

# **Physicochemical effects of different pruning techniques and different amounts of Municipal Solid Waste compost on Sauvignon blanc wines in Quinta do Gradil (Lisbon Wine Region)**

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## Abstract

The aim of this work is to analyze the effects of the mechanical pruning technique and the use of municipal solid waste compost in Sauvignon blanc wines.

For this reason, the experimental vineyard was divided into four blocks, in which the different treatments were used for two consecutive years and a strip-plot experimental design was used.

The vinification was a classical white vinification and the alcoholic fermentation took place in 50L stainless steel vats. At the end of the fermentation a correction to 35mg/L of free sulphur dioxide was made before the bottling of the wines.

Subsequently, the wines were analysed basing on physico-chemical parameters, phenolic composition and chromatic characteristics to assess the effects of the different treatments.

In general, as for the pruning technique, it is possible to say that mechanical pruning led to higher hue values for the wines of 2018 and higher values of clarity for the wines of 2019.

At the same time, mechanical pruning led to a lower 420nm absorbance values in wines from 2019 vintage and it corresponds to a lower yellow colour of the wine.

In any case, all the underlined differences on the final wines were not visible to the naked eye.

The effect of the use of municipal solid waste compost on wine quality is not so clear, the results depend on each vineyard and more detailed analysis must be carried on. For example, the possible heavy metals phytotoxicity from municipal solid waste compost must be analysed.

Keywords: Wine, Sauvignon blanc, Municipal Solid Waste Compost, mechanical pruning, manual pruning.

## Resumo

O objetivo deste trabalho é analisar os efeitos da técnica de poda mecânica e da utilização de composto de resíduos sólidos urbanos em vinhos Sauvignon blanc.

Por este motivo, a vinha experimental foi dividida em quatro blocos, nos quais foram utilizados os diferentes tratamentos durante dois anos consecutivos e em delineamento experimental em faixa.

A vinificação foi uma vinificação clássica branca e a fermentação alcoólica decorreu em cubas de inox de 50L. No final da fermentação foi feita uma correção para 35mg / L de dióxido de enxofre livre antes do engarrafamento dos vinhos.

Posteriormente, os vinhos foram analisados com base em parâmetros físico-químicos, composição fenólica e características cromáticas para avaliação dos efeitos dos diferentes tratamentos.

De uma forma geral, quanto à técnica de poda, pode-se dizer que a poda mecânica levou a maiores valores de matiz para os vinhos de 2018 e maiores valores de clareza para os vinhos de 2019.

Ao mesmo tempo, a poda mecânica levou a valores mais baixos de absorbância de 420 nm nos vinhos da safra 2019 e corresponde a uma cor amarela mais baixa do vinho.

Em qualquer caso, todas as diferenças sublinhadas nos vinhos finais não eram visíveis a olho nu.

O efeito da utilização de composto de resíduos sólidos urbanos na qualidade do vinho não é tão claro, os resultados dependem de cada vinha e devem ser realizadas análises mais detalhadas. Por exemplo, a possível fitotossicidade de metais pesados de compostagem de resíduos sólidos urbanos deve ser analisada.

Palavras-chave: Vinho, Sauvignon blanc, poda mecânica, poda manual, Resíduos Sólidos Municipais Compostados.

## Resumo alargado

O objetivo do presente trabalho é avaliar a influência da técnica de poda e da aplicação de Resíduos Sólidos Municipais Compostados na qualidade de vinhos da casta Sauvignon blanc.

Para fazer isso foram realizados ensaios na vinha da região vitivinícola de Quinta do Gradil pertencente à região de Lisboa e a avaliação do efeito da poda foi feita com base na comparação entre a poda manual (MAN) e a poda mecânica (MEC).

A vinha experimental foi dividida em quatro blocos e, em cada um deles, foram aplicadas diferentes doses de Resíduos Sólidos Municipais Compostados e as duas diferentes técnicas de poda.

As doses de RSMC utilizadas foram três: M1 (5.000 kg/ha), M2 (10.000 kg/ha) e M3 (20.000 kg/ha).

Ao contrário, 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, fenóis totais, fenóis flavonoides e não flavonoides.

Os resultados obtidos nas análises laboratoriais foram analisados estatisticamente através do método ANOVA com testes de Tuckey, através dos softwares R e SPSS.

Analisando estatisticamente os resultados obtidos, destacou-se que a poda mecânica levou a maiores valores de tonalidade e luminosidade nos vinhos finais. Por outro lado, este tipo de poda tem sido associado a valores de absorvância mais baixos de 420nm e isso resulta em uma menor cor amarela dos vinhos.

Na maioria dos vinhos, o valor de fenol total foi superior para os provenientes de vinhas podadas mecanicamente, embora estes resultados não sejam estatisticamente significativos.

O efeito da MSW compost na qualidade do vinho não é tão claro, os resultados dependem de cada vinha, por exemplo, a possível fitotossicidade de metais pesados de compostagem de resíduos sólidos urbanos deve ser analisada. Para todos os parâmetros, a análise estatística mostra que a utilização de diferentes quantidades de composto de resíduos sólidos urbanos na vinha não conduz a diferenças estatisticamente significativas nos vinhos finais.

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.

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# 1. Introduction

Agriculture has always been one of the main activities that characterizes sedentary population and, in the centuries, it has evolved with new kinds and techniques.

Nowadays, since the world population is continuously growing and agriculture is based on the use of natural resources, the topic of sustainable agriculture is increasingly important. Exactly for this reason, in the years, the concept of viticulture has also evolved and, nowadays, sustainable viticulture plays a key role in the production of grapes and wines. Sustainable viticulture, following the OIV definition (2008), means “Global strategy on the grape production and processing systems, incorporating at the same time the economic sustainability, producing quality products, considering requirements of precision in sustainable viticulture, risks to the environment, products safety and consumer health and valuing of heritage, historical, cultural and landscape aspects”. By this definition is meant that the concept of viticulture has to be sustainable from economic, environmental and social points of view.

Speaking about the economic sustainability, it depends from the techniques applied in the vineyard, from the use of substances and, obviously, from the workforce. However, the reduction of the costs can be achieved introducing mechanization, reducing the amounts of herbicides and chemical substances. Among the less sustainable practices from an economic point of view there is certainly pruning for this reason, according to Bates and Morris (2009), a valid way to reduce the number of working hour/ha and, consequently, vineyard management costs, is the introduction of the mechanical pruning technique.

## 2. Aim of the study

The present work it's part of a four-years research project (2018-2021) called IntenSusVITI (PDR 2020-101-032001) its aim is to analyse the effects of mechanical pruning on the vines and municipal solid waste organic compost used in the field, on the Sauvignon blanc wines produced under the different modalities, comparing their physicochemical characteristics.

The experimental vineyards are located in Quinta do Gradil (Lisboa, Portugal) and the differences between the two wines will be underlined by physicochemical parameters analysis, color analysis and phenolic composition.

## **3. Bibliography review**

### **3.1. Grapevine Pruning**

An important technique in viticulture is the winter pruning since, unlike other fruiting crops, grapevines cannot shed crop on their own. By pruning a vine we can regulate the yield, assure the right ripeness and move the clusters' position in order to promote sunlight and airflow (Intrieri et al. 2011). Moreover, the pruning technique determines the future vigour of shoots and profoundly affects the wine quality. So, according to Intrieri et al. (2011), winter pruning is a cultural intervention that consists in the total or partial suppression of the canes with the aim to obtain a certain level of buds and remove up to 95% of the old wood.

#### **3.1.1. Manual Pruning**

According to Zabadal et al. (2002), the manual pruning allows us to maintain on the vine the perfect number of nodes and the ones that have the best potential to produce, controlling clusters' size and number and maintaining the canopy shape (Intrieri et al. 2011).

Doing manually the winter pruning we can choose the number of buds to let on the vine and, if this value is too low the vegetative and the reproductive growth compete for resources, leading to an increase of the vigour and decrease of yield. Contrariwise, if we let an excessive number of buds on the vine this translates into a loss of fertility, with lighter clusters and canes (Keller et al. 2004). From this we understand how important is to have the right number of buds at the pruning time, in order to achieve a vegetative-reproductive balance and have a good photosynthetic leaf area enough to ripen the berries and restore the reserves in the permanent structures of the vine (Keller et al. 2004). The research carried out by Magalhães (2008) shows how the pruning technique has also an influence on the grape quality, due to the probability of fungus diseases and maturation problems in case of a yield excess. However, manual winter pruning also has disadvantages, in terms of working hours and costs.

### **3.1.2. Oenological influences of manual pruning**

Manual pruning leads, in general, to a lower yield than mechanical pruning (Keller et al., 2004) and, since yield determines the source-sink relationship in the vine, it's not surprising that yield affects grape and wine characteristics.

In a research by Pérez-Bermúdez et al. (2015) is underlined how the manual pruning technique can influence the grape's and wine's characteristics. The authors, comparing the effects of different pruning techniques on Tempranillo and Bobal grapevines, concluded that a lighter pruning significantly lowers Brix, Total Phenols index (TPI) and K accumulation.

These tendencies denote that manually pruned vines invest more energies in the formation of shoots at the expense of fruits' and reserves' formation, leading to a delayed fruit ripeness.

In a research by Chapman et al. (2004) is underlined how, in Cabernet sauvignon wines, manual pruning leads to a decrease of vegetable aroma and an increase of fruity aromas. Nevertheless, the authors concluded that more detailed analyzes must be carried out, because these oenological differences may depend on the grapevine variety and other factors.

### **3.1.3. Mechanical Pruning**

With the aim to improve the efficiency of manual pruning, a mechanical pre-pruning can be done. This treatment has the aim to make easier the final manual pruning (Magalhães 2008). In a research conducted by Morris (2008) is specified how the use of agricultural machines can lead to a reduction of production costs between 44% and 61%. The same topic is more specifically analysed in a study by Gatti et al. (2011), who explain that, with the adoption of mechanical pruning, is possible to reduce by 50% to 90% the labor demand, depending on what technique of mechanical pruning is used and on the conditions of soil and vines. In fact, mechanical pruning can be done using selective or non-selective techniques. An example of non-selective pruning techniques is represented by hedge pruning and minimal pruning.

In terms of how the pruning method influences the final wine Pérez-Bermúdez et al. (2015) underlined that, in the wines belonging from both cultivars, mechanical pruning leads to a significant decrease of alcohol levels. This effect could be considered positive since, nowadays, the topic of wines with a lower alcohol content is always more important. In fact minimal pruning techniques could be used to produce wines with lower alcohol without compromising other wine attributes such as colour and phenolics.

### 3.1.3.1. Hedge Pruning

The total mechanical pruning, according to a research by Lopes et al. (2000), consists in hedging and topping the vines' canopy, in order to create a box shaped canopy and, for this reason this technique takes the name of hedge or box pruning.

According to the same authors, hedge pruning can be applied with different severity levels and we have the accumulation of old wood inside of the canopy. This could be a big disadvantage leading to an increase of canopy density with the consequence of a deficient sunlight exposure and high temperature, increasing the probability of fungal development.

But, on the contrary, as Dokoozlian explains (2013), mechanically-pruned vines have more clusters per vine but fewer and smaller berries per bunch and this effect reduces the impact of diseases.

Moreover, due to a greater number of buds per vine, mechanically-pruned vines begin to grow earlier in the season than the hand-pruned vines (Pool et al., 1988).

So, in general, most of these changes in canopy configuration are positive and improve canopy microclimate and fruit composition, but with some exceptions.

In fact, a study on Cabernet Sauvignon vines by Holt et al. (2008), comparing berries from mechanical-pruned and manually-pruned vines concluded that machine berries were smaller and had higher concentrations of anthocyanins, tannins and total phenolics than berries from manual pruning. Surprisingly, however, mechanically-pruned wines also had the lowest quality scores during a panel test, suggesting that lighter berries presumably having a more favorable skin-to-flesh ratio do not necessarily originate better wines. The effect of mechanical hedge pruning on the quality and yield of Cabernet Sauvignon grapes were studied by Lopes et al. (2000). The authors underlined how, at the harvest, both the grapes samples from manually and mechanical pruned vines had non-significant differences regarding sugars level, acidity and anthocyanins content. For this reason they concluded that hedge pruning is a valid pruning technique to reduce production costs without great quality loss in wines.

Therefore, a study by Poni (2004) explains that a bigger number of node per vine can reduce the vigor of the canes generating, after veraison, better conditions for sugar accumulation. Keller et al. (2004) explain how mechanical pruning can be useful to maximize yields maintaining high quality, without reaching the breaking load in the production curve. On the other hand, however, in a study by Gatti et al. (2011), it's emphasized that some grape varieties are not able to adapt to mechanical pruning, and this translates into a grape overproduction with consequent loss of quality.

Hedge pruning also has an effect on the vigor and vegetative expression of the vines. In fact, the greater number of shoots per vine is associated with a lower unit weight of these. The effects of increased yields have different effects on the characteristics of the grapes. For example, in a study by Intrieri et al. (2001), it's underlined how in some cases the increase of yield lead to a slight reduction in the sugar content.

### **3.1.3.2. Minimal Pruning**

Australian researchers (Clingeffer, 1988 and Possingham, 1996) studied an alternative mechanical technique to manual pruning, called minimal pruning, which allows to carry out a low severity pruning with rapid speed and low cost. In fact, minimal pruning only needs between 5 and 10 working hr/ha and removes more or less 10% of old wood from the vine. According to Morris (2008) the definition of minimal pruning technique is a mechanical skirting of the vines to a certain level above the soil. However, a research by Clingeffer (1988) has shown that the success of minimal pruning technique in wet and cold countries depends on post-fruitset mechanical crop thinning to limit an excessive yield. So generally, minimal pruning resulted more adapted to warm conditions and very sensitive to environmental conditions.

In a research by Poni et al. (2000) the development of the canopy was studied with the aim to analyse the main differences between a manually-pruned vineyard and a mechanical minimally-pruned vineyard, both of Chardonnay variety. The authors show how minimally pruned vines have a higher number of shoots but, at the same time, shoots are smaller and with a reduced leaf area.

Regarding the clusters, it's underlined how, in minimally-pruned vines, the number of clusters is higher but they have less berries per cluster and smaller berries. Poni et al (2000) concluded that minimal pruning leads to a yield increase between 20% and 40% compared to manual pruning, and this increase is not to be underestimated, especially in countries where the average yield is quite low, such as Portugal. However, in the research is underlined how in minimally-pruned vines ripening has been delayed of one week more or less.

The final result of this research indicates minimal pruning as a valid technique to improve the performance of Chardonnay vines under the experimental conditions.

Reading a study by Clingeffer (1988) it's clear how, by a practical point of view, minimal pruning is a valid technique for wine production because it maximizes the production and it is adapted for mechanical harvest.

### **3.1.4. Oenological influences of mechanical pruning**

A research by Botelho et al. (2020a), focused on the effects of mechanical pruning on yield and grape's composition of Syrah variety, underlined how the mechanical pruning leads to a higher sugar production per vine but the results of the study didn't underlined an increment of alcohol content in the final wines. Moreover, the same study underlined how, in terms of anthocyanins content and total phenols, no significant differences were observed between manual and mechanical pruning techniques.

On the contrary Castro et al. (2010), analysing the Dão region wines and comparing those produced by manually and mechanically pruned vines, concluded that mechanical pruning technique lead to a slight reduction in alcohol content in the Alfrocheiro variety wines.

The same effect was noticed in a study by Pérez-Bermudez et al. (2014). The research analysed Bobal and Tempranillo wines and showed that both cultivars' responses to mechanical pruning were identical since it provoked only one significant effect: reduced wine alcohol levels.

This could be a potential new benefit for the production of wines with lower alcohol without compromising other wine attributes such as color and phenolic compounds.

Chapman et al. (2004) conducted a study to investigate the effects of yield manipulation on the sensory attributes of Cabernet Sauvignon wines. Interestingly, a trained panel evaluated and found that the wines from the mechanically-pruned vines had diminished veggie aromas and enhanced fruity aromas for two consecutive vintages. Nevertheless, the authors concluded that more testing was required to determine whether the differences can be perceived by less experienced consumers and how sensory attributes affect wine preferences.

In a study by Archer and Van Schalkwyk (2007) is underlined how different pruning methods have a non-significant effect on the investigated varieties from an oenological point of view. Nevertheless, the same study analyses how, in general, mechanical pruning leads to a significantly increase of yield, while bunch mass, berry mass and berry volume decreased. The decrease in berry size and volume is the result of a higher number of bunches per vine and it leads to an increase of colour in the skins and wines of Cabernet Sauvignon variety.

Another oenological effect of pruning which was highlighted by the authors Archer and Van Schalkwyk (2007) regards the colour of Chenin blanc wines, in fact the wines from mechanical pruned vines had a colour improvement and this is caused by a greater synthesis of flavonoids during the ripening phase. The same effect was observed on Pinotage wines, in which has been noticed that mechanical pruning has given greater colored wines.

This increase in of colour as the berry volume decreases it's caused by a higher sunlight penetration into the bunches during the ripening period, due to the presence of smaller berries (Dokoozlian, 1990). Moreover, with mechanical pruning a greater number of buds is left on the plant and this means that there will be a greater budding of the buds in the upper part of the canopy. All this translates into a greater exposure of the bunches to light and it leads to a higher phenols and anthocyanins synthesis (Kaan Kurtural et al., 2012). The same research underlined how, the grapes from mechanical pruning treatments had a lower pH than those from manually pruned vines and this tendency was carried forward into the wine. This pH decrease is due to higher shade levels in the bunch zone of these vines. The authors Archer and Van Schalkwyk (2007) also analyzed the differences of the wines from the aromatic point of view and they concluded that those from manually pruned vines showed a most pronounced vegetative character and an increase of citrus-like aroma. In spite of this, in any case more detailed analyzes must be carried out, because these oenological

differences may depend on the grape variety and on the ripening conditions such as, for example, the exposure of the clusters, the weather and so on. Also a study by Kaan Kurtural et al. (2012) analyzed the effects of different pruning techniques on canopy architecture and berry composition. The research compared the canopy architecture of manually-pruned vines and mechanically-pruned vines and concluded that, in both the years of the experiment, the number of shoots was higher in mechanically-pruned vines than in manually-pruned vines. Also the number of clusters was analyzed and, according to the authors, it was higher for the mechanically-pruned vines in both years.

Nevertheless, the research didn't underline an effect of these results on measured microclimate variables. Berry and cluster weight were also unaffected by the treatments applied. It was expected that berry size at harvest would be smaller with mechanical box-pruning technique (Zabadal et al., 2002) compared with the manual pruning treatment. However, there was no difference in berry weights with the treatments applied.

The same study, analyzing the oenological effects of different pruning techniques, underlined a slightly increase of juice pH for the manual pruning technique in one of the two analyzed years.

The total acidity and the phenolic compounds' content of Cabernet Sauvignon were unaffected by the treatments applied in either year of the study.

According to another study by the authors Kaan Kurtural et al. (2015) on Zinfandel grape variety, the mechanical pruning increases accumulation of anthocyanins, flavonols, flavan-3-ols and total tannins in the berries compared to traditional manual pruning technique.

The oenological effects of mechanical pruning on "Alfrocheiro" vine grape variety were analyzed by authors Cruz et al. (2011). The authors underlined small differences in the grape parameters and wines between mechanical and manual pruning techniques. The alcoholic content tends to be superior with manual pruning treatment. Regarding color parameters minor differences were found in color intensity just in one year over three.

### **3.2. Use of organic amendments in the vineyard**

Concerning vine's vigour, Lopes et al. (2000) and Cruz et al. (2011) affirmed that the mechanically pruned vines, after different years of pruning, show a tendency to lose vigour which is, in part, related to a low soil fertility. So, the use of organic amendments in the vineyard could be, in this case, a right choice. According to Bugg and Van Horn (1988) the organic matter in the soil is important because it is a source of nutrients for the soil. Moreover, according to the same study, the organic matter has a crucial role in the regulation of soil's pH and it increases aeration, soil stability, pore size and the water-holding capacity of the soil.

In a research project in the Loire, the authors Morlat and Chaussod (2008) studied the effects of the organic amendments added in a vineyard. In the study the addition of organic amendments was analysed focusing on its effect on soil's water content and soil density. The authors concluded that

the addition of organic amendments in the vineyard leads to an increase of the water-holding capacity and a decrease of soil density. According to Pinamonti et al. (2008), who studied the use of municipal solid waste compost in viticulture, the use of MSW compost increases the concentrations of heavy metals such as Ni, Pb, Cd, and Cr in soils, musts and grapevine leaves. Nevertheless, the study didn't highlight symptoms of phytotoxicity from heavy metals in the vines.

Nowadays, there are several organic amendments that can be used in agriculture, coming from different sources. A good sustainable choice, in a world with an always bigger population, would be the use of Municipal Solid Waste Compost (MSWC) in order to reuse a big amount of rubbish. This kind of organic amendment was studied in Naples by Fagnano et al., who evaluated the possibility of recycling waste and use it as an organic amendment after composting. This study was conducted on lettuce fields and the authors concluded that the soil fertilization with compost made from the organic fraction of urban wastes has positive effects on the fixation of Carbon in stable soil organic and, moreover, it leads to an increase in yield and quality.

The source of organic matter traditionally used in Portuguese viticulture is cattle and poultry manure. However, nowadays different sources of organic matter are increasingly available, as the sludge from wastewater treatment (WWTP sludge) and the Municipal Solid Waste Compost (MSWC), whose agricultural use improves physical properties of the soil and provides essential nutrients to plants (Amlinger et al., 2003; Varennes, 2003).

A study by Calleja-Cervantes et al., 2015 highlighted how, the continuous long-term application of organic amendments belonging to MSW, increases the microbial activity and the soil's quality. In a research by Botelho et al. (2020c), is explained how the use of municipal solid waste compost in the vineyard modifies the yield components and the vine growth.

The authors underlined an increase in vine capacity and dry matter production after three years of treatment. Moreover, the same study underlined that also the Ravaz index was increased, showing a higher carbohydrate amount directed to reproductive growth, instead of vegetative growth.

### **3.2.1. Oenological influences of use of organic amendments in the vineyard**

In a study by Schmidt et al. (2014), the oenological effect of the use of organic compost in the vineyard's soil was analyzed. The authors observed that the pH was significantly higher in the musts from vineyards treated with organic amendment, which could be due to an earlier ripening (Hellman, 2004). Moreover, in the years in which the pH of the must wasn't significantly higher, the total acidity of the must still showed an increment.

Another research conducted by Alañon et al. (2016) is focused on the Influence of organic compost on polyphenolic composition of Cabernet Sauvignon wines. In the study, the authors concluded that the use of organic amendment in the vineyard can lead to higher values of volatile and total acidity as well as free and total SO<sub>2</sub>.

Regarding flavanoids, quercetin and myricetin, the authors underlined that these are the most susceptible compounds to amendment treatments, in fact they showed higher concentrations in the wines from the vineyards treated with organic amendment. This is an important fact due to the importance of flavonoids for wine quality. In fact they contribute to wine color by the formation of copigmentation complexes with anthocyanins, improving the color stability of wine during and after ageing (Gómez-Mínguez et al., 2006).

Moreover, the same study underlines how the organic amendments also has an impact on the accumulation of anthocyanic compounds, increasing their quantity in the grape and in the final wines. A study from Delgado et al. (2006) analysed the effect of application of an organic amendment with nitrogen and potassium on anthocyanin composition and chromatic features of Tempranillo grapes. The authors concluded that the nitrogen and potassium soil fertilization resulted increasing the content of these nutrients in the vine's leaves at bloom. As the authors explain, nitrogen and potassium availability for the vines is an important factor during phenolic ripening, in fact this treatment significantly increased the anthocyanin content on the grape skin at the harvest.

Nevertheless, the authors underlined that, with an average content of potassium in the vine, excessive nitrogen supply can reduce the synthesis of polyphenols in the berry skin, affecting the metabolic pathways of anthocyanins and enhancing the degradation of polyphenols during the final part of berry ripening. So the authors concluded that there is a strong nitrogen x potassium interaction which can modify the total polyphenols content, the anthocyanin composition and the copigmentation of anthocyanins in Tempranillo grapes and in the final wines.

A study by Botelho et al. (2020c) is focused on the effect of the use of different organic amendments on vegetative and reproductive growth. In this research the authors analysed how the interaction between mechanical pruning and fertilization can influence the vine growth. The study, conducted on "Syrah" grape vine variety, underlined that both of these techniques influence vine's growth.

In fact, regarding the effect of the organic amendments on reproductive growth, significant differences were observed in 2013 and 2014 in terms of cluster weight. In the first year the use of Sludge fertilizer leads to a higher cluster weight than control and, in 2014 Sludge was significantly higher than Bioc and control. In 2012 and 2015 there were no significant differences in cluster weight between organic amendments.

Yield was significantly increased by the organic amendments since the second year of the trial. Over all, Sludge was the organic amendment with a faster effect on yield, while Municipal Solid Waste Compost and Manure took more time to take effect.

The authors underlined that the use of different organic amendments increased the cluster number per vine just with the mechanical pruning technique and just with the use of Municipal Solid Waste Compost, Sludge and Manure. For this reason they concluded that this result is related to the higher budburst in these treatments.

Regarding the vegetative growth, the same research affirms that Sludge and Municipal Solid Waste Compost treatments were those where shoot and pruning weight were higher, while control and Manure were the lowest. Shoot weight and shoot number per mechanically-pruned vine was increased by organic amendments only in the last two years of the trial, probably due to the significant residual effect of the cumulative applications. On the other hand, considering the use of different fertilizers on manually-pruned vines, also found significant increases the shoot weight but not in shoot number per vine.

## **4. Materials and Methods**

### **4.1. Experimental design**

The present research project started doing an experimental design based on a Sauvignon Blanc vineyard in Quinta do Gradil, Lisboa. The vineyards were divided in four blocks, each of them divided in eight parts, with the aim to study the effects of two different pruning techniques (manual pruning and mechanical pruning) and the effect of the use of Municipal Solid Waste Compost (MSWC) as organic fertilizer. Each block was treated with four different amounts of MSWC and in this way, have been obtained thirty two combinations of treatments to analyse. The IntenSusVITI research project is a four-year project started in 2018 and, in the first year, the treatment with organic amendment wasn't considered. In this way have been obtained eight wines for the 2018 vintage and thirty two for the 2019 vintage.

Regarding the canopy management, that of the manually-pruned vines was organized in unilateral cordons with a vertical shoot positioning, while that of mechanically-pruned vineyards had unilateral cordons with sprawl vegetation. The vineyards were organized with a 2.6 meters distance interrow and 1 meter distance on the row.

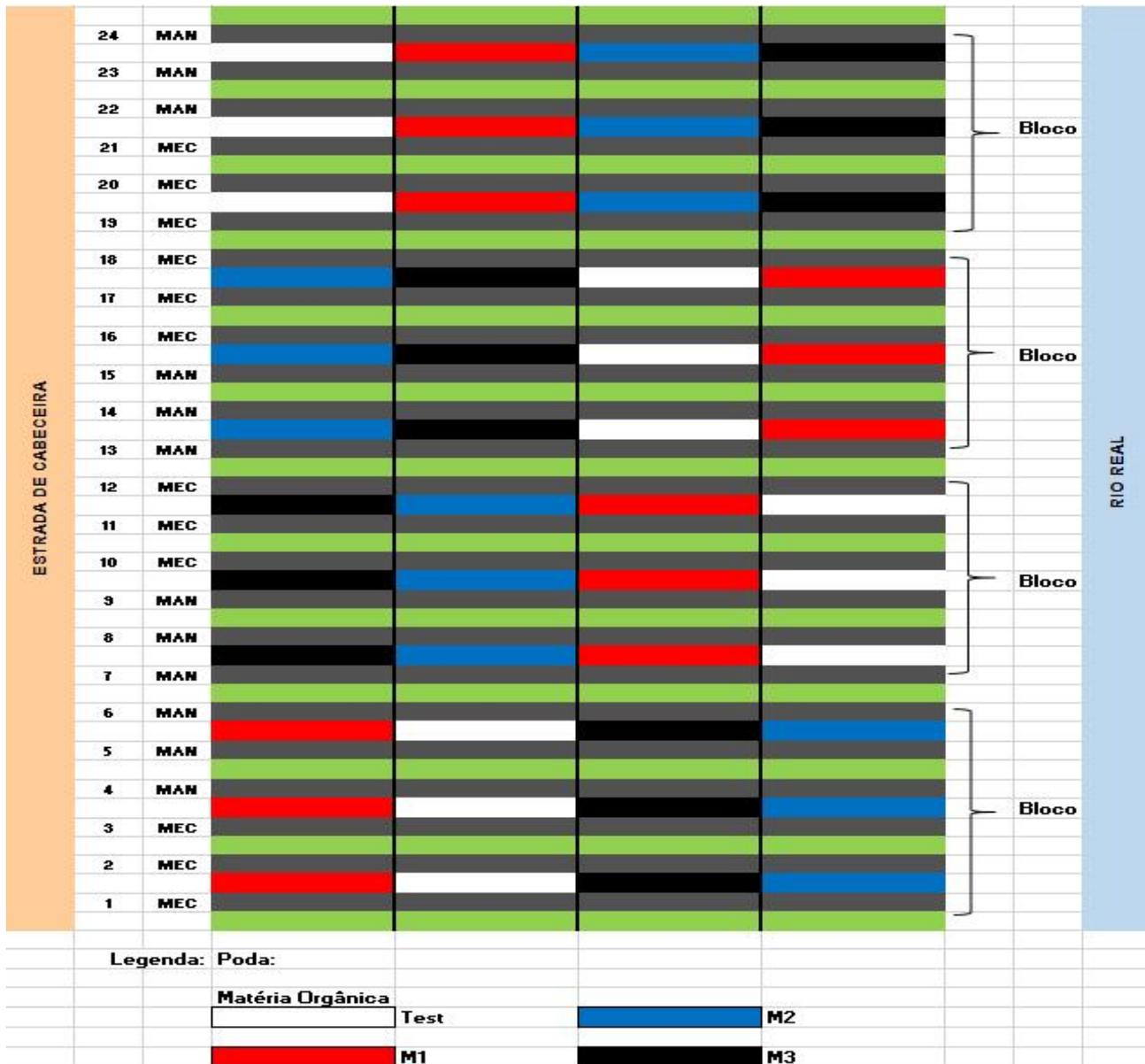
The experimental design is strip-plot with 4 replications. The pruning factor had two treatments: manual pruning (MAN) and mechanical pruning (MEC).

The second factor is the organic matter added to the soil, having been dosed 4 different amounts: "Test" (without addition of MSWC), "M1" (with the addition of 5.000 kg/ha of MSWC), "M2" (with the addition of 10.000 kg/ha of MSWC) and "M3" (with the addition of 20.000 kg/ha of MSWC).

The MSWC was spread over the soil and incorporated with a light disk harrow, before bud burst.

All the informations are summarized in the figure 1 below. So the experimental design is the same for both years, but in 2018 we considered that there were no effects of the use of MSWC, since it was the first year of application. For this reason the grapes from different MSWC treatments were mixed and the grapes were separated only by pruning treatment.

Figure 1 - Experimental design



MAN – Manual pruning; MEC – Mechanical pruning; M1 – 5.000 kg/ha of MSWC;  
M2 – 10.000 kg/ha of MSWC; M3 – 20.000 kg/ha of MSWC; TEST– Without MSWC;

## 4.2. Grape variety, maturation, harvest and vinification

This study has the aim to compare the effects of mechanical and manual pruning on wines from two different harvests (2018 and 2019). The used grapevine variety for this research project is Sauvignon Blanc, one of the most unique grapevine variety in the world, rich in suggestive aromas of intense olfactory and gustatory impact. According to Sivilotti et al. (2017) Sauvignon Blanc gives the best

results in siliceous soils such as those of the Loire, prefers fresh climates, while in countries with warm climates, such as in California, it is often fermented and aged in wood.

The grapes used for this study were grown in Quinta Do Gradil, a winery near the Serra de Montejunto, in the Lisbon wine region. The western region of Portugal has a great potential for grape cultivation, due to the presence of both sea and hills.

At the perfect maturation point a manual harvest of the grapes was performed and then the fermentation took place in the experimental winery at Instituto Superior de Agronomia (Lisbon). First of all, in both years, a de-stemming and crushing process was applied on grapes.

Then the grapes were pressed in a manual press adding 50 mg/L of SO<sub>2</sub>. This phase was followed by that of clarification of the must, which occurred naturally by static sedimentation. The clarification lasted twenty-four hours and, for the musts of the 2018 vintage, it took place at 16 Celsius degrees, while for those of the 2019 vintage at 23 Celsius degrees.

After the clarification phase, the must was inoculated with AEB "Fermol Arome Plus" dry active yeasts and the "Fermolplus Integrateur" fermentation activator was added in doses of 20 g/L. This activator is a complete nutrient based on thiamine and yeast autolysates and has the task of promoting an optimal fermentative course. The alcoholic fermentation took place into 50 L stainless-steel tanks at controlled temperatures, respectively of 18 Celsius degrees for the wines of the 2018 vintage and 24 Celsius degrees for those of the 2019 vintage. During the whole fermentation period different parameters of the wines were controlled, such as density and temperature, in order to monitor the decrease in sugars and the increase in ethanol in the fermenting must (Table 1).

At the end of the alcoholic fermentation a new analysis took place in order to control total and free SO<sub>2</sub>. If the SO<sub>2</sub> was too low, a corrections was made and then the bottling took place. During the vinification only one replica was made for each wine and after the bottling process physico-chemical analysis on the wines were performed.

Table 1- Example of monitored parameters during alcoholic fermentation

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
19/09	1.089	24.0
22/09	1.086	18.5
23/09	1.071	18.5
24/09	1.056	18.5
25/09	1.038	18.5
26/09	1.023	18.5
27/09	1.010	18.5
28/09	1.000	18.5
29/09	0.996	18.5
30/09	0.993	22.0
01/10	0.992	23.0

### 4.3. Physicochemical Analysis

With the aim to analyse the main characteristics of the wines, physicochemical and colour analysis have been made. Physicochemical analysis are in oenology, nowadays, a fundamental tool to control the wine's quality, to certificate the wines and improve the winemaking process. Last but not least, physicochemical analysis are fundamental to control the development of spoilage phenomena in wines.

During the ripening, until the harvest, the maturation of the grape was controlled in order to monitor the progress of the ripening. The parameters controlled in this phase were weight of a hundred berries (g), °Brix, Potential Alcohol Content (% Vol), pH and Total Acidity (g/tartaric acid/L). It is precisely speaking about the characteristics of the wines that, in this case, the physicochemical analysis allowed us to compare different wines of the same variety underlining the main differences and the effect of different pruning technique.

The analysis for the present work were made in the oenology laboratory of Instituto Superior de Agronomia and all parameters were analyzed according to official OIV methods and, in some cases, according to other official methods.

#### a) pH

The pH of the different wines was measured following the official OIV method, which allows us to measure the exact pH analysing the difference of potential between two electrodes immersed in the wine (OIV 2015).

#### b) Total Acidity (g/L of tartaric acid)

The total acidity is an important parameter in wines and, in this case, it was determined by titration with bromothymol blue as indicator, following the official OIV method (2015).

#### c) Volatile Acidity (g/L of acetic acid)

As the total acidity, the volatile acidity is a quite important value in a wine, because it allows us to have informations on the sanitary state of the wine and to monitor the wine conservation. Moreover, the value of volatile acidity is an indicator of possible microbiological spoilage. The volatile acidity is a quality factor, for this reason there is a legal limit that, in case of white wines, is 1,08 g of acetic acid/L (IVV 2015). The volatile acidity value of a wine includes all the steam-distillable acids but, due to the importance of acetic acid, this parameter is usually expressed in grams of acetic acid for liter. The official OIV method (2015) for the volatile acidity's determination consists in the separation of volatile acids from the wine by distillation and a following titration with sodium hydroxide.

#### d) Sulphur Dioxide (mg/L)

The sulphur dioxide is used in oenology for its contemporary antioxidant and antiseptic actions. Despite these positive effects on wine, it should be remembered that its use must still be limited, both for the negative effects on health and for organoleptic reasons. For this reason there is a legal limit for this compound and, for the white wines, is 210 mg/L. Sulphur dioxide is present in two forms in wines: free SO<sub>2</sub> and combined SO<sub>2</sub>.

The first one sulphur dioxide form has positive properties such as antiseptic (mainly the molecular sulphur dioxide), antioxidant (bisulphite ion) and it inhibits enzyme activity and it is determined by titration with iodine solution.

The second form, the combined SO<sub>2</sub>, is determined by titration with iodine solution after an alkaline hydrolysis. In this way, to obtain the total value of SO<sub>2</sub> in a wine, we can sum the free SO<sub>2</sub> and the combined SO<sub>2</sub> (OIV 2015).

#### e) Alcohol content (% Vol)

Since the wine has a boiling point lower than water and higher than ethanol, we can exploit it to measure the alcohol content of a wine using ebulliometry. In a study by Zoecklein et al. (1999) is explained how this method allows us to obtain the percentage of ethanol, linking it with the difference between these temperatures.

#### f) Reducing Substances (g/L of reducing sugars)

The value of reducing substances allows us to confirm if the alcoholic fermentation is finished in fact, with a reducing substances value lower than 2 g/L, the wine could be considered dry.

In order to obtain this value, we have to follow, according to the official OIV method (2015), two steps. The first step is called clarification and it consists in the elimination of reducing substances other than sugars. After this, there is the second step, which consists in heating the sample in order to achieve the boiling point and the reduction of sugars with an alkaline solution of a copper salt. Later we need to titrate the copper in excess obtaining the value of reducing substances.

#### g) Wine's color

The wine's color an important characteristic, specially during the sensorial analysis because it can influence the consumers' choice and it gives us informations about the applied vinification technique. In the present work, in order to evaluate in the best way the color of all the analysed wines, two different methods were used: The official OIV method and the CIE Lab method.

For the first method a spectrophotometer is used, with the aim to determine the color intensity and tonality of the wines. The official OIV method (2015) is based on the use of three measurements for each sample at three wavelengths, 420, 520 and 620 nm. But in our case, having a white wine, we just analysed the 420nm absorbance.

In general, the color intensity (I) is calculated by the following expression:

$$I \text{ (a.u.)} = A_{420} + A_{520} + A_{620}$$

For the tonality (T) is used the following expression:

$$T = A_{420} / A_{520}$$

These expressions are valid for a red wine, in which we have an influence of the lecture at 520 and 620 nm. In this case, since we have white wines, we just measured the absorbance at 420nm to obtain the color intensity of our wines. The second method used in the present work for the determination of chromatic characteristics was the CIE Lab method, which allows us to determine different characteristics of a wine, such as clarity, chroma and hue (OIV 2006). This method is important because allows us to calculate the total colorimetric difference and the difference in hue between two samples.

This analytic method starts clarifying the wine by centrifugation at 3500 rpm for ten minutes, then a measurement of absorbance in a range between 380 and 780 nm is applied. In this way the device gives us back three values called "L", "a" and "b" for each sample.

Following the official protocol, with the "L", "a" and "b" coordinates is possible to calculate the clarity, the chroma (C) and the hue (H) with the following calculations:

$$\text{Clarity} = L$$

$$C = (a^2 + b^2)^{1/2}$$

$$H = \text{tg} (b/a)$$

#### h) Total phenols (mg/L of gallic acid)

Total phenols in wine were calculated with the method by Ribereau-Gayon (1970), which consists in a direct spectrophotometric lecture of the diluted wine sample with a wavelength of 280 nm. For this reason, the amount of total phenols of the wine is given by the following expression:

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

#### i) Flavonoids and non-flavonoids phenols (mg/L of gallic acid)

Following a scientific method by Kramling and Singleton (1969), we are able to divide the total phenols of the wine in flavonoid phenols such as catechins, epicatechins, flavonols, anthocyanins and condensed tannins, and non-flavonoid phenols such as phenolic acids (cinamic and benzoic) and volatile phenols. During this work, the method by Kramling and Singleton (1969) was used for the determination of flavonoid and non-flavonoid phenols. This scientific method consists in the direct lecture of the sample at a wavelength of 280 nm after a reaction of with formaldehyde and hydrochloric acid. This reaction takes place in a dark place and it takes 24 hours.

### **4.4. Statistical Analysis**

In a scientific work statistical analysis have a crucial role to understand the statistical significance of the obtained values during all the analysis.

This is precisely why, the statistical analysis of this work was carried out with the specific SPSS statistical software, with the aim to highlight all the differences existing in the various samples and understand how the pruning technique, the application of the MSW organic amendment and the interaction between them could influence the final wine's quality.

All the treatments were analysed with a split-plot design by an ANOVA variance test with the Tuckey test. To determine significant statistic differences between each treatment the p value was analysed and the symbol \* was used to indicate 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.

In case of significant values, letters "a", "b" and "ab" were used to indicate the significative values: "a" means the higher value, "b" the lower value and "ab" the similarity between values.

## **5. Results and Discussion**

In this chapter the results obtained from the physicochemical, phenolic and color analyzes will be shown. In order to make the presentation of the data easier to understand, the latter will be presented in chronological order. For this reason, the results of the analyzes carried out during the ripening phase will be presented first, followed by those of the analyzes carried out at the harvest and, finally, those of the analyzes on wines.

## 5.1. Grape and must's Analysis

The Sauvignon blanc grapes have been analyzed during the maturation involving sugar-acidic parameters. Then the harvest date was decided according to those values, reaching the technological maturity.

## 5.2. Wines' Analysis

In order to analyse the data in the best way, we can divide the wines' analysis' results in two categories: "physicochemical analysis" and "color and phenolic composition analysis".

### 5.2.1. Physicochemical Analysis of the Wines

Since all the analysis were made in February 2020, for all the wines from both 2018 and 2019 vintage the physicochemical parameters were analyzed before the bottling. Instead, regarding the phenolic composition and color's analysis, they were made after bottling for 2018 vintage's wines and before bottling for 2019 vintage's wines.

Moreover, since the 2018 was the first year of the research project, we hadn't organic amendment's use in the vineyard and for this vintage only the results deriving from the different pruning techniques applied are taken into consideration (tables 2 and 3).

Table 2 –Physicochemical analysis' results vintage 2018 with manual pruning

Sample	Alcohol (% Vol.) (±0.1)	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	pH	Free sulphur dioxide (mg/L)	Total sulphur dioxide (mg/L)	Sugars (g/L)
MAN B1	12.6	8.70	0.35	3.17	39	105	0.3
MAN B2	13.0	9.00	0.40	3.14	47	118	0.4
MAN B3	13.2	8.70	0.33	3.15	43	115	0.3
MAN B4	12.8	8.90	0.32	3.16	46	110	0.5

MAN – manual pruning; B – block's number;

Table 3 –Physicochemical analysis' results vintage 2018 with mechanical pruning

Sample	Alcohol (% Vol.) ( $\pm$ 0.1)	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	pH	Free sulphur dioxide (mg/L)	Total sulphur dioxide (mg/L)	Sugars (g/L)
MEC B1	13.5	8.30	0.50	3.14	40	125	0.4
MEC B2	13.4	8.40	0.54	3.15	34	110	0.5
MEC B3	13.5	8.70	0.49	3.13	33	105	0.3
MEC B4	13.7	8.30	0.44	3.16	31	98	0.4

MEC – mechanical pruning; B – block's number;

The indicated values of free and total SO<sub>2</sub> in the tables 2 and 3 above are adjusted after that a correction to 35 mg/L of free SO<sub>2</sub> has been done.

On the contrary, for the 2019 vintage's wines, the differences were analyzed for each individual block, also taking into account the influence of the organic amendment (tables 4 and 5).

Also in this case a correction to 35 mg/L of free SO<sub>2</sub> has been done.

Table 4 –Physicochemical analysis' results vintage 2019 with manual pruning

Sample	Alcohol (% Vol.) ( $\pm$ 0.1)	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	pH	Free sulphur dioxide (mg/L)	Total sulphur dioxide (mg/L)	Sugars (g/L)
MAN B1 M1	14.6	8.30	0.33	3.36	42	140	1.4
MAN B1 M2	14.5	8.70	0.35	3.33	43	155	1.5
MAN B1 M3	14.4	9.30	0.27	3.31	40	130	1.4
MAN B1 TEST	14.1	9.30	0.28	3.20	44	135	2.0
MAN B2 M1	14.3	8.90	0.33	3.29	36	135	0.2
MAN B2 M2	14.3	9.20	0.34	3.23	52	155	0.9
MAN B2 M3	14.8	9.00	0.41	3.21	48	145	0.1
MAN B2 TEST	14.8	8.00	0.11	3.26	36	135	0.3
MAN B3 M1	14.8	8.90	0.34	3.26	37	145	0.2
MAN B3 M2	14.3	9.30	0.30	3.30	39	125	0.1
MAN B3 M3	14.3	8.90	0.27	3.27	50	150	0.2
MAN B3 TEST	14.8	9.40	0.40	3.28	42	170	0.3
MAN B4 M1	14.2	9.50	0.35	3.24	39	140	0.1
MAN B4 M2	14.6	9.20	0.33	3.28	42	145	0.2
MAN B4 M3	14.6	10.10	0.31	3.30	46	140	0.2
MAN B4 TEST	14.0	8.70	0.27	3.30	49	130	0.1

MAN – manual pruning; B – block's number; M – sample's number; TEST – sample without organic amendment;

Table 5 –Physicochemical analysis’ results vintage 2019 with mechanical pruning

Sample	Alcohol (% Vol.) ( $\pm 0.1$ )	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	pH	Free sulphur dioxide (mg/L)	Total sulphur dioxide (mg/L)	Sugars (g/L)
MEC B1 M1	15.1	8.60	0.32	3.21	41	125	0.2
MEC B1 M2	14.6	8.70	0.20	3.20	40	135	0.1
MEC B1 M3	14.6	8.40	0.29	3.33	44	135	0.1
MEC B1 TEST	14.1	8.70	0.37	3.25	44	140	0.2
MEC B2 M1	14.8	8.40	0.38	3.30	32	125	0.1
MEC B2 M2	14.9	8.40	0.41	3.25	38	140	1.1
MEC B2 M3	15.1	8.30	0.41	3.24	42	145	0.1
MEC B2 TEST	14.5	8.40	0.37	3.25	40	135	0.2
MEC B3 M1	15.7	6.30	0.56	3.31	43	165	0.3
MEC B3 M2	14.6	9.30	0.40	3.26	40	120	0.1
MEC B3 M3	14.4	8.70	0.34	3.27	44	135	0.2
MEC B3 TEST	14.9	8.90	0.62	3.23	42	130	0.1
MEC B4 M1	14.4	8.60	0.38	3.27	32	140	0.1
MEC B4 M2	14.9	9.00	0.40	3.26	36	145	0.1
MEC B4 M3	14.4	8.70	0.38	3.29	46	140	0.2
MEC B4 TEST	14.0	9.60	0.35	3.11	41	145	0.2

MEC – mechanical pruning; B – block’s number; M – sample’s number; TEST – sample without organic amendment;

All the obtained results from a physico-chemical analysis, indicated in the tables 2, 3, 4 and 5 above, can be considered normal values for Sauvignon blanc wines. In fact, wines belonging to Sauvignon blanc grape variety are characterized by high values of total acidity.

### 5.2.2. Statistical Analysis of Physicochemical parameters’ results

The statistical analysis doesn’t underline significant differences in the values of volatile acidity for the treatments “pruning” and “fertilizer”, for this reason all the graphics for the non-significant treatments are in the “annex” part of the present work.

In the table 6 below are summarized all the statistical analysis’ results for the physicochemical parameters of 2019 wines. The table 6 below underlines that there is not a statistical significance for the previously mentioned treatments and values, so the different treatments didn’t lead to a statistically-significant differences in the final wines of 2019 vintage.

Table 6 – Statistical Analysis of the 2019 wines’ physicochemical parameters’ results

Treatment	Volatile acidity (g/L of acetic acid)	Total acidity (g/L of tartaric acid)	pH	Alcohol (% v/v)	Sugars (g/L)
MAN	0.46	8.98	3.27	14.38	0.54
MEC	0.49	8.52	3.25	14.58	0.42
<b>Sig</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>
M1	0.51	8.43	3.28	14.73	0.40
M2	0.48	8.77	3.26	14.54	0.46
M3	0.44	8.82	3.26	14.62	0.43
Test	0.47	8.92	3.25	14.41	0.39
<b>Sig</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>
P * FERT	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>

MAN – manual pruning; MEC – mechanical pruning; M1 – 5.000 kg/ha of MSWC; M2 – 10.000 kg/ha of MSWC; M3 – 20.000 kg/ha of MSWC; Test – Without MSWC; \* – p-value between 0.05 – 0.01; \*\* – p-value between 0.01 – 0.001; \*\*\* – p-value lower than 0.001; ns – not significant

In the table 6 above we can notice that both the pruning technique and the use of different amounts of municipal solid waste compost didn't lead to statistically significant differences in the final wines. Moreover, looking at the obtained values, we can notice higher values of total acidity in the wines belonging to manually-pruned vines. This could happen because manual pruning technique leads to a lower yield. In this way the vine has a higher crop and a lower yield and it can synthesize higher amounts of substances (acids, sugars, phenols, and so on).

### 5.2.3. Analysis of the wines’ color and phenolic composition

For the wines from both 2018 and 2019 vintage the chromatic characteristics and the phenolic composition were determined in February 2020, so after the bottling for 2018 wines and before the bottling for 2019 wines. Regarding the chromatic characteristics two methods have been used. The first method is the CIE Lab, obtaining for each sample three coordinates called “L”, “a” and “b” (in the tables 7, 8 and 9 below).

Table 7 – CIE Lab method's coordinates vintage 2018

<b>Sample</b>	<b>L</b>	<b>a</b>	<b>b</b>
MAN B1	99.34	-0.43	3.05
MAN B2	99.11	-0.40	2.79
MAN B3	99.22	-0.41	3.17
MAN B4	99.15	-0.47	2.96
MEC B1	99.20	-0.30	3.12
MEC B2	99.25	-0.23	2.62
MEC B3	99.31	-0.28	2.66
MEC B4	99.30	-0.28	2.62

MAN – manual pruning; MEC – mechanical pruning; B – block's number;

Table 8 – CIE Lab method's coordinates vintage 2019 with manual pruning

<b>Sample</b>	<b>L</b>	<b>a</b>	<b>b</b>
MAN B1 M1	98.38	-0.21	3.67
MAN B1 M2	98.67	-0.31	3.44
MAN B1 M3	98.77	-0.39	3.28
MAN B1 TEST	98.85	-0.49	3.41
MAN B2 M1	98.92	-0.36	3.58
MAN B2 M2	98.87	-0.38	3.47
MAN B2 M3	98.23	-0.20	4.00
MAN B2 TEST	97.59	-0.59	2.70
MAN B3 M1	98.26	-0.20	3.92
MAN B3 M2	98.82	-0.27	3.52
MAN B3 M3	98.68	-0.29	3.42
MAN B3 TEST	98.97	-0.25	3.16
MAN B4 M1	98.99	-0.29	3.16
MAN B4 M2	98.64	-0.52	4.00
MAN B4 M3	98.10	-0.35	4.10
MAN B4 TEST	98.46	-0.41	4.19

MAN – manual pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

Table 9 – CIE Lab method's coordinates vintage 2019 with mechanical pruning

<b>Sample</b>	<b>L</b>	<b>a</b>	<b>b</b>
MEC B1 M1	99.06	-0.26	3.40
MEC B1 M2	99.14	-0.20	3.15
MEC B1 M3	98.86	-0.32	3.63
MEC B1 TEST	99.17	-0.19	2.81
MEC B2 M1	98.70	-0.17	3.34
MEC B2 M2	98.83	-0.26	3.46
MEC B2 M3	98.54	-0.37	3.76
MEC B2 TEST	98.70	-0.24	3.63
MEC B3 M1	98.78	-0.26	3.39
MEC B3 M2	99.00	-0.25	3.44
MEC B3 M3	99.00	-0.33	3.33
MEC B3 TEST	98.78	-0.24	3.69
MEC B4 M1	98.44	-0.26	3.40
MEC B4 M2	98.67	-0.26	3.47
MEC B4 M3	98.75	-0.27	3.28
MEC B4 TEST	98.66	-0.23	3.75

MEC – mechanical pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

As we can notice from the preview tables (7, 8 and 9), the analysed values of the “a” parameter by the cieLab method underlined lower values for the wines belonging to manual-pruned vines compared with those belonging to mechanical-pruned vines.

Since the “a” value indicate the colour of the wine in a range from green (-a) to red (+a), we can say that the wines from mechanical pruning had a higher red colour. This method allows us to obtain, following some calculations, three parameters of clarity, chroma (C) and hue (H) for each wine's sample (in the tables 10, 11 and 12 below).

Table 10 – CIE Lab method's results vintage 2018

<b>Sample</b>	<b>Clarity</b>	<b>Chroma (C)</b>	<b>Hue</b>
MAN B1	99.34	3.08	0.12
MAN B2	99.11	2.82	0.13
MAN B3	99.22	3.19	0.13
MAN B4	99.15	2.99	0.11
MEC B1	99.20	3.13	0.18
MEC B2	99.25	2.63	0.20
MEC B3	99.31	2.67	0.17
MEC B4	99.30	2.63	0.20

MAN – manual pruning; MEC – mechanical pruning; B – block's number;

Table 11 – CIE Lab method's results vintage 2019 with manual pruning

<b>Sample</b>	<b>Clarity</b>	<b>Chroma (C)</b>	<b>Hue</b>
MAN B1 M1	98.38	3.67	0.31
MAN B1 M2	98.67	3.44	0.19
MAN B1 M3	98.77	3.43	0.15
MAN B1 TEST	98.85	3.45	0.12
MAN B2 M1	98.92	3.59	0.17
MAN B2 M2	98.87	3.49	0.16
MAN B2 M3	98.23	4.00	0.36
MAN B2 TEST	97.59	2.76	0.08
MAN B3 M1	98.26	3.92	0.35
MAN B3 M2	98.82	3.52	0.23
MAN B3 M3	98.68	3.43	0.21
MAN B3 TEST	98.97	3.16	0.22
MAN B4 M1	98.99	3.17	0.19
MAN B4 M2	98.64	4.03	0.13
MAN B4 M3	98.10	4.11	0.21
MAN B4 TEST	98.46	4.20	0.18

MAN – manual pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

Table 12 – CIE Lab method's results vintage 2019 with mechanical pruning

<b>Sample</b>	<b>Clarity</b>	<b>Chroma (C)</b>	<b>Hue</b>
MEC B1 M1	99.06	3.40	0.23
MEC B1 M2	99.14	3.18	0.28
MEC B1 M3	98.86	3.73	0.20
MEC B1 TEST	99.17	2.83	0.26
MEC B2 M1	98.70	3.35	0.35
MEC B2 M2	98.83	3.46	0.23
MEC B2 M3	98.54	3.77	0.18
MEC B2 TEST	98.70	3.63	0.27
MEC B3 M1	98.78	3.39	0.23
MEC B3 M2	99.00	3.44	0.24
MEC B3 M3	99.00	3.34	0.17
MEC B3 TEST	98.78	3.69	0.27
MEC B4 M1	98.44	3.40	0.23
MEC B4 M2	98.67	3.47	0.24
MEC B4 M3	98.75	3.28	0.21
MEC B4 TEST	98.66	3.74	0.29

MEC – mechanical pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

In the tables 13 and 14 below we can find the chromatic differences between the wines from the manually-pruned vineyards and those from mechanically pruned vineyards. These differences have been analysed with the CIE Lab method but, since the results were clearly non-significative, they haven't been analysed from a statistical point of view.

Table 13 – Chromatic differences in 2018 wines

<b>Sample</b>	<b><math>\Delta E</math></b>	<b><math>\Delta H</math></b>
MAN B1 VS MEC B1	0.20	0.14
MAN B2 VS MEC B2	0.27	0.12
MAN B3 VS MEC B3	0.53	0.04
MAN B4 VS MEC B4	0.41	0.13

MAN – manual pruning; MEC – mechanical pruning; B – block's number;  $\Delta E$  – Colorimetric difference;  $\Delta H$  –Difference in hue;

Table 14 – Chromatic differences in 2019 wines

<b>Sample</b>	<b><math>\Delta E</math></b>	<b><math>\Delta H</math></b>
MAN B1 M1 VS MEC B1 M1	0.42	0.59
MAN B1 M2 VS MEC B1 M2	0.71	0.47
MAN B1 M3 VS MEC B1 M3	0.38	0.22
MAN B1 TEST VS MEC B1 TEST	0.72	0.10
MAN B2 M1 VS MEC B2 M1	0.36	0.17
MAN B2 M2 VS MEC B2 M2	0.13	0.11
MAN B2 M3 VS MEC B2 M3	0.41	0.16
MAN B2 TEST VS MEC B2 TEST	1.48	0.47
MAN B3 M1 VS MEC B3 M1	0.74	0.12
MAN B3 M2 VS MEC B3 M2	0.20	0.47
MAN B3 M3 VS MEC B3 M3	0.33	0.10
MAN B3 TEST VS MEC B3 TEST	0.56	0.05
MAN B4 M1 VS MEC B4 M1	0.60	0.00
MAN B4 M2 VS MEC B4 M2	0.59	0.27
MAN B4 M3 VS MEC B4 M3	1.05	0.09
MAN B4 TEST VS MEC B4 TEST	0.52	0.12

MAN – manual pruning; MEC – mechanical pruning; B – block's number; M – Sample's number;  $\Delta E$  – Colorimetric difference;  $\Delta H$  –Difference in hue; TEST – sample without organic amendment

The second used method to analyse the chromatic characteristics of the wines is the official OIV method (2015) and the obtained results are presented in the tables 15, 16 and 17 below.

Table 15 – Color of 2018 wines with OIV method

<b>Sample</b>	<b>ABS 420nm</b>
MAN B1	0.068
MAN B2	0.086
MAN B3	0.092
MAN B4	0.090
MEC B1	0.090
MEC B2	0.084
MEC B3	0.085
MEC B4	0.083

MAN – manual pruning; MEC – mechanical pruning; B – block's number;

Table 16 – Color of 2019 wines with manual pruning with OIV method

<b>Sample</b>	<b>ABS 420nm</b>
MAN B1 M1	0.094
MAN B1 M2	0.096
MAN B1 M3	0.113
MAN B1 TEST	0.101
MAN B2 M1	0.099
MAN B2 M2	0.098
MAN B2 M3	0.111
MAN B2 TEST	0.100
MAN B3 M1	0.105
MAN B3 M2	0.099
MAN B3 M3	0.088
MAN B3 TEST	0.084
MAN B4 M1	0.088
MAN B4 M2	0.105
MAN B4 M3	0.113
MAN B4 TEST	0.106

MAN – manual pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

Table 17 – Color of 2019 wines with manual pruning with OIV method

<b>Sample</b>	<b>ABS 420nm</b>
MEC B1 M1	0.097
MEC B1 M2	0.093
MEC B1 M3	0.098
MEC B1 TEST	0.084
MEC B2 M1	0.100
MEC B2 M2	0.094
MEC B2 M3	0.097
MEC B2 TEST	0.087
MEC B3 M1	0.095
MEC B3 M2	0.092
MEC B3 M3	0.092
MEC B3 TEST	0.094
MEC B4 M1	0.089
MEC B4 M2	0.090
MEC B4 M3	0.091
MEC B4 TEST	0.092

MAN – manual pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

It's possible to find a relation between the “b” values of the cieLab method and the 420nm absorbance values in the final wines.

In fact, the cieLab method underlined higher values of “b” in the wines belonging to manual-pruned vines and, since the “b” value indicates a variation of colour between blue (-b) and yellow (+b), we can affirm that manual pruning led to a higher yellow colour in the wines.

Also the analysis of 420nm absorbance underlined higher values (higher yellow colour) in the wines from manual-pruned wines so, comparing the two methods, we can affirm that manual pruning led to higher yellow colour in the final wines.

After the chromatic characteristics, analysis of phenolic composition have been done, obtaining the results in the table 18, 19 and 20 below.

Table 18 – Results of phenols' analysis of 2018 wines

<b>Sample</b>	<b>Total phenols (mg/L of gallic acid)</b>	<b>Non-flavonoids (mg/L of gallic acid)</b>	<b>Flavonoids (mg/L of gallic acid)</b>
MAN B1	165.90	86.60	79.40
MAN B2	162.50	88.50	73.90
MAN B3	179.30	90.10	89.20
MAN B4	157.40	83.30	74.10
MEC B1	178.00	87.20	90.70
MEC B2	177.60	94.70	89.90
MEC B3	166.50	87.20	79.20
MEC B4	170.30	89.30	81.00

MAN – manual pruning; MEC – mechanical pruning; B – block's number;

Table 19 – Results of phenols' analysis of 2019 wines with manual pruning

<b>Sample</b>	<b>Total phenols (mg/L of gallic acid)</b>	<b>Non-flavonoids (mg/L of gallic acid)</b>	<b>Flavonoids (mg/L of gallic acid)</b>
MAN B1 M1	168.80	68.80	100.00
MAN B1 M2	185.10	67.20	117.90
MAN B1 M3	164.00	61.40	102.60
MAN B1 TEST	168.30	61.40	106.90
MAN B2 M1	175.10	60.50	114.60
MAN B2 M2	189.60	69.50	120.10
MAN B2 M3	199.00	72.50	126.50
MAN B2 TEST	186.40	63.90	122.58
MAN B3 M1	188.50	64.90	123.60
MAN B3 M2	177.10	64.90	112.20
MAN B3 M3	182.10	64.30	117.70
MAN B3 TEST	189.90	67.30	122.50
MAN B4 M1	167.50	58.90	108.50
MAN B4 M2	179.10	66.50	112.60
MAN B4 M3	179.10	67.20	111.80
MAN B4 TEST	185.10	68.50	116.60

MAN – manual pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

Table 20 – Results of phenols' analysis of 2019 wines with mechanical pruning

Sample	Total phenols (mg/L of gallic acid)	Non-flavonoids (mg/L of gallic acid)	Flavonoids (mg/L of gallic acid)
MEC B1 M1	182.00	70.20	111.70
MEC B1 M2	211.40	55.60	155.80
MEC B1 M3	188.80	62.90	125.90
MEC B1 TEST	224.60	66.60	158.00
MEC B2 M1	181.90	61.90	120.00
MEC B2 M2	188.50	67.80	120.80
MEC B2 M3	202.20	73.70	128.50
MEC B2 TEST	173.30	63.50	109.80
MEC B3 M1	194.00	67.80	126.20
MEC B3 M2	184.30	85.90	118.50
MEC B3 M3	174.90	64.90	109.90
MEC B3 TEST	188.50	65.60	122.90
MEC B4 M1	181.90	69.50	112.40
MEC B4 M2	182.20	68.50	113.70
MEC B4 M3	188.50	70.80	117.70
MEC B4 TEST	170.90	62.70	108.20

MEC – mechanical pruning; B – block's number; M – Sample's number; TEST – sample without organic amendment

#### 5.2.4. Statistical Analysis of the wines' color and phenolic composition results

The table 25 below summarizes the results of the statistical analysis for the color and the phenolic composition of the 2018 vintage wines. From the table we can see how the pruning technique is the unique treatment that influenced in a statistically-significant way the values of the final wines. In fact, the statistical analysis underlined a net increment of the hue's value in the wines from mechanical-pruned vines (table 21 and figure 2 below).

The same effect was noticed by Archer and Van Schalkwyk (2007) in a research about The effects of pruning methods on the oenological performance of Chenin blanc variety. In fact, the authors concluded that the colour of Chenin blanc wines was improved by mechanical pruning.

The same study (Archer and Van Schalkwyk, 2007) underlines mechanical pruning leads to a significantly increase of yield, while bunch mass, berry mass and berry volume decreased. The decrease in berry size and volume is the result of a higher number of bunches per vine and it leads to an increase of colour in the skins and wines.

Nevertheless, it's possible that this difference in the hue's values of the final wines depends on different not analysed factors, such as the berry exposure to the sunlight.

Table 21 – Statistical Analysis of the 2018 wines' color and phenolic composition results

Treatment	Total phenols (mg/L of gallic acid)	Non-flavonoids (mg/L of gallic acid)	Flavonoids (mg/L of gallic acid)	Abs 420nm	Clarity	Chroma	Hue
MAN	166.3	87.1	79.2	0.084	99.2	3.02	0.120
MEC	173.1	89.6	83.5	0.085	99.3	2.77	0.188
<b>Sig</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>**</b>

MAN – manual pruning; MEC – mechanical pruning; \* – p-value between 0.05 – 0.01; \*\* – p-value between 0.01 – 0.001; \*\*\* – p-value lower than 0.001; ns – not significant

Looking at the table 21 above, it's possible to underline a correlation between the values of total phenols (in particular flavonoids) and hue in the final wines. In fact, phenolic acids are the responsible of the colour in a white wine. From the mentioned table we can notice that, with higher values of total phenols (with mechanical pruning) we obtained higher values of hue. So, the amount of phenols in the final wine also influenced the angle of tonality.

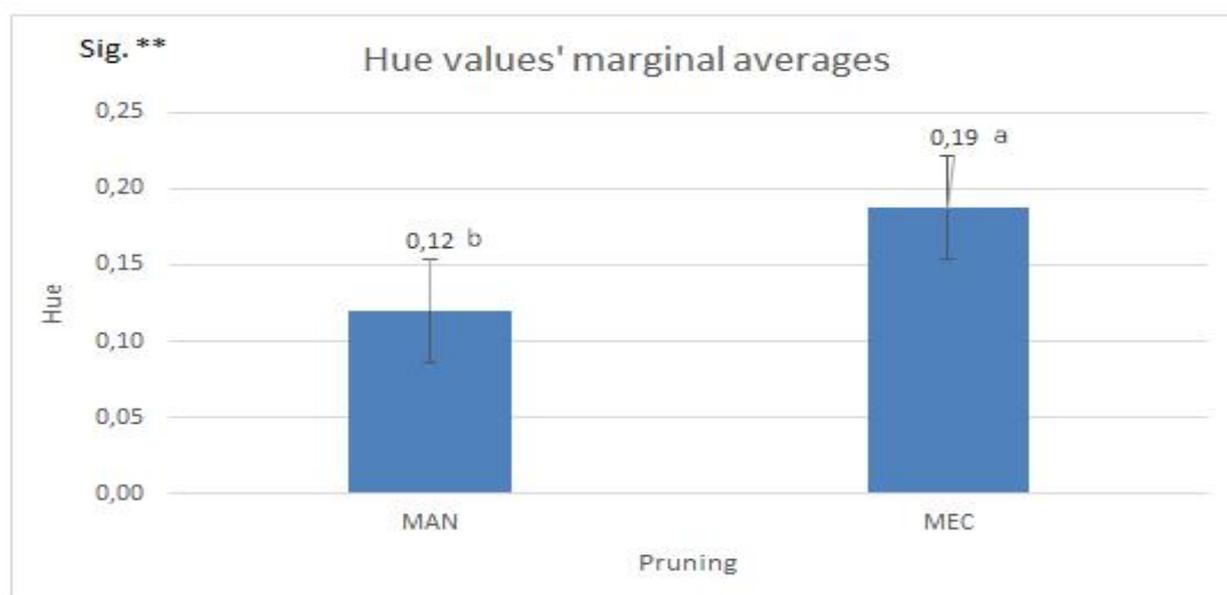


Figure 2 – Effect of Pruning on hue's values of 2018 wines

MAN – manual pruning; MEC – mechanical pruning; \*\* – p-value between 0.01 – 0.001;

The table 21 below doesn't underline a statistically-significative difference in the analysed values for the other treatments. In the table 22 below all the results from the statistical analysis for the color and the phenolic composition of the 2019 vintage wines are summarized.

Table 22 – Statistical Analysis of the 2019 wines' color and phenolic composition results

Treatment	Total phenols (mg/L of gallic acid)	Non-flavonoids (mg/L of gallic acid)	Flavonoids (mg/L of gallic acid)	Abs 420nm	Clarity	Chroma	Hue
MAN	180.3	65.5	114.8	0.100	98.5	3.59	0.204
MEC	188.6	66.1	122.5	0.093	98.8	3.44	0.243
<b>Sig</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>**</b>	<b>**</b>	<b>n.s.</b>	<b>n.s.</b>
M1	180.0	65.3	114.6	0.100	98.6	3.49	0.258
M2	187.2	65.7	121.4	0.100	98.8	3.50	0.213
M3	184.8	67.2	117.6	0.100	98.6	3.64	0.211
Test	185.9	64.9	120.9	0.094	98.7	3.43	0.211
<b>Sig</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>
P * FERT	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>

MAN – manual pruning; MEC – mechanical pruning; M1 – 5.000 kg/ha of MSWC; M2 – 10.000 kg/ha of MSWC; M3 – 20.000 kg/ha of MSWC; Test – Without MSWC; \* – p-value between 0.05 – 0.01; \*\* – p-value between 0.01 – 0.001; \*\*\* – p-value lower than 0.001; ns – not significant

The statistical analysis found a statistically-significative difference in the values of 420nm absorbance and clarity depending by the pruning technique. In fact, the wines from the manually-pruned vineyards showed a higher value of 420nm absorbance (figure 3) which means a higher yellow colour of the wine.

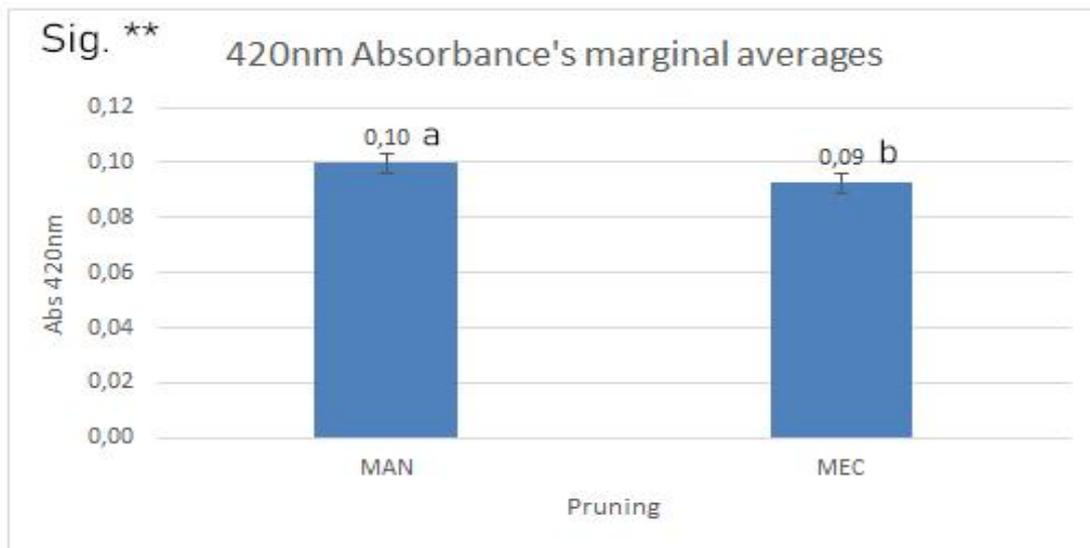


Figure 3 – Effect of Pruning on 420nm Abs values of 2019 wines

MAN – manual pruning; MEC – mechanical pruning; \*\* – p-value between 0.01 – 0.001;

On the contrary, the mechanical pruning treatment lead to a higher value of clarity in the final wines (figure 4).

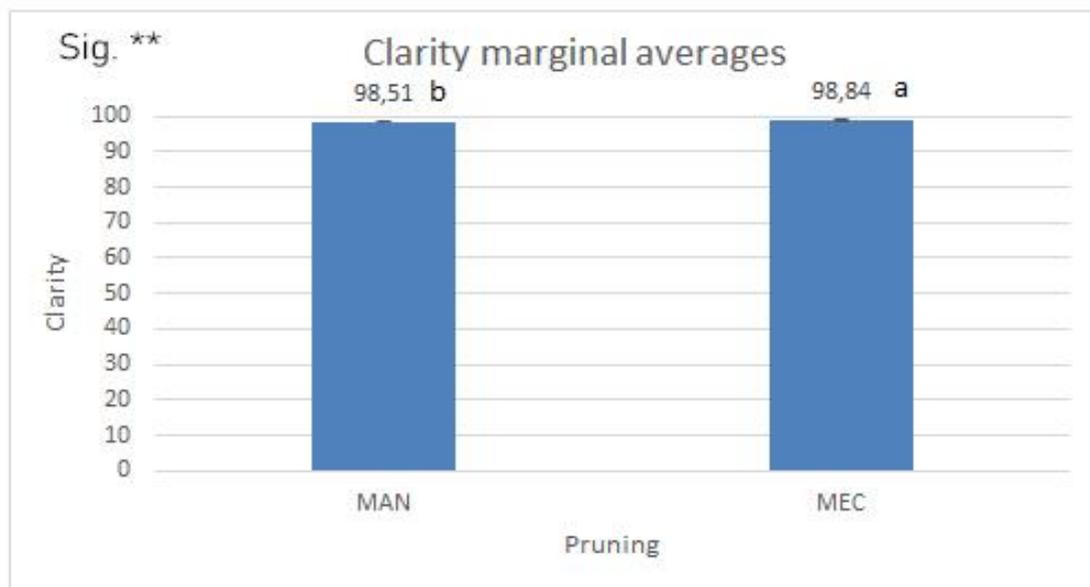


Figure 4 – Effect of Pruning on clarity values of 2019 wines

MAN – manual pruning; MEC – mechanical pruning; \*\* – p-value between 0.01 – 0.001;

## 6. Conclusions

The aim of the present work was to evaluate the effects of different pruning techniques and the use of municipal solid waste compost on Sauvignon blanc wines quality. The analysed parameters were the physicochemical parameters, the phenolic composition and the chromatic characteristics of the wines. In general, about the pruning technique, it is possible to affirm that mechanical pruning led to higher values of hue for the 2018 vintage's wines and higher values of clarity for the 2019 vintage's wines. At the same time, mechanical pruning led to a lower value of 420nm absorbance in 2019 vintage's wines and it means a lower yellow colour of the wine.

So, regarding the colour of the final wines, we can affirm that the manual pruning led to a higher yellow colour of the wine with both cieLab method and 420nm absorbance's measurement.

The effect of organic amendments in wine quality is not so clear, the results depend upon each vineyard. Concerning the physicochemical parameters, the statistical analysis shows that the use of different amounts of municipal solid waste compost in the vineyard doesn't lead to statistically significant differences in the final wines.

In general, we can conclude that the decision between manual pruning or mechanical pruning must be taken considering the type of wine to produce, because the differences in the results are not extremely high. Nevertheless, the mechanical pruning allows us to increase the yield maintaining an high quality of the wine and decreasing the vineyard's management costs. In fact, mechanical pruning is a very positive technique to reduce the management costs of the vineyard without having repercussions on the quality of the grapes and the final wine. Furthermore, this pruning technique allows us to have an increase in yield and this is a very important factor, especially in wine-growing regions with low productivity such as, for example, the wine-growing region of Lisbon.

Speaking about the use of organic amendments in the vineyard it's possible to affirm that, in general, they can be a good alternative to chemical fertilizers.

However, its addition must be carefully made and only after analyzing the nutritional needs of the plants.

Also the possible heavy metals phytotoxicity must be analysed, in fact the municipal solid waste compost is rich in heavy metals and it could cause a phytotoxicity for the plant.

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## 8. Annex

Annex 1- Monitored parameters during alcoholic fermentation: 2018 vintage, block 1, manual pruning

<b>Data</b>	<b>Density (kg/dm<sup>3</sup>)</b>	<b>Temperature (°C)</b>
19/09	1,089	24
22/09	1,086	18,5
23/09	1,071	18,5
24/09	1,056	18,5
25/09	1,038	18,5
26/09	1,023	18,5
27/09	1,010	18,5
28/09	1,000	18,5
29/09	0,996	18,5
30/09	0,993	22
01/10	0,992	23

Annex 2- Monitored parameters during alcoholic fermentation: 2018 vintage, block 2, manual pruning

<b>Data</b>	<b>Density (kg/dm<sup>3</sup>)</b>	<b>Temperature (°C)</b>
19/09	1,088	24
22/09	1,088	18,5
23/09	1,075	18,5
24/09	1,059	18,5
25/09	1,044	18,5
26/09	1,027	18,5
27/09	1,016	18,5
28/09	1,003	18,5
29/09	0,997	18,5
30/09	0,993	22
01/10	0,992	23

Annex 3- Monitored parameters during alcoholic fermentation: 2018 vintage, block 3, manual pruning

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
19/09	1,090	24
22/09	1,090	18,5
23/09	1,078	18,5
24/09	1,062	18,5
25/09	1,046	18,5
26/09	1,029	18,5
27/09	1,017	18,5
28/09	1,005	18,5
29/09	0,997	18,5
30/09	0,993	22
01/10	0,992	23

Annex 4- Monitored parameters during alcoholic fermentation: 2018 vintage, block 4, manual pruning

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
19/09	1,087	24
22/09	1,086	18,5
23/09	1,073	18,5
24/09	1,057	18,5
25/09	1,039	18,5
26/09	1,023	18,5
27/09	1,010	18,5
28/09	1,001	18,5
29/09	0,995	18,5
30/09	0,992	22
01/10	0,992	23

Annex 5- Monitored parameters during alcoholic fermentation: 2018 vintage, block 1, mechanical pruning

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
19/09	1,091	24
22/09	1,091	18,5
23/09	1,081	18,5
24/09	1,072	18,5
25/09	1,064	18,5
26/09	1,058	18,5
27/09	1,046	18,5
28/09	1,037	18,5
29/09	1,028	18,5
30/09	1,020	18,5
01/10	1,011	18,5
02/10	1,005	18,5
03/10	1,000	18,5
04/10	0,997	18,5
05/10	0,993	22
06/10	0,992	22

Annex 6- Monitored parameters during alcoholic fermentation: 2018 vintage, block 2, mechanical pruning

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
19/09	1,090	24
22/09	1,090	18,5
23/09	1,082	18,5
24/09	1,073	18,5
25/09	1,068	18,5
26/09	1,061	18,5
27/09	1,051	18,5
28/09	1,041	18,5
29/09	1,031	18,5
30/09	1,023	18,5
01/10	1,014	18,5
02/10	1,007	18,5
03/10	1,001	18,5
04/10	0,998	18,5
05/10	0,994	22
06/10	0,993	22
07/10	0,992	22

Annex 7- Monitored parameters during alcoholic fermentation: 2018 vintage, block 3, mechanical pruning

<b>Data</b>	<b>Density (kg/dm<sup>3</sup>)</b>	<b>Temperature (°C)</b>
19/09	1,089	24
22/09	1,089	18,5
23/09	1,081	18,5
24/09	1,072	18,5
25/09	1,066	18,5
26/09	1,058	18,5
27/09	1,048	18,5
28/09	1,038	18,5
29/09	1,028	18,5
30/09	1,020	18,5
01/10	1,011	18,5
02/10	1,005	18,5
03/10	1,000	18,5
04/10	0,997	18,5
05/10	0,994	22
06/10	0,992	22

Annex 8- Monitored parameters during alcoholic fermentation: 2018 vintage, block 4, mechanical pruning

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
19/09	1,091	24
22/09	1,091	18
23/09	1,082	18,5
24/09	1,074	18,5
25/09	1,067	18,5
26/09	1,059	18,5
27/09	1,048	18,5
28/09	1,038	18,5
29/09	1,027	18,5
30/09	1,019	18,5
01/10	1,010	18,5
02/10	1,004	18,5
03/10	0,999	18,5
04/10	0,996	18,5
05/10	0,993	22
06/10	0,992	22

Annex 9- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, manual pruning, test (without MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,095	23
15/09	1,067	25
16/09	1,036	25
17/09	1,016	25
18/09	1,000	25
19/09	0,994	24
20/09	0,993	24
21/09	0,993	24

Annex 10- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, manual pruning, L (5.000 kg/ha MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,097	23
15/09	1,065	25
16/09	1,030	25
17/09	1,010	25
18/09	0,998	24
19/09	0,993	24
20/09	0,992	24
21/09	0,992	24

Annex 11- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, manual pruning, M (10.000 kg/ha MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,097	23
15/09	1,084	25
16/09	1,056	25
17/09	1,029	25
18/09	1,011	25
19/09	0,998	24
20/09	0,993	24
21/09	0,992	24

Annex 12- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, manual pruning, H (20.000 kg/ha MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,097	23
15/09	1,066	25
16/09	1,037	25
17/09	1,015	25
18/09	1,010	25
19/09	0,995	24
20/09	0,992	24
21/09	0,992	24

Annex 13- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, mechanical pruning, Test (without MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,093	23
15/09	1,074	25
16/09	1,047	25
17/09	1,029	25
18/09	1,011	25
19/09	1,000	24
20/09	0,995	24
21/09	0,992	24
22/09	0,992	24

Annex 14- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, mechanical pruning, L (5.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,099	23
15/09	1,077	25
16/09	1,051	25
17/09	1,030	25
18/09	1,015	25
19/09	1,004	25
20/09	0,997	24
21/09	0,993	24
22/09	0,992	24

Annex 15- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, mechanical pruning, M (10.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,096	23
15/09	1,074	25
16/09	1,052	25
17/09	1,035	25
18/09	1,021	25
19/09	1,010	25
20/09	1,001	24
21/09	0,996	24
22/09	0,992	24
23/09	0,992	24

Annex 16- Monitored parameters during alcoholic fermentation: 2019 vintage, block 1, mechanical pruning, H (20.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,100	23
15/09	1,072	25
16/09	1,043	25
17/09	1,023	25
18/09	1,009	25
19/09	1,000	25
20/09	0,994	24
21/09	0,992	24
22/09	0,992	24

Annex 17- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, manual pruning, Test (without MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,095	23
15/09	1,072	25
16/09	1,045	25
17/09	1,029	25
18/09	1,014	25
19/09	1,004	25
20/09	0,997	24
21/09	0,994	24
22/09	0,992	24
23/09	0,992	24

Annex 18- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, manual pruning, L (5.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,098	23
15/09	1,066	25
16/09	1,037	25
17/09	1,020	25
18/09	1,005	25
19/09	0,996	24
20/09	0,993	24
21/09	0,992	24
22/09	0,992	24

Annex 19- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, manual pruning, M (10.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,101	23
15/09	1,066	25
16/09	1,026	25
17/09	1,006	25
18/09	0,997	24
19/09	0,992	24
20/09	0,992	24

Annex 20- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, manual pruning, H (20.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,100	23
15/09	1,076	25
16/09	1,028	25
17/09	1,006	25
18/09	0,997	24
19/09	0,992	24
20/09	0,992	24

Annex 21- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, mechanical pruning, Test (without MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,099	23
15/09	1,076	25
16/09	1,054	25
17/09	1,037	25
18/09	1,021	25
19/09	1,010	25
20/09	1,001	25
21/09	0,995	24
22/09	0,992	24
23/09	0,992	24

Annex 22- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, mechanical pruning, L (5.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,098	23
15/09	1,070	25
16/09	1,055	25
17/09	1,040	25
18/09	1,023	25
19/09	1,011	25
20/09	1,002	24
21/09	0,996	24
22/09	0,992	24
23/09	0,992	24

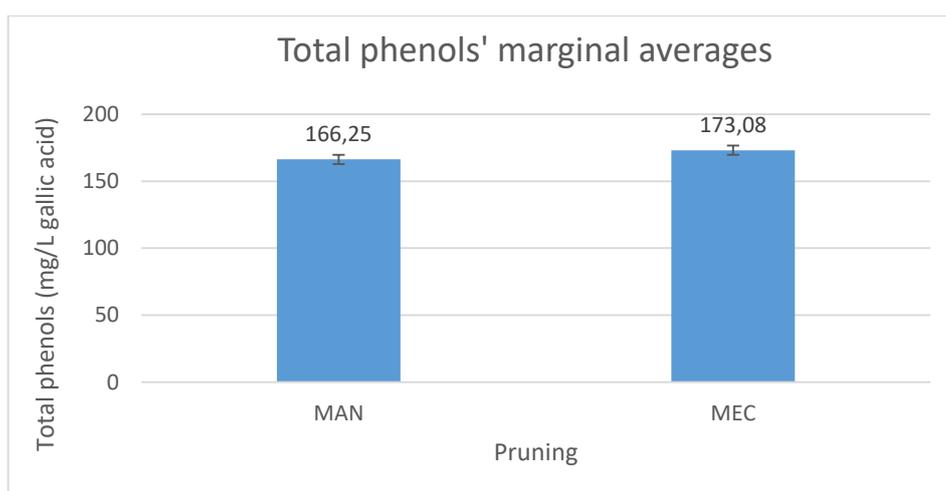
Annex 23- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, mechanical pruning, M (10.000 kg/ha of MSWC)

<b>Data</b>	<b>Density (kg/dm3)</b>	<b>Temperature (°C)</b>
13/09	1,102	23
15/09	1,080	25
16/09	1,057	25
17/09	1,039	25
18/09	1,024	25
19/09	1,012	25
20/09	1,002	24
21/09	0,994	24
22/09	0,992	24
23/09	0,992	24

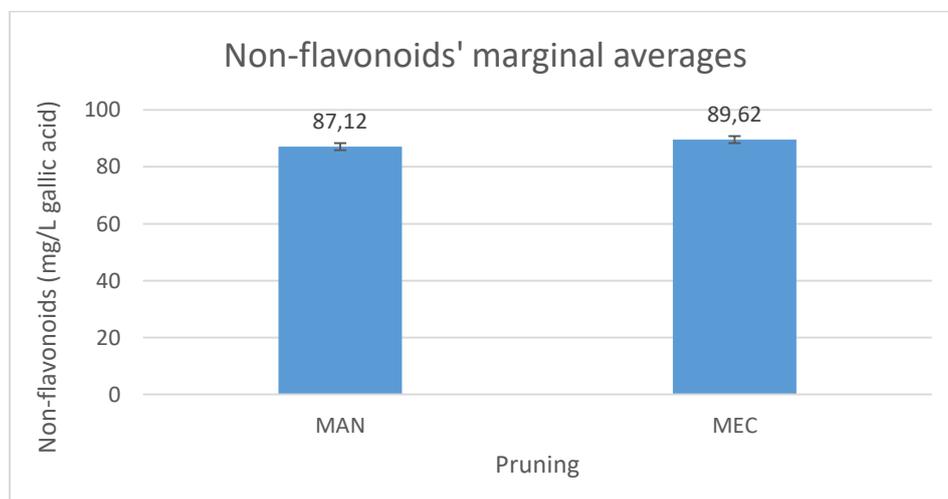
Annex 24- Monitored parameters during alcoholic fermentation: 2019 vintage, block 2, mechanical pruning, H (20.000 kg/ha of MSWC)

Data	Density (kg/dm <sup>3</sup> )	Temperature (°C)
13/09	1,103	23
15/09	1,068	25
16/09	1,040	25
17/09	1,021	25
18/09	1,008	25
19/09	0,999	24
20/09	0,994	24
21/09	0,992	24
22/09	0,992	24

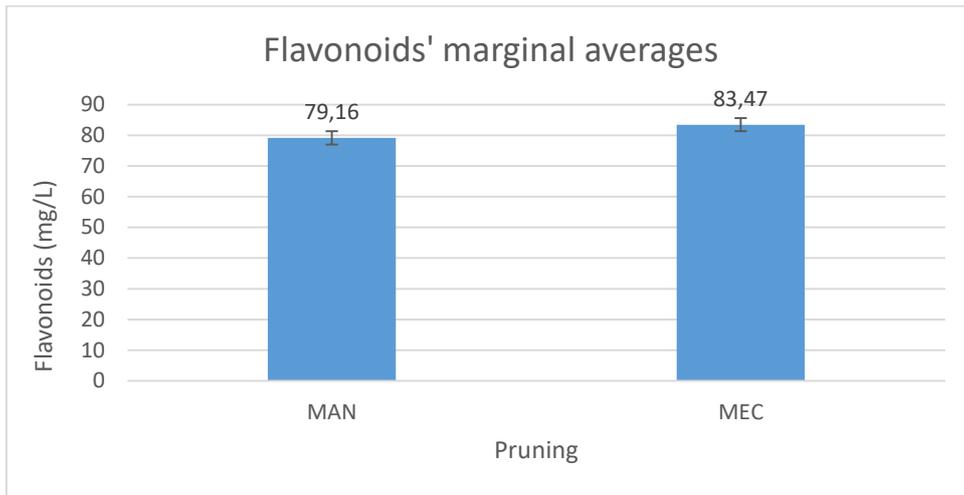
Annex 25- Effect of Pruning on total phenols' values of 2018 wines



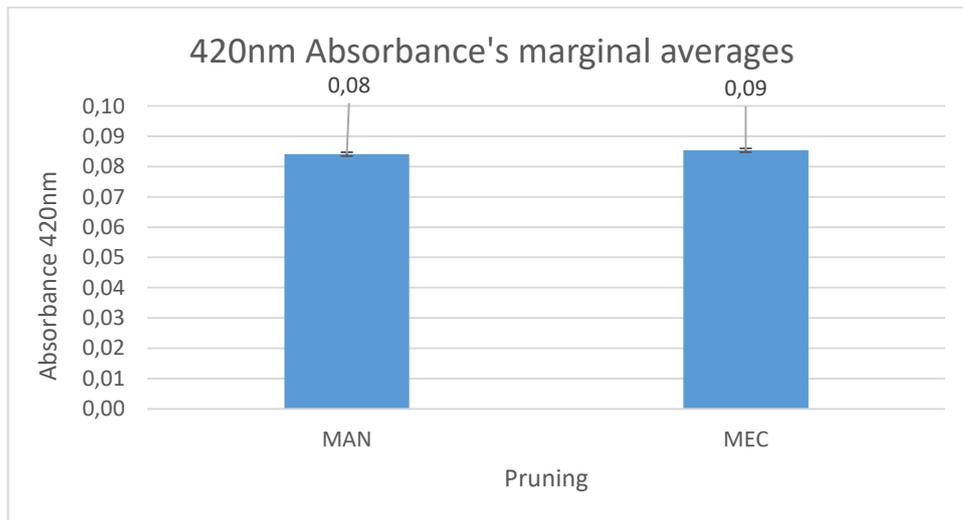
Annex 26- Effect of Pruning on non-flavonoids' values of 2018 wines



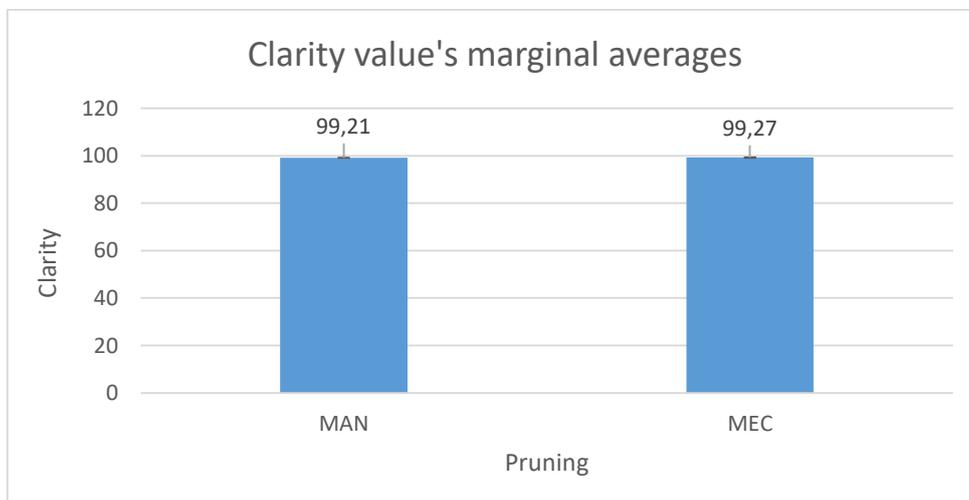
Annex 27- Effect of Pruning on Flavonoids' values of 2018 wines



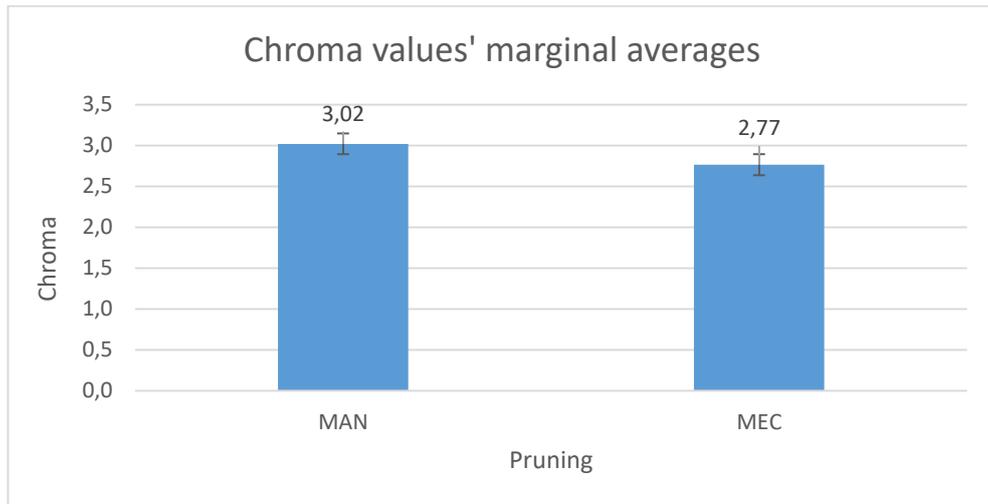
Annex 28- Effect of Pruning on 420nm absorbance values of 2018 wines



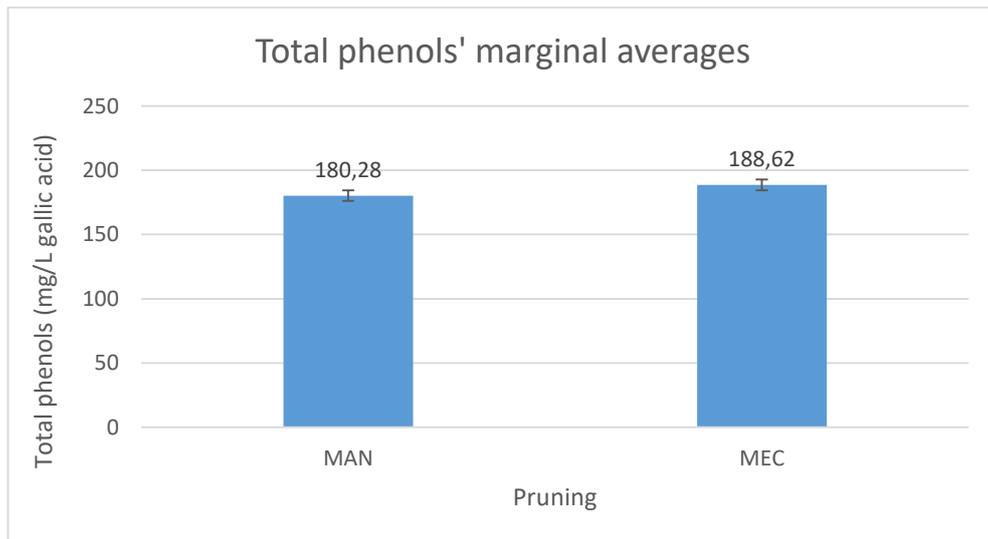
Annex 29- Effect of Pruning on Clarity values of 2018 wines



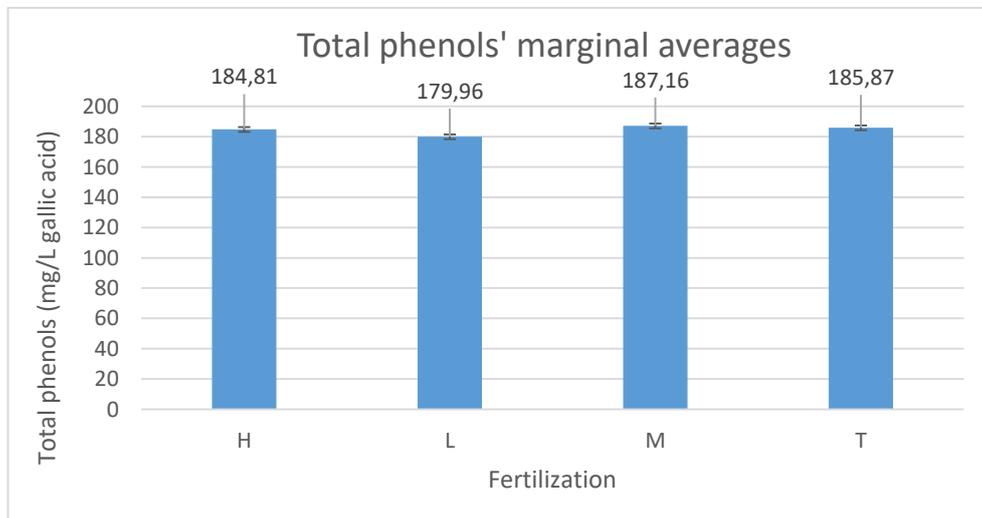
Annex 30- Effect of Pruning on Chroma values of 2018 wines



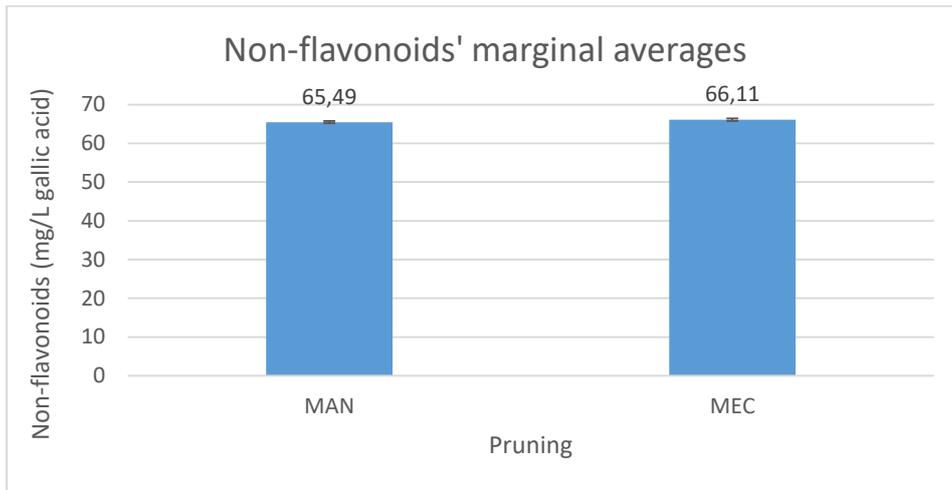
Annex 31- Effect of Pruning on Total phenols' values of 2019 wines



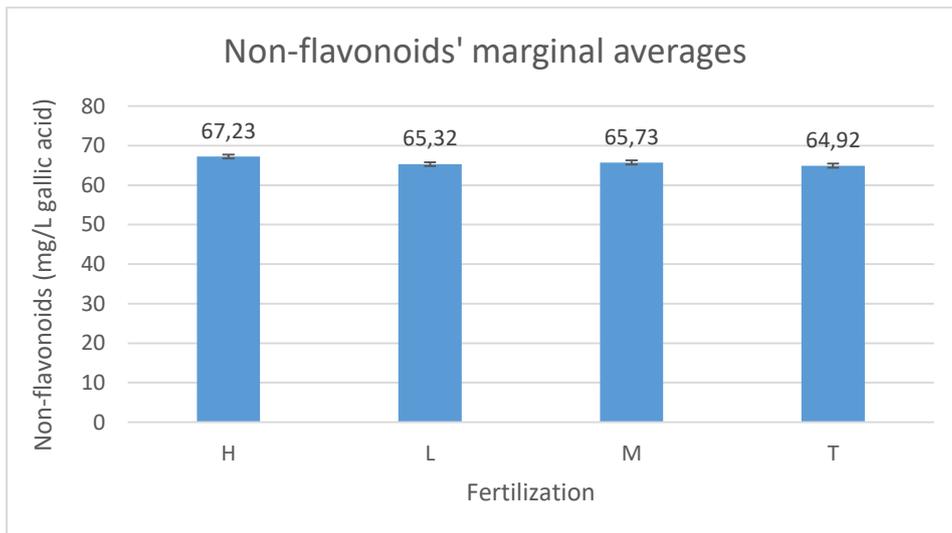
Annex 32- Effect of fertilization on Total phenols' values of 2019 wines



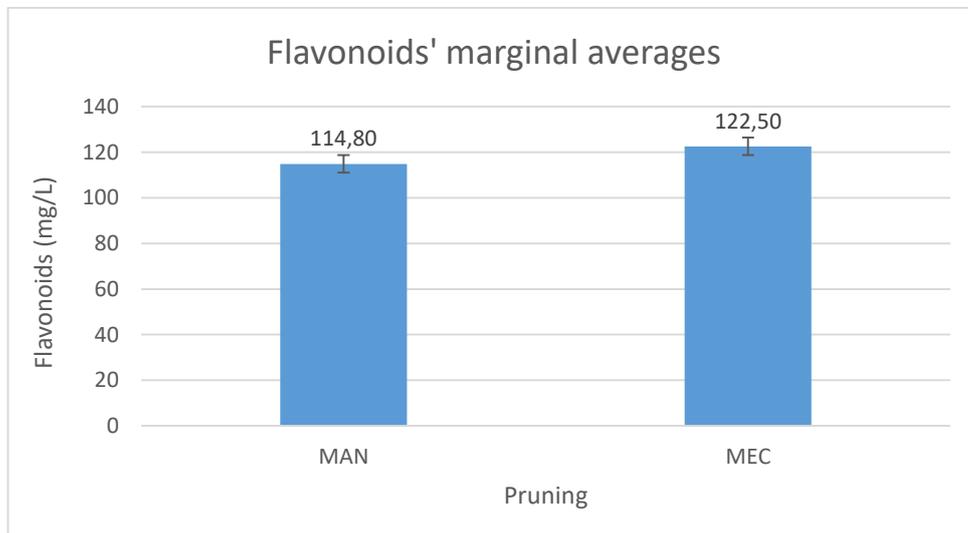
Annex 33- Effect of Pruning on non-flavonoids' values of 2019 wines



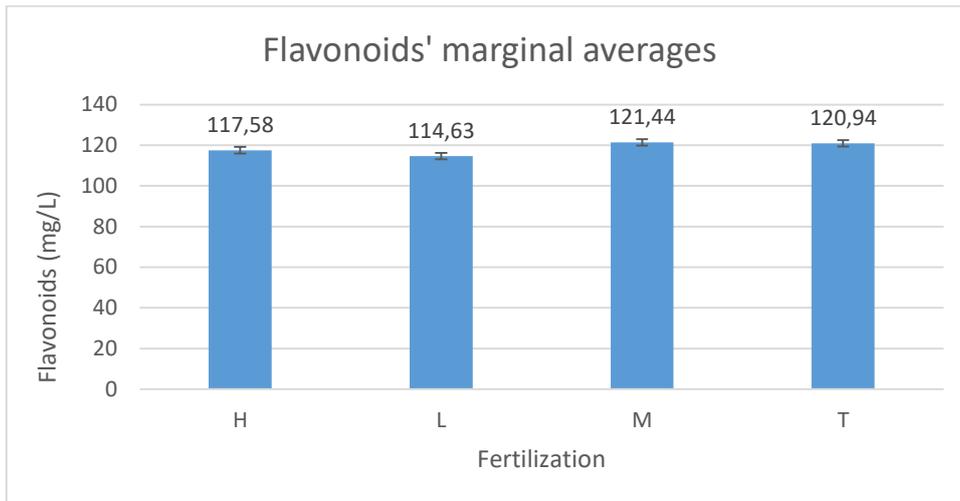
Annex 34- Effect of fertilization on non-flavonoids' values of 2019 wines



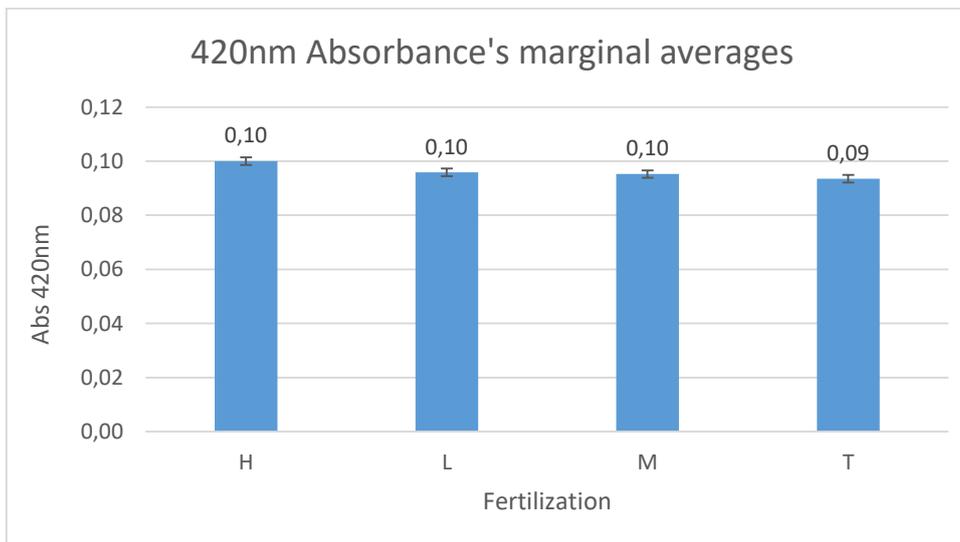
Annex 35- Effect of Pruning on flavonoids' values of 2019 wines



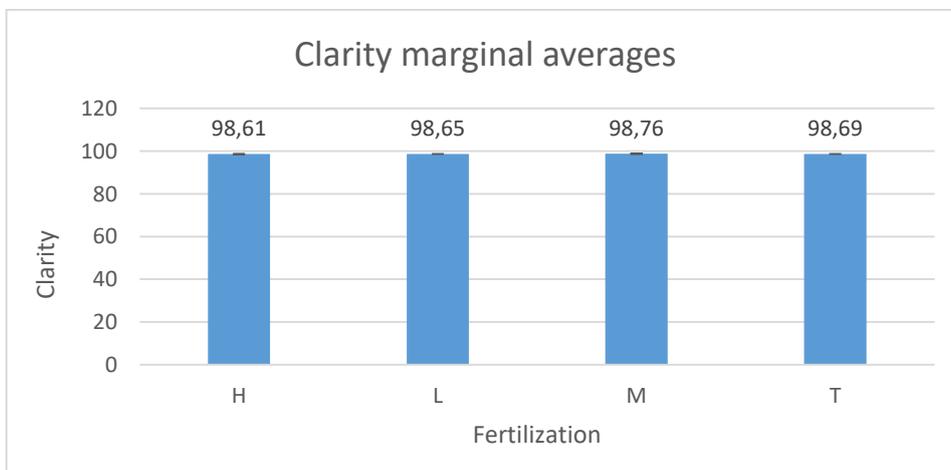
Annex 36- Effect of fertilization on flavonoids' values of 2019 wines



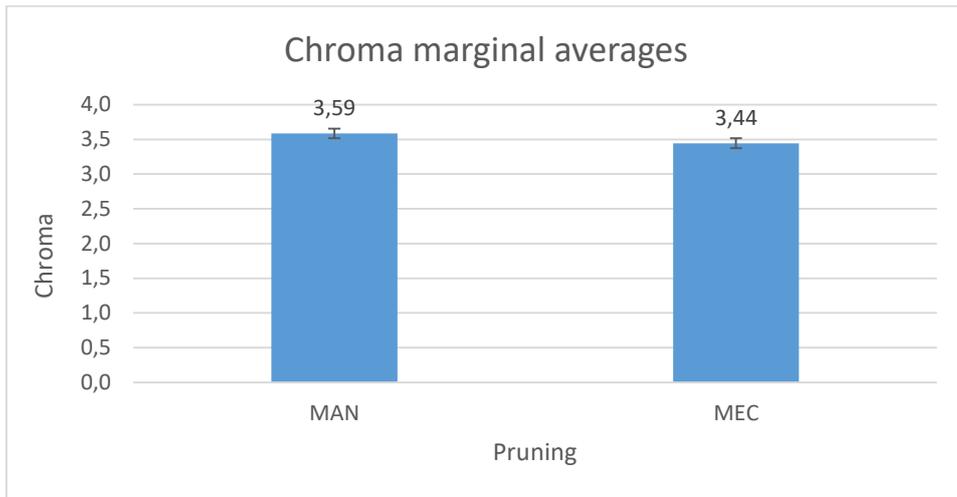
Annex 37- Effect of fertilization on 420nm absorbance values of 2019 wines



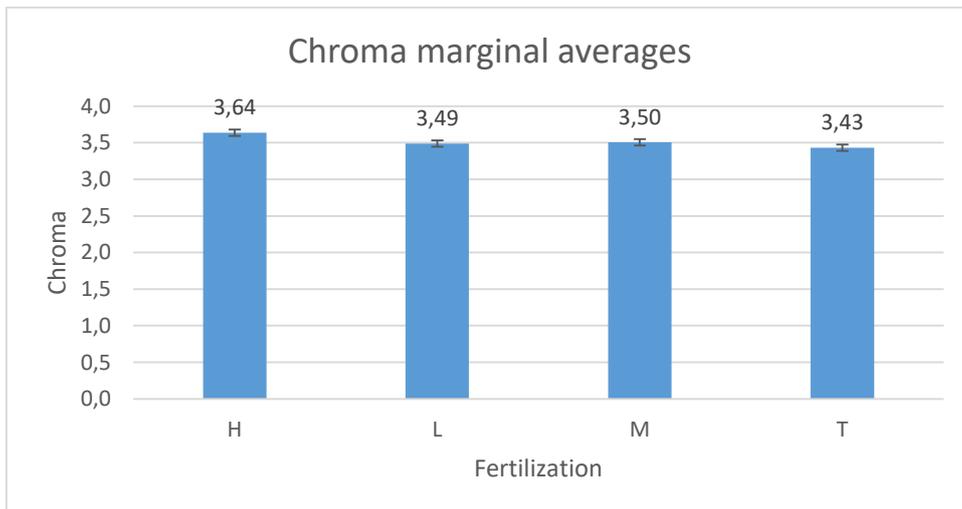
Annex 38- Effect of fertilization on clarity values of 2019 wines



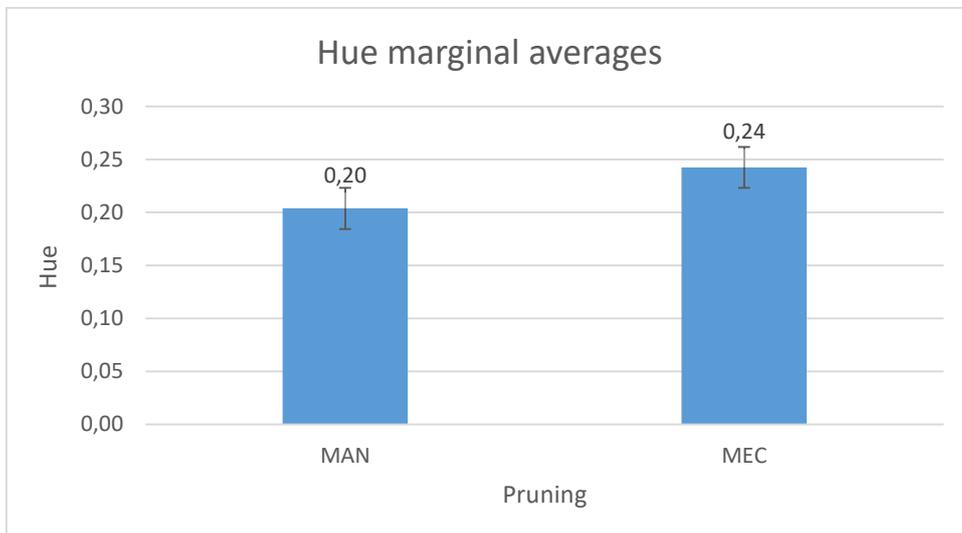
Annex 39- Effect of pruning on chroma values of 2019 wines



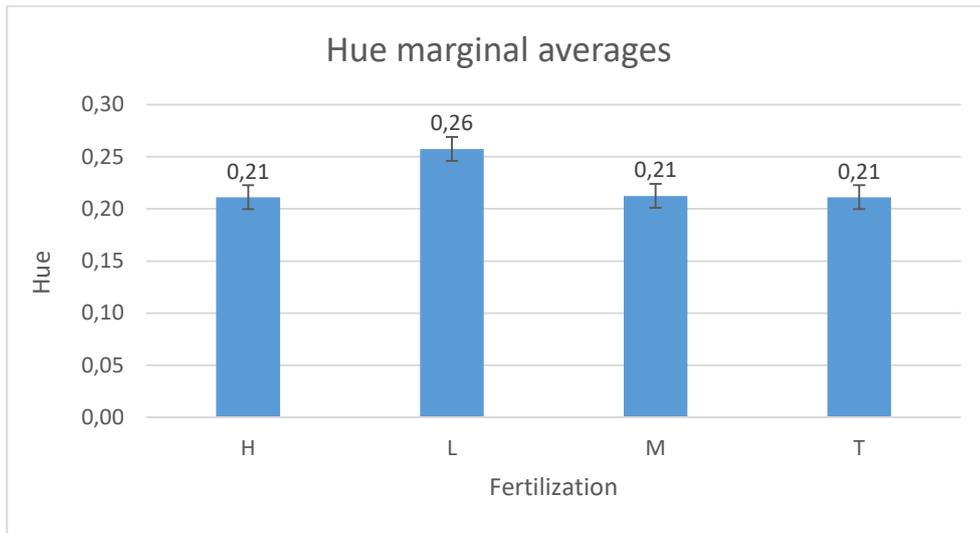
Annex 40- Effect of fertilization on chroma values of 2019 wines



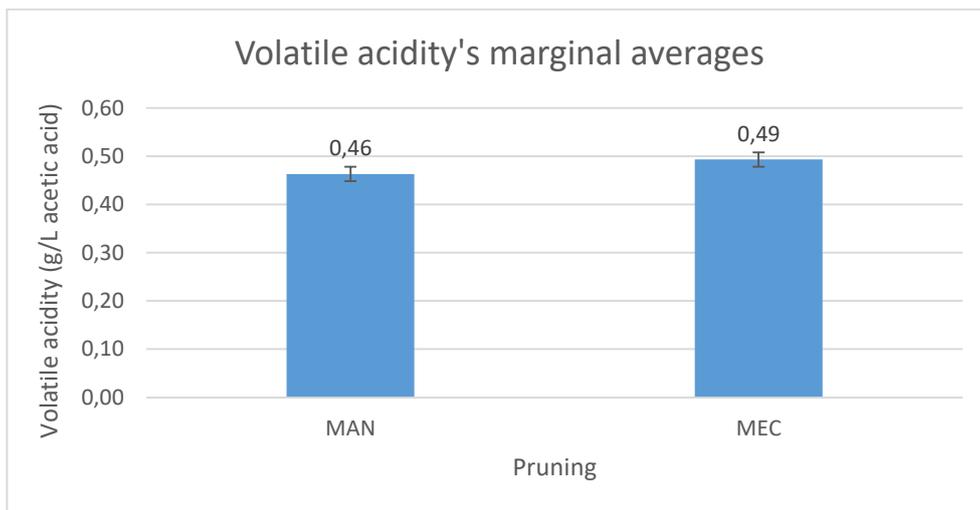
Annex 41- Effect of pruning on hue values of 2019 wines



Annex 42- Effect of fertilization on hue values of 2019 wines



Annex 43- Effect of pruning on volatile acidity's values of 2019 wines



Annex 44- Effect of fertilization on volatile acidity's values of 2019 wines

