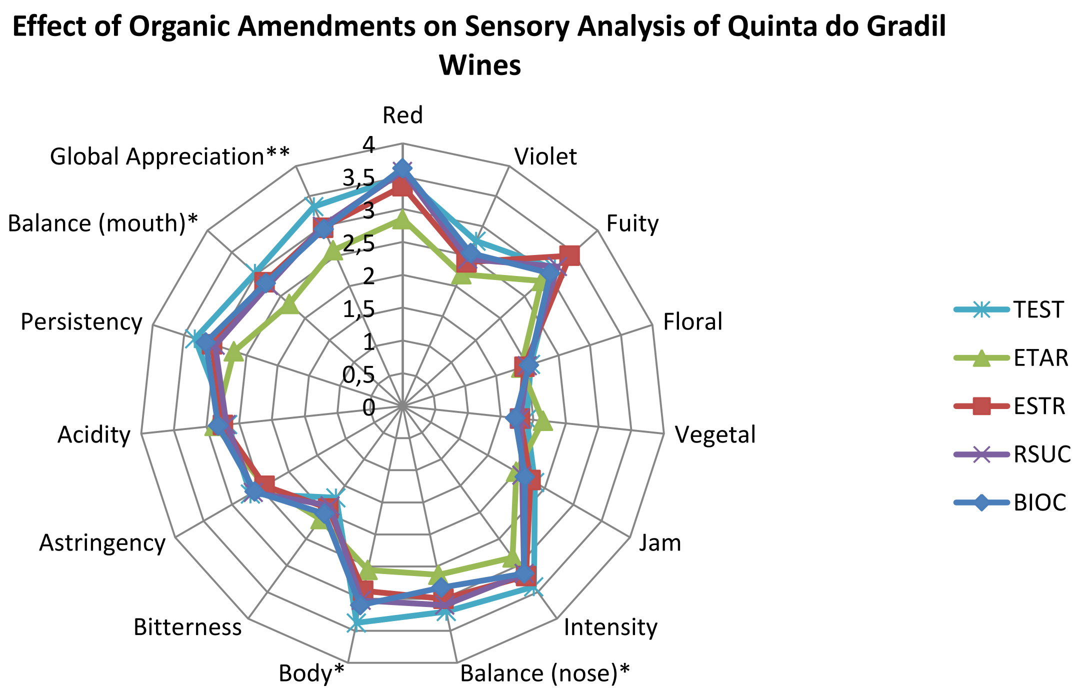


Figure 5 – Effect of Organic Amendments on Sensory Analysis of Quinta do Gradil Wines

Table 59 - Effect of Organic Amendments on Sensory Analysis of Herdade de Rio Frio Wines

General Attribute	Tasting Attribute	Organic Amendment (average values)					*Sig.*
		TEST	ETAR	ESTR	RSUC	BIOC	
Color	Red	2.92	3.17	4.00	3.33	3.08	ns
	Violet	1.75	1.92	2.17	1.92	1.58	ns
Aroma	Fruity	2.92	3.00	1.92	3.00	2.92	ns
	Floral	1.58	1.42	1.08	1.25	1.58	ns
	Vegetal	1.67	1.58	2.17	1.83	1.83	ns
	Jam	1.50	1.67	1.17	1.83	1.50	ns
	Intensity	3.00	3.25	2.83	3.00	2.83	ns
	Balance (nose)	2.75 a	2.92 a	1.42 b	2.67 a	2.67 a	*
Taste	Body	2.33	2.50	2.17	2.67	2.42	ns
	Bitterness	1.58	1.50	2.33	1.58	1.42	ns
	Astringency	2.00	2.42	2.42	2.00	2.25	ns
	Acidity	2.58	2.50	2.33	2.58	2.67	ns
	Persistency	2.42	2.50	2.25	2.42	2.75	ns
	Balance (mouth)	2.58	2.67	1.75	2.83	2.67	ns
Global Appreciation		2.50 ab	2.83 a	1.33 b	2.75 ab	2.67 ab	*

* p-value between 0.05 – 0.01; ** p-value between 0.01 – 0.001; *** p-value lower than 0.001; ns: not significant; MAN – manual pruning; MEC – mechanical pruning; TEST – control plot; ETAR – sewage sludge; ESTR – cattle manure; RSUC – municipal solid waste compost; BIOC – biochar

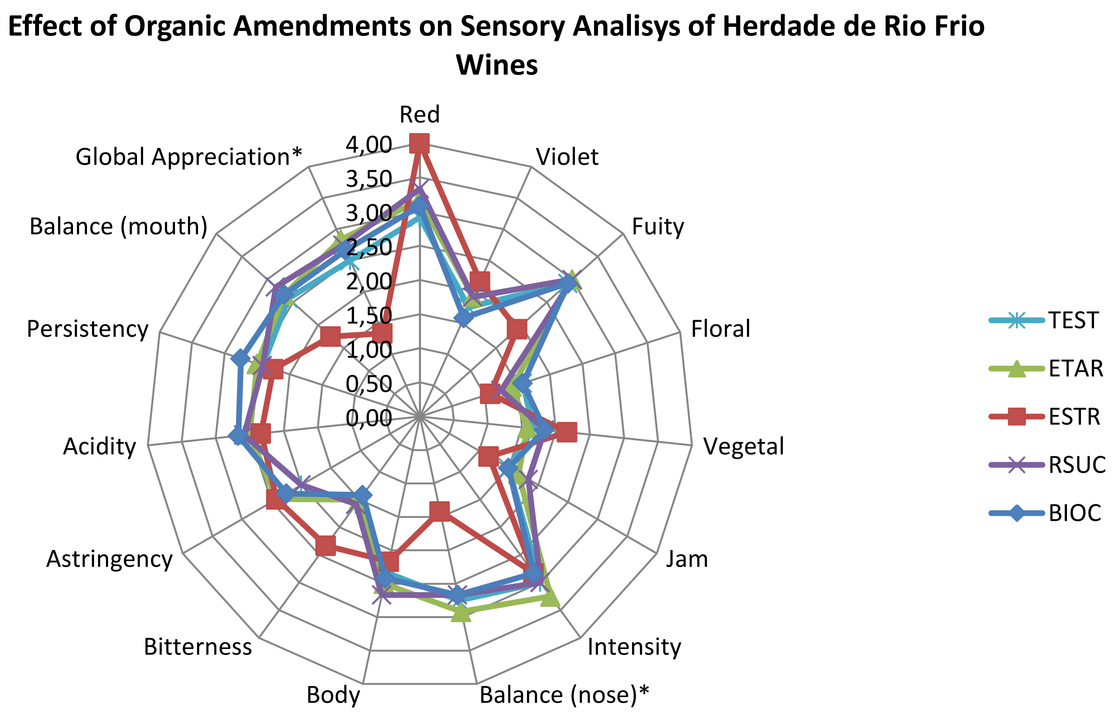


Figure 6 - Effect of Organic Amendment on Sensory Analysis of Herdade de Rio Frio Wines

In Quinta do Côro, the pruning technique used led to statistically different results only for two attributes – astringency and balance in the mouth, in which manual pruning originated more astringent wines but with more balance (Figure 1). This astringency result is according to the higher values of tannin power and total phenols found in wines from manually pruned vines. Since the overall differences are not significant, the global appreciation of the wines is similar, it is possible to affirm that the pruning technique did not influence wine quality in a perceived level by the tasting panel (Table 55). In Quinta do Gradil manual pruning led to wines with more body, more balance and with higher global appreciation which means that in this terroir, tasters prefer wines from manually pruned vines (Figure 3). Once more, this is according to the values determined in laboratory analysis in which manual pruning led to wines with more total phenols and tannin power. In both cases, color attributes were highly scored when manual pruning was applied, which can be explained by the fact that mechanically pruned vines had more canes and leaves that shaded the clusters leading to a smaller concentration of phenolic compounds. In Quinta do Côro the aroma characteristics have higher scores when manual pruning was applied while in Quinta do Gradil the perception of the vegetative aroma is higher for wines resultant from mechanically pruned vines (Table 57), which can be explained by the fact that these grapes may have been less mature than the ones originated from manual pruning. In what taste attributes are concerned, in Quinta do Côro mechanical pruning led to wines with more bitterness, acidity and astringency while in Quinta do Gradil manual pruning resulted in wines with higher scores for all the attributes. The same results are presented by Herderich et al (2006), in their study the wines from mechanically pruned vines received the lower scores in the sensory analysis.

Organic amendments affected wine quality, in what sensory analysis is concerned especially in Quinta do Gradil (Table 58) and Herdade de Rio Frio (Table 59). In Quinta do Côro (Table 56) the results were not statistically different however if the absolute values are considered it is possible to see that the wines from control plots received the higher scores for the majority of the attributes, including the wine's global appreciation (Figure 2). In this winery the wines from plots amended with ETAR and ESTR were the less appreciated by the tasters, this is in agreement to the findings of Morlat and Symoneaux (2008) and with the results presented by Kaushal (2014). In Quinta do Gradil wines from control plots and the ones from plots amended with BIOC registered four attributes that were influenced by the organic amendments - balance in the nose, body (volume in the mouth), balance in the mouth and global appreciation (Figure 4). These differences in the body of the wine perceived by the tasters may be explained by the higher total phenols, polymerized molecules and tannin power found in wines treated with BIOC and from the control plot. In all of these attributes, the control plots originated wines with the best scores while ETAR led to wines less appreciated. Korboulewsky et al. (2004) also found that ETAR application to the soil led to wines less appreciated by the tasters once it increased the animal aroma and decreased the fruity one diminishing olfactory quality. In Herdade de Rio Frio balance in the nose and global appreciation were significantly different from the other characteristics, in which ESTR and ETAR were the best scored, respectively (Figure 5), contrary to what happened in the other wineries.

5. Conclusions

The aim of the present work was to evaluate the effects of mechanical pruning and the addition of organic amendments on Syrah wines quality. That evaluation was based upon physicochemical parameters determination, chromatic characteristics results, mineral and heavy metals analysis complemented by sensory analysis in order to see if the differences found in analytical procedures were translated in tasters' perception. The wines came from three different wineries located in three different *terroirs*, which means that the effect of the treatments applied and the results obtained are specific for these locations.

In general, about the pruning technique it is possible to affirm that manual pruning led to higher results in the studied parameters for both Quinta do Côro and Quinta do Gradil. In both wineries, manual pruning originated more alcoholic wines, with higher pH and volatile acidity. All the parameters normally related to red wine quality such as total anthocyanin content, color intensity, tannin power and total phenols have higher results for this type of pruning technique. This means that in these cases, mechanical pruning led to wines with lower quality. Mineral analysis showed that mechanical pruning led to less accumulation of N, K, Fe and Cu in the wines but the Ca levels were higher for this type of pruning. The heavy metals and rare earth elements results showed that for some elements as As, Sr, Ba, V mechanical pruning led to wines with higher concentrations while manual pruning increased Rb, Y, Na, Co, Zn, Ga content. The sensory analysis showed that manual pruning resulted in wines with more color confirming the results obtained by chromatic analysis. These wines had more fruity, floral and jam aromas than the ones originated from mechanically pruned vines, as well as more intensity, balance and persistency which led to higher global appreciations.

The effect of organic amendments in wine quality is not so clear, the results depend upon each vineyard. In the physicochemical analysis it is possible to state that ETAR was the amendment that originated wines with less alcohol content in Quinta do Côro and Quinta do Gradil. For the other parameters, the results show that different organic amendments led to higher results in each parameter. After analyzing the chromatic characteristics it is possible to conclude that BIOC and the control plots originated wines with higher color intensity unlike ETAR that led to lower results, these are in accordance to sensory analysis. The control plots also originated wines with higher total anthocyanin concentration in Quinta do Côro and Quinta do Gradil, however in Herdade de Rio Frio ESTR was the amendment that conducted to higher color intensity and higher total anthocyanin levels. In the mineral analysis ETAR was the responsible for the higher accumulation of N in the three vineyards and it also increased the Ca levels in Quinta do Côro and Quinta do Gradil. In what K is concerned the organic amendment that originated wines with a higher concentration of this element was ESTR in Quinta do Côro and Herdade de Rio Frio. The Fe and Cu levels did not show any trend related to the application of the organic amendments. The heavy metals analysis show that for the three wineries, ETAR was the organic amendment that led to higher accumulation of these elements in wine and BIOC also influenced this accumulation in Herdade de Rio Frio. In sensory analysis, except for Herdade de Rio Frio, the control plots originated wines more appreciated by the tasters and with higher global appreciation. In general some results are statistically different however in a practical point of view those differences may not be distinguished, that is why only a few attributes were significantly different in sensory analysis while in laboratory essays almost all parameters resulted in significant differences.

The decision between manual pruning or mechanical pruning must be taken considering the type of wine to produce. These results show that mechanical pruning led to wines less appreciated by the consumers, but the differences in the results are not extremely high. The vinegrowers must take into account all the costs in their productions and the savings that could be achieved by the use of the mechanical pruning and decide after analysis of all the factors. In order to compete with the other producing countries, maybe the strategy can pass through a division in the vineyards – top quality vines that originate top quality wines pruned manually and the other ones pruned by machine.

Organic amendments can be a good alternative to chemical fertilizers, not only it add minerals to the soils but also organic matter. However, its addition must be carefully made and only after analyzing the nutritional needs of the plants. In some years, it may be best to add a certain type of organic amendment that is not needed in other years. Vinegrowers must always be aware of the state of their vineyards in order to take the best decision possible based upon all the information they can have access to. Further study is necessary, specially to understand the implications of the additions of organic amendments in the soils because the reactions are slow and the practical results take years to be noticed.

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7. Annex

Annex 1: Assimilable Nitrogen Values obtained in Must Analysis in Quinta do Gradil and Herdade de Rio Frio

	Quinta do Gradil	Herdade de Rio Frio
Treatment	Assimilable Nitrogen (mg/L)	Assimilable Nitrogen (mg/L)
MAN TEST	149,6	77,0
MAN ETAR	185,5	105,0
MAN ESTR	165,4	82,3
MAN RSUC	175,0	87,5
MAN BIOC	148,8	74,4
MEC TEST	71,8	
MEC ETAR	131,3	
MEC ESTR	78,8	
MEC RSUC	105,0	
MEC BIOC	83,1	

Annex 2: Limits of detection (LOD) and Limits of Quantification (LOQ) for semi-quantitative method of analysis for heavy metals (Catarino et al. 2006 and Catarino et al. 2011)

Element	LOD	LOQ
Li (µg/L)	0,003	-
Be (µg/L)	0,003	-
Na (mg/L)	-	-
Mg (mg/L)	-	-
Al (mg/L)	0,00008	-
V (µg/L)	0,003	-
Mn (mg/L)	0,000003	-
Co (µg/L)	0,001	-
Ni (µg/L)	0,02	-
Zn (mg/L)	0,0002	-
Ga (µg/L)	0,004	-
As (µg/L)	0,005	-
Se (µg/L)	0,02	-
Rb (mg/L)	0,000001	-
Sr (µg/L)	0,004	-
Y (ng/L)	-	-
Cd (µg/L)	0,001	-
Cs (µg/L)	0,0004	-
Ba (µg/L)	0,01	-
La (ng/L)	-	80,0
Ce (ng/L)	-	5,6
Pr (ng/L)	-	2,2
Nd (ng/L)	-	4,6
Sm (ng/L)	-	4,0
Eu (ng/L)	-	2,6
Gd (ng/L)	-	4,6
Tb (ng/L)	-	2,0
Dy (ng/L)	-	4,1
Ho (ng/L)	-	2,0
Er (ng/L)	-	3,9
Tm (ng/L)	-	1,9
Yb (ng/L)	-	3,1
Lu (ng/L)	-	1,8
Tl (µg/L)	0,0006	-
Pb (µg/L)	0,006	-

Annex 3: Sensory Analysis Score Card

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Ficha de Prova para os vinhos tintos do projeto Fertilpoda

Data: Sessão:

Prove os vinhos pela ordem apresentada e classifique os diferentes atributos utilizando as seguintes escalas:

Para **Cor**, **Aroma** e **Gosto**: 1. Inexistente 2. Pouco Intenso(a) 3. Medianamente intenso(a) 4.Intenso(a) 5. Muito Intenso(a)

Para **Equilíbrio** (Aroma e Gosto) e **Apreciação Global**: 1. Medíocre 2. Satisfatório 3. Bom 4. Muito Bom 5. Excelente

		VINHOS/ CÓDIGOS												
COR	VERMELHO													
	VIOLÁCEO													
AROMA	FRUTADO													
	FLORAL													
	VEGETAL													
	COMPOTA													
	INTENSIDADE													
	EQUILÍBRIO													
GOSTO	CORPO													
	AMARGO													
	ADSTRINGÊNCIA													
	ACIDEZ													
	PERSISTÊNCIA													
	EQUILÍBRIO													
APRECIAÇÃO GLOBAL														

Observações