



# Physicochemical and sensory analysis of Sauvignon Blanc wines from mechanically pruned vines in Quinta do Gradil treated with municipal solid waste compost

Pedro Manuel Fernandes da Silva

Dissertação para obtenção de Grau Mestre em

Engenharia de Viticultura e Enologia

Orientador: Jorge Manuel Rodrigues Ricardo da Silva

Júri:

Presidente: Sofia Cristina Gomes Catarino, Professora Auxiliar, Instituto Superior de Agronomia da Universidade de Lisboa

Vogais: Manuel José de Carvalho Pimenta Malfeito Ferreira, Professor Associado com agregação, Instituto Superior de Agronomia da Universidade de Lisboa

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



UNIVERSIDADE De lisboa

# Agradecimentos

Agradeço ao professor Jorge Ricardo da Silva e ao Engenheiro Manuel Botelho por me facilitarem a escolha do tema desta dissertação, pelo apoio na planificação e execução da análise sensorial, pela possibilidade de visitar as vinhas que estudei, e por todos os esclarecimentos que me forneceram durante a fase de escrita da tese.

Agradeço ao staff da adega experimental do ISA, em particular ao senhor António, por rececionar as uvas e vinificar todos os vinhos ao longo dos anos do projeto, e por me auxiliarem durante a componente prática da dissertação.

Agradeço a todo o staff do laboratório por todo o apoio que me deram durante as análises laboratoriais, assim como nos dias das análises sensoriais.

Agradeço aos profissionais da fileira do vinho com que me cruzei ao longo dos anos, por me terem acolhido e possibilitado aplicar os conhecimentos adquiridos, por me transmitirem o seu próprio conhecimento e experiência e por despertarem o gosto pela enologia e pela viticultura.

Agradeço aos meus colegas do ISA, assim como aos de Coimbra, por todos os bons momentos que tive a oportunidade de passar durante a licenciatura e o mestrado.

E em último, agradeço à minha família por me terem possibilitado concluir os meus estudos, por me terem apoiado incondicionalmente ao longo de todas as etapas pelas quais passei e passamos.



## Resumo

Para averiguar o efeito da técnica de poda (poda manual vs poda mecânica em sebe) e da dose de resíduos sólidos municipais compostados aplicada na qualidade dos vinhos da casta Sauvignon Blanc, foram estudados vinhos elaborados a partir de uvas provenientes da Quinta do Gradil (Região IGP Lisboa) dos anos 2018, 2019 e 2020.

Para esse efeito foram executadas análises físico-químicas aos vinhos, ao seu teor de compostos fenólicos, e às suas caraterísticas cromáticas. Adicionalmente os vinhos foram sujeitos a análise sensorial.

Os resultados da componente laboratorial dos vinhos dos anos 2018 e 2019 foram recolhidos por Liggio (2020), sendo incluídos nesta dissertação para averiguar se as diferenças identificadas são consistentes ao longo dos anos.

A poda mecânica em sebe resultou consistentemente em vinhos de maior título alcoométrico volúmico e menor acidez total. O teor de compostos fenólicos dos vinhos não demonstrou diferenças significativas mediante os fatores estudados. No que toca aos parâmetros de cor não existiu coerência entre vinhos de anos diferentes, em 2018 e 2019 os vinhos de poda manual foram considerados mais esverdeados e com menor luminosidade e em 2020 foram considerados mais amarelados, com maior intensidade de cor e mais luminosos.

O painel de prova classificou os vinhos quanto à fase visual e à fase olfativa como sendo medianos (exceto para os descritores "frutado" e "herbáceo", que foram melhor classificados do que os restantes descritores), e relativamente à fase gustativa como sendo de qualidade superior à média, particularmente para os parâmetros "acidez", "intensidade" e "volume". O painel teve dificuldade em distinguir os diferentes fatores em estudo, mas quando os distinguiu, os vinhos de poda manual obtiveram sempre melhores classificações. Em relação ao uso de resíduos sólidos municipais compostados, estes raramente afetaram a qualidade do vinho, tanto ao nível químico como a nível de análise sensorial.

Palavras-Chave: Poda Mecânica, Sauvignon Blanc, Resíduos Sólidos Municipais Compostados, Vinho, Análise Sensorial

# Abstract

In order to evaluate the impact of the pruning methodology (manual vs mechanical hedge pruning) adopted and of the dose of municipal solid waste compost used upon Sauvignon Blanc wine quality, wines made from grapes of Quinta do Gradil (IGP Lisboa region) from years 2018, 2019 and 2020 were studied.

Physicochemical analysis were carried out, as well as analysis to their phenolic composition and to their chromatic parameters. The wines were also subjected to sensory analysis.

The laboratorial results for the wines of years 2018 and 2019 were collected by Liggio (2020), and they are included in this dissertation in order to evaluate whether the differences observed are consistent through different years or not.

Mechanical hedge pruning wines consistently had higher alcohol by volume and lower total acidity. The phenolic composition of the wines was not affected by the factors being studied. The colour parameters did not show any pattern related to the factors being studied: in 2018 and 2019 the manual pruning wines were greener in tone and darker, and in 2020 they were more yellow in tone, had higher luminosity and higher colour intensity.

The tasting panel described the wines as being average in the visual and olfactory stages of the tasting (except for the "fruity" and "herbaceous" aroma descriptors, which scored higher than the other aroma descriptors) and above average in the taste stage, especially for the "acidity", "intensity" and "volume" parameters. The panel was generally not able to distinguish the wines by their pruning methodology or by the dose of Municipal Solid Waste Compost that was used, however, when there were significant differences regarding the pruning methodology adopted, the manual pruning wines had higher scores. The dose of Municipal Solid Waste Compost rarely affected wine quality at the chemical level and at the sensory analysis level.

Keywords: Mechanical Pruning, Sauvignon Blanc, Municipal Solid Waste Compost, Wine, Sensory Analysis

# **Resumo Alargado**

De forma a averiguar o efeito da técnica de poda (poda manual vs poda mecânica em sebe) e da dose de resíduos sólidos municipais compostados (RSMC) aplicada (foram aplicadas quatro doses, a 5000kg/ha, 10000 kg/há, 20000 kg/ha, e ainda as parcelas em que não houve qualquer aplicação de resíduos sólidos municipais compostados) na qualidade dos vinhos da casta Sauvignon Blanc, foram estudados vinhos elaborados a partir de uvas provenientes da Quinta do Gradil (Região IGP Lisboa) dos anos 2018, 2019 e 2020. No primeiro ano, 2018, não foi possível estudar o efeito do uso de resíduos sólidos municipais compostados municipais compostados, uma vez que o impacto do uso de corretivos orgânicos não tem efeitos imediatos, e tratava-se do primeiro ano em que estes foram aplicados na vinha.

Para esse efeito foram executadas diversas análises físico-químicas aos vinhos, em particular aos parâmetros clássicos, ao teor de compostos fenólicos e às caraterísticas cromáticas. Adicionalmente os vinhos foram sujeitos a análise sensorial. As análises físico-químicas clássicas consistiram na determinação do pH, da acidez total, do título alcoométrico volúmico, da acidez volátil, dos teores de dióxido de enxofre livre e total, e das substâncias redutoras. Para analisar a composição fenólica dos vinhos foi determinada a concentração de fenóis totais, a concentração de fenóis flavonoides e a concentração de fenóis não flavonoides. As caraterísticas cromáticas determinadas foram a absorvância a 420 nm e a determinação das coordenadas no espaço CIElab, concretamente as coordenadas L\* (luminosidade), a\* (eixo vermelho/verde), b\* (eixo azul/amarelo), e a partir destes, o valor de C\*, saturação, e H\*, tonalidade. De forma a comparar os resultados obtidos pelo método CIElab pelos vários vinhos, ainda se calculou o valor de  $\Delta$ E. A análise sensorial foi executada em dois dias, por painéis distintos em cada dia, de 9 e 11 provadores experientes, respetivamente.

A parcela estudada foi dividida em 4 blocos, em que cada bloco contempla os dois sistemas de poda e as quatro modalidades de correção orgânica, resultando em 32 unidades experimentais "bloco\*poda\*RSMC" distintas. Cada unidade experimental foi vindimada e vinificada em separado, e após a vindima procederam-se a análises laboratoriais, cujos resultados correspondem à média de 2 repetições, e a análise sensorial por painel de prova. Os resultados foram posteriormente analisados estatisticamente através do método ANOVA a dois fatores, e de testes Tukey de separação de médias.

Os resultados das análises físico-químicas demonstram que os vinhos derivados de videiras podadas mecanicamente apresentaram consistentemente maiores valores de título alcoométrico total e de menor acidez total. A dose aplicada de corretivo orgânicos nunca resultou em diferenças significativas ao nível dos resultados das análises físico-químicas.

No que toca à composição fenólica, os fatores estudados nunca afetaram a concentração de fenóis totais, de fenóis flavonoides ou de fenóis não-flavonoides. Em relação aos valores de absorvância a 420nm, existiram diferenças significativas entre os tipos de poda adoptados para os vinhos do ano 2019, em que os vinhos de poda manual obtiveram valores superiores aos de poda mecânica.

Segundo os resultados obtidos no método ClElab, no geral, os vinhos podem ser descritos como sendo vinhos amarelos pálidos. Relativamente ao sistema de poda adotado, verificaram-se diferenças significativas nos três anos – no ano de 2018, existiram diferenças significativas ao nível da coordenada a\* e tonalidade, que indicam que os vinhos de poda manual tendem a ser mais esverdeados; no ano de 2019 os vinhos de poda manual foram novamente considerados mais esverdeados e com menor valor de luminosidade (existiram diferenças significativas ao nível dos parâmetros L\*, a\* e tonalidade) e no ano de 2020, contrariando os anos anteriores, os vinhos de poda manual foram considerados mais amarelados, com maior intensidade de cor e com valores maiores de luminosidade (diferenças significativas ao nível dos parâmetros L\*, b\* e saturação). Verificaram-se ainda diferenças significativas ao nível da interação entre os dois fatores em estudo para os vinhos do ano 2019 para a coordenada a\* e para a tonalidade, em que é notório um decréscimo do valor de a\* com o aumento da dose de RSMC para os vinhos de poda mecânica, mas para a tonalidade não é tão evidente a interação entre fatores que é identificada pela ANOVA. Nos vinhos de 2020 existiram ainda diferenças significativas ao nível da dose de RSMC usada para a tonalidade, entre o bloco testemunha e o bloco M1, correspondente à dose de 5000kg/ha, em que o bloco testemunha resultou em vinhos de maior tonalidade, o que significa que são mais amarelados e menos avermelhados.

A análise sensorial foi dividida em três fases distintas: a visual, a olfativa e a gustativa. Os vinhos de 2018 e 2019 foram categorizados como sendo vinhos de intensidade cromática baixa a média, em que predominam os tons esverdeados em relação aos amarelados, enquanto os vinhos de 2020 obtiveram valores de intensidade cromática similares, mas foram considerados mais amarelados do que esverdeados. O painel de prova nunca distinguiu os vinhos segundo os fatores estudados. No estágio olfativo da prova avaliou-se a intensidade aromática geral, a intensidade aromática de alguns descritores específicos à casta Sauvignon Blanc e o equilíbrio entre os aromas identificados. No geral, os vinhos forma considerados de intensidade aromática mediana, com predominância de aromas "frutados" e "herbáceos", mas com pouca intensidade aromática em descritores tipicamente associados à casta, como "toranja", "maracujá" ou "xixi de gato". No ano de 2018 os resultados indicam a existência de diferenças significativas para os descritores "xixi de gato" e "herbáceo", em que os vinhos de poda manual são considerados como de maior intensidade aromática. No ano de 2019 o descritor "equilíbrio" apresentou diferenças significativas relativas à dose de RSMC, contudo essas diferenças foram identificadas pelo método ANOVA mas não pelo teste Tukey, logo não são significativas o suficiente para que seja possível distinguir quais as doses que de facto diferem entre si. No ano de 2020 existiram diferenças significativas para o descritor "intensidade", em que os vinhos de poda manual foram considerados como sendo de maior intensidade aromática do que os de poda mecânica.

Na fase de prova gustativa foi pedido aos provadores para avaliar vários parâmetros típicos de analise sensorial, nomeadamente "intensidade", "acidez", "volume", "persistência" e "equilíbrio" e ainda para que dessem uma apreciação global do vinho provado. No geral, os provadores avaliaram os parâmetros como medianos, exceto para a acidez, que consistentemente obteve valores acima da média. Os resultados dados pelos provadores não apresentaram diferenças significativas entre os dois tipos de poda estudados ou entre as doses de RSMC usadas na vinha.

Cada viticultor deve adotar as metodologias que forem mais adequadas para cada parcela que explora. A poda mecânica em sebe é uma alternativa viável à poda manual para a generalidade dos casos, uma vez que o painel de provadores não distinguiu os vinhos com facilidade. Ainda assim, a poda manual continua a apresentar a vantagem de permitir ao podador definir exatamente a carga, mediante o rendimento e qualidade que pretende, logo continua a ser indispensável para elaborar vinhos de gama alta. Em relação ao uso de corretivos orgânicos, em particular os RSMC, estes podem complementar o protocolo de fertilizações anual, ainda que o viticultor deva sempre usá-los apenas após conhecer o estado nutricional da sua vinha, após proceder a todas as análises necessárias para esse efeito. O seu impacto sobre a qualidade do vinho permanece pouco claro e deve ser objeto de estudos futuros.

# Index

1. Introduction1
2. Bibliographic Review
2.1. Pruning
2.1.1 Mechanical Pruning3
2.1.2 Hedge pruning
2.2. Organic amendments
2.2.1 Municipal Solid Waste Compost (MSWC)6
2.3. Sauvignon Blanc7
2.3.1 Generalities7
2.3.2. Varietal aroma
2.3.3. Sauvignon Blanc vinification11
2.4. Quinta do Gradil and IGP Lisboa region12
2.5 Objectives
3. Materials and Methods
3.1. Experimental Design
3.2. Maturation, Harvest and Vinification17
3.3. Chemical analysis18
3.4. Quantitative descriptive sensory analysis21
4. Results and Discussion
4.1. Physicochemical parameters23
4.2. Phenolic Composition and colour analysis of wines
4.3. Sensory Analysis
5. Conclusions
6. Bibliographical references
7. Annexes

# **Table Index**

Table 1 – Summary of aromatic descriptors, detection thresholds and concentrations of the main
volatile thiols that are found in wine10
Table 2 – Bioclimatic indexes of Lisbon wine region (Winkler index, Huglin heat sum index and fresh
nights index) and Global solar radiation13
Table 3 – Soil composition without the application of organic amendments
Table 4 – Must analyses results. The analyses were performed in the 4 <sup>th</sup> of September 2020
Table 5 – Fermentation Control of block 1, mechanical pruning, no organic amendment wine
Table 6 – Effect of pruning system upon the physicochemical characteristics of the wines from the
harvest of 2018. (Adapted from Liggio, 2020)
Table 7 – Effect of pruning system and Municipal Solid Waste compost upon the physicochemical
characteristics of the wines from the harvest of 2019 (adapted from Liggio, 2020)
Table 8 – Effect of pruning system and Municipal Solid Waste compost upon the physicochemical
characteristics of the wines from the harvest of 202025
Table 9 – Effect of pruning system over phenolic composition and chromatic characteristics of the
wines from year 2018 (adapted from Liggio, 2020)
Table 10 – Effect of pruning system and Municipal Solid Waste Compost over phenolic composition
and chromatic characteristics of the wines from year 2019 (adapted from Liggio, 2020)
Table $11 - \Delta E$ values for the wines from year 2019, comparing average values of wines from mechanical
pruning to manual pruning (adapted from Liggio, 2020)
Table 12 – $\Delta E$ values verified for the wines of year 2019, comparing average values of different MSWC
doses of application (with the same pruning system)
Table 13 – Effect of pruning system and Municipal Solid Waste Compost on phenolic composition and
chromatic characteristics of wines from year 2020
Table 14 – $\Delta E$ values verified for the wines of year 2020, comparing average values of wines from
manual pruning to average values of wines from mechanical pruning (with the same dose of MSWC
application)
Table 15 – $\Delta E$ values verified for the wines of year 2020, comparing average values of different MSWC
doses of application (with the same pruning system)
Table 16 – Colour intensity evaluation values obtained in sensory analysis, in a scale of 1 to 5, of the
wines from year 2018
Table 17 – Aroma descriptors evaluation values, in a scale of 1 to 5, of the wines from year 2018 39
Table 18 – Taste descriptors evaluation values and global appreciation values, in a scale of 1 to 5, of
the wines from year 2018

Table 19 – Colour intensity evaluation values obtained in sensory analysis, in a scale of 1 to 5, of the
wines from year 2019
Table 20 – Aroma descriptors evaluation values, in a scale of 1 to 5, of the wines from year 2019 41
Table 21 – Taste descriptors evaluation values and global appreciation values, in a scale of 1 to 5, of
the wines from year 2019 42
Table 22 – Colour intensity evaluation values obtained in sensory analysis, in a scale of 1 to 5, of the
wines from year 2020 43
Table 23 – Aroma descriptors evaluation, in a scale of 1 to 5, of the wines from year 2020 44
Table 24 – Taste descriptors evaluation values and global appreciation values, in a scale of 1 to 5, of
the wines from year 2020 45
Table 25 – Coding used during the panel tasting for the wines of year 2018
Table 26 – Coding used during the panel tasting for the wines of year 2019
Table 27 – Coding used during the panel tasting for the wines of year 2020
Table 28 – Free and total sulphur dioxide of 2020 wines, measured before bottling
Table 29 – Results of Physicochemical analyses of the wines from 2018 (adapted from Liggio, 2020)63
Table 30 – Colour intensity, expressed in absorvance at 420nm, of wines form year 2018 (adapted from
Liggio, 2020)
Table 31 – Phenolic Composition of wines from 2018 (adapted from Liggio, 2020)
Table 32 – CIElab method results for 2018 wines (adapted from Liggio, 2020)
Table 33 – Chromatic Difference between mechanical pruning and manual pruning of 2018 wines
(adapted from Liggio, 2020)
Table 34 – Results of Physicochemical analyses of the wines from 2019 (adapted from Liggio, 2020).
Table 25 Calcur intensity overcessed in abcomono at 120nm of the wines of year 2010 (adapted
fram Lingin 2020)
Table 26 Dependie Composition of wines from wear 2010 (adapted from Liggie 2020)
Table 36 – Phenolic Composition of Wines from year 2019 (adapted from Liggio, 2020)
Table 37 – Cleiab coordinates for 2019 wines (adapted from Liggio, 2020)
Table 38 – Chromatic differences verified in 2019 wines, between different pruning systems but from
The same block and Municipal Solid Waste Compost modality (adapted from Liggio, 2020)
Table 39 – Results of the physicochemical analyses of 2020 wines
Table 40 – Colour Intensity, expressed in absorvance at 420 nm, of the Wines from 2020
Table 41 – Phenolic Composition of 2020 wines $72$
Table $42 - Cleap coordinates for 2020 wines$
Table 43 – Chromatic differences verified in 2020 wines, between different pruning systems but from
the same block and Municipal Solid Waste Compost modality74

Table 44 -	Chromatic	differences	verified in	2020 wines,	between	different	Municipal	Solid	Waste
Compost r	nodalities, b	ut from the	same block	k and same pr	uning syst	ems			75

# Figure Index

Figure 1 – Minimally pruned canopy
Figure 2 – Vine Stripper by Langlois
Figure 3 – Mechanical hedge pruning vineyard4
Figure 4 – 2-Metoxi-3-alquilpirazines present in grapes and wines of Sauvignon Blanc
Figure 5 – Experimental design of the mechanical pruning and organic amendment assay in Quinta do
Gradil16
Figure 6 – Average values for volatile acidity, according to the adopted pruning system, for years 2018,
2019 and 2020
Figure 7 – Average values of total acidity, according to the adopted pruning system, for years 2018,
2019 and 2020
Figure 8 – Average values for Alcohol by Volume (%vol.), according to the adopted pruning system, for
years 2018, 2019 and 2020
Figure 9 – Average values for pH, according to the adopted pruning system, for years 2018, 2019 and
2020
Figure 10 – Average values for reducing substances, according to the adopted pruning system, for years
2018, 2019 and 2020
Figure 11 – Average values of a* coordenate (CIElab method) for 2019 wines
Figure 12 – Average values of Hue (H*) for 2019 wines
Figure 13 – Tasting Sheet used in the wine tasting61

# 1. Introduction

Agriculture marked the start of sedentary human civilization. Through agriculture it became possible to produce food at a surplus, enabling the development of cities. Agriculture, and the other economic activities that make up the primary sector, used to make up around 35% of the total working force in Portugal in 1974, however, the primary sector now represents only around 5% (Pordata, 2021). Despite the much lower number of workers, agriculture, forestry and fishing produced 4179 Million euros worth of gross domestic product in 2018, whereas in 1974 the same economic activities produced 210,2 Million euros, meaning that in around 40 years the contribution towards the country's GDP increased 20-fold (Pordata, 2021).

Currently, one of the biggest contributors to the GDP generated by agriculture in Portugal is the wine industry. Portuguese table wines made up around 975 Million euros in the domestic market in 2018 and all categories of wine generated 800 Million euros in revenue in exports in 2018, according to the IVV yearbook of 2018 (IVV, 2018).

Nowadays agriculture is tasked not only with being economically sustainable, but also with being environmentally sustainable. The International organization of vine and wine (OIV) describes sustainable viticulture as "a global strategy on the scale of the grape production and processing systems, incorporating at the same time the economic sustainability of structures and territories, 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, ecological and landscape aspects." (Castelluci, 2008).

In viticulture, the most expensive operations are harvesting and pruning – there have been major strides in lowering the cost of harvesting by the introduction of mechanical harvesters, which are commonly used in all countries, but pruning remains a costly manual operation.

Mechanization of pruning has been studied since the 1970's (Poni et al., 2016), and is widely used in some new world countries such as Australia, where around 65% of all vineyards are mechanically pruned (Clingeleffer, 2013).

One of the bigger burdens that mechanical pruning must overcome in order for it to be adopted by wine companies and cooperatives, is the allegations that mechanical pruning lowers wine quality. The working theory for this dissertation is that by increasing soil fertility it is possible to ameliorate the

plant's auto regulation mechanisms that change grape composition, allowing for there to be an increase in production without compromising berry composition.

# 2. Bibliographic Review

# 2.1. Pruning

Pruning is a cultural intervention that aims to influence the physiological behaviour of the plant through the removal of any living organ, except bunches or inflorescences. Pruning can be categorized according to the season in which it is carried out, winter pruning and summer pruning, the first being performed during vegetative rest and the second during the active phase of the vegetative cycle, and according to the age of the vine at the time of pruning, dividing the pruning into formation pruning (performed in the first years of the plant's life) and fruiting pruning (for adult vines) (Magalhães, 2015).

The mains goals of pruning are: manipulating the shape of the vine, defining an adequate bud load that is compatible with vine capacity, reducing production fluctuations year-to-year, and defining the quantity and quality of the year's production (Magalhães, 2015).

Pruning has a depressive effect not only on production but also on vine capacity, which is defined as the capacity of the vine to develop its roots and trunk and forming new branches, leaves and fruits. An effective pruning must manage to reduce the least amount of vine capacity, so that the effect on production is not too severe (Magalhães, 2015).

Manual forms of pruning allow the worker to define a precise amount of buds to keep for next year, as well as their spatial location, allowing for a homogenous distribution of the canopy, that maximizes the amount of exposed leaf area. Manual pruning can be carried out with conventional pneumatic or electrical scissors, the last two reducing the time required to prune per hectare (Magalhães, 2015).

Winter pruning is very demanding in time and labour force, making it the most expensive and time consuming cultural activity in viticulture, which is why it's mechanization has been studied for decades, the first studies having been conducted in the 1970's (Poni et al., 2016). Nowadays, in Australia around 65% of all vineyards are mechanically pruned (Clingeleffer, 2013).

## 2.1.1 Mechanical Pruning

Mechanical pruning can reduce labour requirements in 54 to 70% (Gatti et al., 2011), and reduce the total duration of pruning per hectare by up to 93% (Pezzi et al., 2013). Mechanized pruning can be applied to a lot of different training systems and resulting in a number of different bud loads per vine.

In Australia, the MPCT system (minimal pruning of cordon trained vines) was developed as a way to reduce production costs (figure 1). It consists in not pruning the vines during the dormant period, opting instead in performing shoot hedging during the period of vegetative growth, in order to prevent contact the bunches and branches form coming in to contact with the soil (Smart & Robinson, 1991) (Clingeleffer, 1988). Its advantages are the reduction of pruning costs, ease in mechanized harvest, vigour control and maximizing of production (Clingeleffer, 1988).



Figure 1 – Minimally pruned canopy

#### Source: Poni et al., 2016

Some preliminary assays have been made into mechanizing long cane pruning. Some operations, such as the selection of the new cane and it's tying to the wires, remain manual operations, however the removal of dead wood, which is a non-selective and time consuming operation, is a good opportunity for mechanization. Two machines have been developed: the "vine stripper" (developed by Walter Langlois) (figure 2) that functions by pulling the excess canes into two counter rotating disks that will shred the wood and then distribute it across the inter-row, acting as mulch; and the "cane pruner" (developed by Klima) that functions by raising the fruiting wires, cutting the excess wood, and then spreading the debris as mulching over the middle of the row (Poni et al., 2016).



Figure 2 – Vine Stripper by Langlois

#### Source: Poni et al., 2016

Poni et al. (2016) applied hedge pruning to vines driven by a single wire at about 1.6m in height, without any catch wires above the cordon. This training system allows the machine to make all the cuts in close proximity to the cord, allowing for better control over the number of buds left and reducing the need for subsequent manual corrections. In certain situations it is possible that up to 90% of the year's living organs are formed above the cord, which facilitates its removal by the machine during winter pruning, thus resulting in less accumulation of old wood in the fruiting area, improving the microclimate of the canopy compared to other training systems.

The vines used for this dissertation are hedge pruned, vertical shoot positioned (VSP) vines (figure 3). The application of this type of pruning system on this training system has been studied for several years (Lopes et al., 2000) (Botelho et al., 2021) (Castro et al., 2010).



Figure 3 – Mechanical hedge pruning vineyard

## 2.1.2 Hedge pruning

One common result of hedge pruning is the large increase in bud load (Gatti et al., 2011). The increase in bud load leads to compensation mechanisms by the plant, such as a decrease in the bud burst rate, fertility index, bunch weight and vigour. Other observed results are the early development of the leaf area (although the final size of the canopy tends to be similar to that observed in vines pruned manually), higher values of leaf surface directly exposed to the sun and reduction in the accumulation of sugar in the berry (Castro et al., 2010) (Botelho et al., 2020) (Clingeleffer, 1988) (Lopes et al., 2000) (Poni et al., 2016).

Botelho et al. (2020) and Lopes et al. (2000) describe that vines subject to hedge pruning tend to be more efficient in their use of synthesized carbohydrates, favouring reproductive growth over vegetative growth, because although they tend to have a lower leaf to fruit ratio, but increased yields and relatively small reductions in the concentration of sugar in the berry. The reductions in sugar content can be significant, Castro et al. (2010) detected differences in alcohol content of 1.5% vol. in the Alfrocheiro variety between manual pruning (Bilateral Royat) and mechanical hedge pruning.

According to Gatti et al. (2010) the reduction in berry sugar content is a result of over cropping, and only occurs when vine capacity is exceeded. In certain situations where vine capacity is assured and there is good production potential, there is a minimization of the auto regulation mechanizations described previously (lighter bunches, lower bud burst rates and lower fertility rates) without the negative changes of berry composition (Gatti et al, 2011) (Lopes et al., 2000) (Botelho et al., 2021).

Sauvignon Blanc, the grape variety being studied, is a relatively early variety, therefore, in a climate change scenario characterized by raising average annual temperature the ability to successfully grow this variety and achieve reasonable berry composition by maturation will be profoundly affected, therefore all methodologies that delay the growth cycle ought to be explored (van Leeuwen et al., 2019). When vines are pruned with two to three leaves the growth cycle is significantly delayed, meaning that the maturation period will be carried out under cooler temperatures. Since mechanical pruning is significantly faster than manual pruning, it allows viticulturists to delay pruning of early varieties without compromising the schedule of operations. Additionally, the changes promoted by mechanical pruning, such as increased yields and lower leaf to fruit ratio, also contribute in delaying maturation (Clingeleffer, 1988).

## 2.2. Organic amendments

The drastic increase in bud load means that auto regulation mechanisms by the plant play a role of critical importance in defining production and berry composition, and the auto regulation mechanisms are influenced by, among other factors, soil fertility (Botelho, 2020).

One of the limiting factors of viticulture in Portugal is the low fertility of soils, which is a direct result of low organic matter content (Santos, 2012). This is a result of the Mediterranean climate that is characterized by long periods of high temperatures and drought during spring and summer, which will only become more aggravated by climate change (Fraga et al., 2012). The loss of organic matter content in soil can be mitigated by introducing cover crops or by reducing the amount of soil tillage operations, however it is also possible to use organic amendments to increase organic matter content.

The availability of conventional organic amendments, such as cow and poultry manure, has been decreasing, which is part of the reason for why organic matter replenishing practices have been decreasing. Synthetic and "novel" organic amendments, such as municipal solid waste compost (MSWC) are becoming increasingly more available, allowing for the replacement of conventional organic amendments (Botelho et al., 2020). "Novel" organic amendments allow viticulturists to avoid using synthetic fertilizers, which are typically more harmful to the environment (Tangolar et al., 2020). The use of MSWC as an organic amendment ought to be studied and applied due to its high organic matter and macronutrient content as well as high availability (Hargreaves et al., 2008) (Pinamonti, 1998).

## 2.2.1 Municipal Solid Waste Compost (MSWC)

Soil amending with MSWC upgrades the physical properties of soil significantly, resulting in increased porosity, increasing available water, lowering erosion and increasing soil stability (Pinamonti, 1998). Garcia-Gil et al. (2004) observed that continuous use of MSWC improved humic acid composition of soil in a nine year experiment, verifying significant increases at 20 t/ha and at 80 t/ha, with minimal difference between the two rates of application studied.

Enzymatic activity in soil appears to be influenced by the composition of MSWC, Garcia-Gil et al. (2000) detected significant decreases in phosphatase and protease activity which were attributed to high heavy metal content, while Perucci (1990) detected an increase in activity for the same enzymes.

In terms of productivity and berry composition, Gaiotti et al. (2017) applied 4 t/ha of pruning waste compost which resulted in slight increases in yield and unaffected berry composition in comparison to the control where no fertilization was applied; Mugnai et al. (2013) did a nine year study comparing a synthetic fertilizer with green waste compost and observed small differences in yield and Brix degree between the two fertilizers; Pinamonti (1998) used MSWC as mulch in the implementation of a vineyard and did not find significant differences in vigour or production and Botelho et al. (2020) applied 16,4 t/ha of MSWC and obtained very significant increases in production (in comparison to the control without any fertilization), less sugar berry content, and an increase in vine capacity (that the author calculated with the formula: total dry mass=0,2\*yield + 0.5\*pruning wood weight). MSWC is at least as effective as animal manures in increasing soil fertility without a noticeable decrease in quality of berry composition. The most concerning factor related to the use of MSWC in vineyards is the high heavy metal concentration that it can have (Pinamonti, 1998). Pinamonti (1998) found the concentrations of Nickel and Cadmium to be significantly higher in vine leaf analysis for plants treated with MSWC, and he also found higher concentrations of Copper, Cadmium and Chromium in musts derived from plants treated with MSWC.

MSWC have higher concentrations of heavy metals greater than what is observed in agricultural soils, but generally they are inferior to the limits established by PAS 100 BSI (British Standards Institution Publicly Available Specification) (Smith, 2009). The aerobic composting process increases the complexation of heavy metals present in the organic waste, and moreover, when the MSWC are used in the soil, they establish very strong bonds with the solid phase of the soil, greatly limiting their solubility and bioavailability. As such, the heavy metals present in the MSWC are less incorporated by crops than the heavy metals present in other biological waste, such as water treatment plant sludge (Smith, 2009). The application of MSWC in agricultural crops constitutes a methodology that allows to increase the fertility of the soil without compromising the food safety of the final product (Smith, 2009).

## 2.3. Sauvignon Blanc

## 2.3.1 Generalities

The Sauvignon Blanc grape variety is a very challenging variety both in the vineyard, where it's compact bunches are susceptible to *Botrytis cinerea* and powdery mildew attacks, and in the cellar, where the choices made by the winemaker during fermentation and aging will have a profound impact upon the wine's flavours and aromas (Robinson et al., 2012). The variety can be used to make wines of exceptional quality, being one of the most planted varieties in the world with 123000 ha currently (OIV, 2017).

Robinson et al. (2012) describes the wines that can be produced with this grape variety in the different viticultural regions of the world, describing California's monovarietals as being "softer, richer, with short aging in oak, and with an aroma of melon", the wines from New Zealand as wines of "a lot of production and fast consumption, almost without any aging, aggressively fruity, pungent and with marked acidity", dry monovarietal wines from the region of Sancerre (belonging to the region of Loire), in France as either being "acidic, very dry, with (vague) green fruit aroma", the characteristic the author ascribes to the more common ones, and "having an austere, intriguing character, with a lot of sense of place, being imbued by the particular permutations of and dispositions of flint, limestone and clay that underlie each vineyard", for the finest wines.

The aroma descriptors of the variety can be divided in two main classes, the more "herbaceous" ones, namely grass, asparagus, green pepper, tomato leaf, peas, boxwood, cassis and cat's pee; and the "tropical fruit" aromas, particularly grapefruit, melon, passion fruit and citrus fruit aromas (Coetzee & du Toit, 2012).

When it comes to colour, phenolic compounds are the most important compounds. Mechanical harvesting tends to decrease their concentration in wine, since the mechanical damage inflicted will expose the berry to being degraded by free radicals. Other techniques will have the opposite effect, such as maceration (Olejar et al., 2015). The use of antioxidant substances ought to be controlled, since oxidation of phenolic compounds will cause "browning" of white wines. The use of sulphur dioxide (SO<sub>2</sub>) creates an oxygen free environment that protects and maintains wine aroma and also will result in wines with lower values of colour intensity. Ascorbic acid must be used with care, because if SO<sub>2</sub> is fully oxidized, ascorbic acid will start releasing hydrogen peroxide, a strong oxidant (Cojocaru & Antonce, 2016).

## 2.3.2. Varietal aroma

Sauvignon Blanc has been the subject of several papers and currently its typical aromas are attributed to methoxypyrazines and volatile thiols, the first being responsible for the herbaceous aromas and the last for tropical fruit aromas (Coetzee & du Toit, 2012).

Methoxypyrazines are chemically defined as heterocyclic aromatic rings, containing two atoms of nitrogen, one R group (which is what defines the methoxypyrazine), one methoxy group (figure 4). In

Sauvignon Blanc (as well as other grape varieties, such as Semillon and Cabernet Sauvignon) three types of methoxypyrazines can be identified: 2-Metoxi-3-isobutilpirazine (ibMP), 2-Metoxi-3-isopropilpirazine (ipMP) and 2-Metoxi-3-Sec-Butilpirazine (sbMP) (Marais, 1994).





#### Source: Marais, 1994

Each methoxypyrazine contributes different aromas, ibMP imbues wines with green pepper aromas, ipMP with pea/asparagus, and the contribution is moderated by each methoxypyrazine's detection threshold (around 2 ng/l for ibMP) and by the concentration it is in. Typically ibMP plays a large role in defining the herbaceous aroma in Sauvignon Blanc wine since it is the methoxypyrazine that is found in higher concentration, around seven times the concentration of ipMP and even more than sbMP (Marais, 1994). Vineyards in cooler climates tend to have higher concentrations of methoxypyrazines than vineyards in warmer climates, and their concentration is high during veraison and decreases very significantly during maturation (Lacey et al., 1991).

The presence of volatile thiols in Sauvignon Blanc was first identified by Darriet et al. (1993) and Tominaga et al. (1996). Tominaga et al. (1998) and Des Gachons et al. (2002) later described the mechanisms by which volatile thiols appear in wines, namely the lysis of non-aromatic precursors (cysteine S-conjugates, glutathione s-conjugates and E-hexen-2-al) during the alcoholic fermentation. Tominaga et al. (2000) identified these compounds in other varieties, cementing the importance of volatile thiols in winemaking. Ferreira (2011) also identified volatile thiols in Portuguese varieties.

Chemically, volatile thiols consist of thiol compounds to which cetone groups, alcohols and esters are added. The ones responsible for Sauvignon Blanc characteristic aromas are 4-Mercapto-4-Methyl-

pentan-2-one (4MMP), 3-Mercaptohexan-1-ol (3MH), 3-Mercaptohenyl acetate (3MHA) and 4-Mercapto-4-metilpentan-2-ol (4MMPOH). Table 1 summarizes the aromatic descriptors of each volatile thiol as well as their detection thresholds (expressed in ng/l) and the range of concentrations in which they can be found in wines. 4MMPOH is omitted from the table due to rarely being in concentrations greater than 55 ng/l, its detection threshold (Coetzee & du Toit, 2012).

Table 1 – Summary of aromatic descriptors, detection thresholds and concentrations of the main volatile thiols that are found in wine

Volatile thiol	Abbreviation	Aromatic Descriptors	Detection thresholds (ng/l)	Concentration in wine (ng/l)
4-Mercapto-4- Methyl-pentan-2- one	4MMP	Boxwood,passionfruitand cassis	0.8	4-40
3- Mercaptohexano- 1-ol	3MH	Grapefruit, maracujá e citrinos	60	26-18000
3-Mercaptohenyl acetate	3MHA	Maracujá, toranja e citrinos	4.2	0-2500

Source: Coetzee & du Toit, 2012

The concentrations of the volatile thiols will dictate whether the aromas are pleasant to the consumer to not, since excessive concentrations of volatile thiols lead to cat's pee aroma (Coetzee & du Toit, 2012).

As referred above, volatile thiols only become present in wine after alcoholic fermentation, since it is during this process that due to the Beta-liase enzyme will act upon the non-aromatic precursors, which are the cysteine S-conjugates (S-3-(hexan-1-ol)-l-cysteine (Cis-3MH) and S-4-(4-methilpentan-2-one)-l-cysteine (Cis-4MMP)), glutathione S-conjugates ((S-3-hexan-1-ol)-glutathione (Glut-3MH) and S-4-(4-methilpentan-2-one)-glutathione (Glut-4MMP)), and E-hexen-2-al. (Coetzee & du Toit, 2012).

In the vineyard, the most important factors in modulating the concentrations of volatile thiol precursors are berry maturation (with ten-fold increases in Glut-4MMP concentration) and *Botrytis cinerea* attacks on the grape bunch (with up to 100 times more concentration of Cys-3MH). Other

factors that have an impact on volatile thiol precursor concentration are mild water stress and low quantities of nitrogen in soil. Another factor to take into account in winemaking is the distribution of volatile thiol precursors in the berry – all precursors are present in the pulp and in the skin, Glut-4MMP, Cys-4MMP and Glut-3MH show similar concentrations in pulp and skin, while Cys-3MH is mostly present in the skin, therefore, maceration while increase volatile thiol precursor concentration in the must (Coetzee & du Toit, 2012).

At the cellar some actions may increase volatile thiol synthesis. The use of some specific yeast strains and higher fermentation temperatures (20°C) contribute to increasing volatile thiol concentration in wine (Coetzee & du Toit, 2012).

## 2.3.3. Sauvignon Blanc vinification

For Sauvignon Blanc, mechanical harvesting will increase volatile thiol concentration (Allen et al., 2011) (Olejar et al., 2015). This increase in volatile thiol concentration is due to the damage that is inflicted upon the grape berry during mechanical harvesting, which will increase volatile thiol precursor migration from the berries to the must.

Cryogenic pre-fermentative maceration (-20°C) will also increase volatile thiol concentration in wine, for the same reason for the increase verified in mechanical harvesting – berry damage. In this case, the damage is caused by the formation of ice crystals inside the berry that will rupture the berry from the inside (Olejar et al., 2015) (Chen et al., 2019).

The pressure that is applied to the grapes during pressing is also of capital importance. According to Patel et al. (2010) wines made from grapes that were pressed at 0, 25 bar or 1 bar will have less than half 3MH and 3MHA than wines made from the must that is extracted from the press before the machine starts to exert pressure to the grapes, and in addition to the increased volatile thiol content there is also less acidity and increased oxidation of polyphenol content.

Sauvignon Blanc is a variety that is particularly susceptible to an oxidative process named "pinking", that consists in the change of the wine colour, with the acquisition of new light pink to salmon-red tonalities (Mel et al., 2014). For pinking to occur the monomeric anthocyanin malvidin-3-O-glucoside must be present in a concentration of at least 0.3 mg/l. According to Andrea-Silva et al. (2014), the occurrence of pinking in already bottled wines is due to the increase in concentration, followed by oxidation, of flavylium cations resulting in the formation of red compounds resistant to colour change caused by wine pH and caused by the bleaching effect of sulphur dioxide. Strategies employed to deal

with pinking are adding sulphur dioxide, using polivinilpolipirrolidone (PVPP) and using ascorbic acid (Andrea-Silva et al., 2014).

Pinking tends to be mainly observed in white wines that were made in a reducing environment, which can be avoided by adding more sulphur dioxide during vinification, and other antioxidants such as ascorbic acids (Andrea-Silva et al., 2014). To avoid increasing the initial concentration of flavonoid content in must it is best to avoid skin contact and in case there is maceration opting for cold maceration (Jackson, 2008).

Another potential strategy to deal with pinking and browning is pre-fermentative hyperoxygenation of the must. By oxidizing the must phenolic precursors will also be oxidized which leads to polymerization and a significant increase in molecular weight which leads to their precipitation (Baiano et al., 2016). Though it is a promising strategy for avoiding the use of sulphur dioxide in winemaking, it is not suitable for this variety, since its aroma compounds, namely volatile thiols, are degraded by oxygen (Jackson, 2008).

Yeast play a very important role in developing the aroma of Sauvignon Blanc, since they are responsible for degrading cysteine S-conjugates, resulting in volatile thiols. According to Dubourdieu et al. (2006), the same must fermented by different strains of *Saccharomyces cerevisiae* will result in wines with different concentrations of volatile thiols, with the yeast strains EG8 and VL3c being the ones responsible for the larger 4MMP concentrations. The paper also affirms that *Saccharomyces bayanus*, which has already been isolated from spontaneous fermentations in the Sancerre region, is particularly prepared for releasing sulphur compounds from grapes of Sauvignon Blanc.

The inoculation of the must with yeasts other than *Saccharomyces* is a strategy to simulate a spontaneous fermentation while avoiding the problems associated with it (Sadoudi et al., 2012). Anfang et al. (2009) reports that co inoculation of *M. pulcherrima* with *S. cerevisiae* increases production of aromatic compounds and that *C. zemplinina* produces high quantities of terpenes and lactones.

# 2.4. Quinta do Gradil and IGP Lisboa region

Quinta do Gradil, the owners of the vineyard being used for this project, is located in IGP Lisboa region, in Cadaval municipality. In accordance to the date collected and processed by IPMA (2021) throughout the years of 1961-1990, the region has CSb climate in accordance to Köppen-Geiger classification,

temperate with dry and mild summer, has 600-800 mm of annual precipitation and average daily temperature of 15-16°C.

The location of Quinta do Gradil has a considerable impact upon the profile of the wines made, the proximity to Montejunto mountain range grants high daily temperature amplitude and the relative proximity to the sea promotes high Atlantic influence resulting in high acidity fresh wines without compromising grape maturation due to lack of sun or temperature. Another factor is the clayey-limestone soils in the region (Carvalho, 2020).

In order to properly characterize the region and measure its potential for viticulture, it is important to measure the bioclimatic indexes most important for viticulture. Table 2 presents the values of the bioclimatic indexes commonly used in viticulture, namely the Winkler index (which consists of the sum of average daily temperatures, for days of basal temperature of 10°C, recorded during the period between April 1<sup>st</sup> and October 30<sup>th</sup>), the Huglin heat sum index (which measures the maximum air temperature, the average air temperature and the duration of day recorded from the 1<sup>st</sup> of April to September 30<sup>th</sup>) and Fresh Night index (which is the average of the nightly minimum temperatures recorded in September) according to date for the years 1971 to 2000, available on the IPMA *web site* (2021).

In addition to the previous bioclimatic indexes that are presented, table 2 also presents the average global solar radiation value for the Cadaval municipality as well as its annual variability, derived from data collected by IPMA from 2001 to 2015 (Cavaco et al., 2016).

Table 2 – Bioclimatic indexes of Lisbon wine region (Winkler index, Huglin heat sum index and fresh nights index) and Global solar radiation

Bioclimatic índex	Value
Fresh Nights index	14-16 (ºC)
Huglin Heat Sum index	1800-2100
Winkler índex	1300-1500 (ºC.day)
Global solar radiation	1600-1700 (kWh/m²)
Annual variability of global solar radiation	4-5(%)

## Source: IPMA, 2021; Cavaco et al., 2016

According to the results in table 2, the fresh nights Index indicates that the region has temperate nights, with a minimum temperature of 14 to 16°C. The Huglin heat sum index's value, that is of 1800-2100, puts the region in class 3, corresponding to a temperate climate, where it is possible to achieve full maturation in most known grape varieties, and also being clearly above the threshold of 1400 established by Huglin, that describes any value lower than 1400 as being insufficient for successful viticulture (Huglin, 1986). The value range of the Winkler Index shows that it is a temperate climate zone. The value range for global solar radiation is relatively in line with the rest of the country (except for the southern regions, where it is higher), however the variability is atypically high, which is mostly due to the region's characteristic cloudiness and to the very frequent occurrence of morning fogs (Cavaco et al., 2016).

Thus, the three indexes and the values for global solar radiation and its annual variability, seem to indicate that it is a temperate region, with an adequate climate for the needs of the vineyard, allowing the development of most grape varieties, including Sauvignon Blanc.

# 2.5 Objectives

This dissertation is part of the research project IntenSusVITI (PDR 2020-101-032001), a 4-year project (2018-2021) whose objective is the intensification of wine production, respecting the environmental, economic and social sustainability of this economic activity.

The goals are to evaluate the physicochemical and sensory effects verified in white wines of the Sauvignon Blanc variety through the mechanization of pruning and the use of Municipal Solid Waste Compost as an organic amendment. The vineyards are located in Quinta do Gradil, located in the IGP Lisboa region, and the differences verified between modalities will be evaluated and quantified through sensory analysis and physicochemical analyses on different wine parameters, namely their phenolic composition, colour (assessed by absorbance at 420 nm and by the CIElab method), alcohol content, total acidity, volatile acidity, reducing substances, pH and free and total sulphur dioxide content.

# 3. Materials and Methods

The present project was developed at Quinta do Gradil, using the Sauvignon Blanc variety, and under the IntenSusVITI project (PDR 2020-101-032001) a 4-year project (2018-2021). The rootstock used in the vineyard is SO4, the compass is 2.6x1m (planting density ≈3846 plants/ha), the vineyard has NE-SW exposure and the vines were planted in 2006.

Two factors were studied: pruning system (mechanical hedge pruning and manual spur pruning), and the application of four different amounts of an organic amendment, namely municipal solid waste compost (MSWC).

## 3.1. Experimental Design

The specific manual pruning system adopted consisted in a unilateral Royat cordon and upward single pane vertical orientation, and mechanical hedge pruning was simulated by manually pruning all wood in a box of 15 cm width and height around the cordon. Both types of pruning are applied manually, so that it is possible to save the pruning wood, enabling the collection of relevant data on the vigour of the vines under study.

For MSWC, four different modalities were tested, "TEST", which is the non - application of MSWC, and three different modalities of MSWC application "M1", "M2" and "M3", which correspond respectively to the application of MSWC in the doses of 5000 kg/ha, 10000 kg/ha and 20000 kg/ha. The organic matter was applied before bud break, having been dispersed on the soil surface (in the line and between the lines), every other line, and later incorporated through a light harrow.

The vineyard where the study was made had 24 lines (that are identified by the colour dark green), and it was divided into four different blocks – each being made up of 6 lines, where the organic amendments were spread across the soil every other line. The first three consecutive lines were mechanically pruned while the last three consecutive lines were manually pruned. The lines from each block are then divided (in length) into four, so that all blocks can contain all organic amendment doses. Figure 5 is a schematic of the vineyard, showing which lines were mechanically pruned and which were manually pruned. The light green identifies the interline spaces where no organic amendment was applied, while the remaining interlines are coded in four different colours along their length: white, red, blue and black. The parts of each interline that are identified by the colour white are the ones that belong to the test modality (no organic amendment application) and the three different modalities

where there is MSWC application are identified by the colours red (M1 modality), blue (M2 modality) and black (M3 modality).



Figure 5 – Experimental design of the mechanical pruning and organic amendment assay in Quinta do Gradil.

Pruning System: MAN –manual pruning; MEC –mechanical pruning.

Table 3 represents the soil composition before any application of MSWC.

Table 3 – Soil composition without the application of organic amendments

Organic Matter (%)	1.28
рН	6.06
E. C. (μS cm <sup>-1</sup> )	46.00
MinN (mg kg <sup>-1</sup> )	3.83
P2O₅ (mg kg <sup>-1</sup> )	66.19
K <sub>2</sub> 0 (mg kg <sup>-1</sup> )	120.5
Cu (mg kg <sup>-1</sup> )	6.96
Mn (mg kg <sup>-1</sup> )	69.10
Zn (mg kg <sup>-1</sup> )	2.24
Fe (mg kg <sup>-1</sup> )	44.99
Ca (cmol kg <sup>-1</sup> )	11.05
Mg (cmol kg <sup>-1</sup> )	6.54
Na (cmol kg <sup>-1</sup> )	0.22
K (cmol kg <sup>-1</sup> )	0.26

Year 2018 was the first year of the project, the first year when MSWC was applied, and therefore no differentiation was made between the different amounts of MSWC. In the following years the grapes were separately processed, resulting in 32 different wines for the years 2019 and 2020, and only 8 wines for the year 2018.

## 3.2. Maturation, Harvest and Vinification

At maturation, some grapes were collected in order to analyse must parameters, namely pH, total acidity, probable alcohol content (measured by the conversion of Brix degree) and assimilable nitrogen. The results are presented in table 4. These analyses were performed by Quinta do Gradil staff.

Pruning	MSWC	Brix Degree	Probable alcohol content	рН	TA (g tartaric acid/ I)	Assimilable Nitrogen (mg/L)
MAN	TEST	21,2	13,2	2,98	6,30	94,06
MAN	M1	20,8	13,0	3,00	6,17	107,19
MAN	M2	20,5	12,8	2,99	6,11	113,75
MAN	M3	21,2	13,2	3,01	6,24	115,94
MEC	TEST	21,5	13,4	3,05	5,63	91,87
MEC	M1	21,5	13,4	3,07	5,74	111,56
MEC	M2	21,1	13,2	3,04	5,64	96,25
MEC	M3	21,2	13,3	3,07	5,83	98,44

Table 4 – Must analyses results. The analyses were performed in the 4<sup>th</sup> of September 2020.

Pruning System: MAN –manual pruning; MEC –mechanical pruning; MSWC application: TEST – no MSWC application; M – application of MSWC; TA–total acidity.

The grapes were harvested via manual harvest on 4<sup>th</sup> September, and were immediately transported to the ISA experimental cellar to be vinified in 25 litre glass bottles. 50 mg/l of sulphur dioxide was added prior to pressing, and one day after pressing the wine was clarified by static decanting prior to the start of the alcoholic fermentation.

After decanting, the must was transferred to another bottle where it was inoculated with a neutral yeast, starting fermentation at room temperature of the cellar (around 24°C). During the fermentation, the temperature and the density of the must were controlled daily. Table 5 shows the fermentation control of the wine corresponding to the mechanical pruning test without adding MSW in block 1. In Annex I, the fermentation controls of the remaining wines can be found.

Date	Density (mg/dm <sup>3</sup> )	Temperature (ºC)
04/set	1093	24
06/set	1089	24
07/set	1071	26
08/set	1050	26
09/set	1030	26
10/set	1017	25
11/set	1005	25
12/set	997	24
13/set	994	24
14/set	993	24
15/set	992	24
16/set	992	24

Table 5 – Fermentation Control of block 1, mechanical pruning, no organic amendment wine

Before bottling, free and total sulphur dioxide content were measured again, in order to correct all wines to 35 mg/l free SO<sub>2</sub>. Exceptionally, the wine that corresponds to block 3, mechanical pruning and no addition of MSWC was corrected to 40 mg/l free SO<sub>2</sub>, due to its high reducing substances value of 2.9 g invert sugar/l. Table 28 in annex III shows the results of total and free sulphur dioxide content for all wines.

## 3.3. Chemical analysis

Chemical analysis represent fundamental tools for monitoring wine quality, are compulsory for the certification process and are important in the winemaking process. They play a large role in all stages of winemaking, from monitoring the maturation in the vineyard, to the fermentation, and also for controlling the aging process of the wine.

The chemical analysis were performed at different stages of the winemaking process: during the maturation in the vineyard there were maturation controls where probable alcohol content, pH and total acidity were determined; the must was analysed shortly after the harvest (pH, total acidity, probable alcohol content and assimilable nitrogen), and then the wines were analysed again after the fermentation at different times, namely after the end of the harvest (October), when volatile acidity, total and free sulphur dioxide content, pH, alcohol by volume, total acidity and reducing sugars were determined, and later in February and March, when free and total sulphur dioxide, phenolic composition, absorvance at 420nm and the CIE lab parameters were determined.

#### a) pH

The pH consists of the proportion of  $H^+$  and  $OH^-$  ions, being lower in acidic media saturated with  $H^+$  ions, and lower in alkaline media with a higher concentration of  $OH^-$  ions. The pH is measured by potentiometry, in accordance with the official OIV method (2015).

#### b) Total Acidity

Total acidity consists of the sum of all titratable acids present in the wine when it is titrated with a base until reaching a pH of 7. Total acidity and pH are very important to determine the acidity of a particular wine and are routinely quantified in winemaking. It will be determined by titration with blue bromothymol as an indicator, following the indications of the official OIV method (2015).

#### c) Volatile Acidity

Volatile Acidity is typically referred to as the "wine thermometer", since high levels of volatile acidity tend to be good indicators of microbiological attack. In addition to the technical purpose of recognizing symptoms of microbiological attack, volatile acidity is also monitored because of the legal limits it is subjected to, in Portugal the limit for dry white wine is 1.08 g / I (IVV, 2016). The volatile acidity will be determined by the OIV method (2015), consisting of the distillation of the wine, followed by the titration of the distillate with sodium hydroxide (NaOH).

#### d) Assimilable Nitrogen

Nitrogen is of capital importance for a successful fermentation, without any formation of off-flavours. The must can either have sufficient nitrogen coming in the grapes, or, in case it is not sufficient, it may be necessary to supplement the must with exogenous nutrients. It can therefore be important to know the concentration of assimilable nitrogen in musts before the beginning of the fermentation. The method employed was the reference method that consists of a formol titration at pH 8 (Skoutelas et al., 2011).

#### e) Sulphur Dioxide

Sulphur dioxide is used in the production of wines mainly for its antioxidant properties, supporting in the protection of the wine against microbiological attack and against oxidation. As it is harmful to human health when consumed in excessive doses, there is a legal limit for white wine with less than 5 g invert sugars /I in Portugal of 200 mg/I (IVV, 2016). In wine, sulphur dioxide is considered to be

present in three distinct forms: total, free and molecular. To determine the concentration of free and total sulphur dioxide the official OIV method (OIV, 2015), which consists of a potentiometric titration with iodine, will be used.

### f) Alcohol by Volume (ABV)

Alcohol by Volume is essential in many aspects, from the commercialization and certification of wine to its organoleptic analysis and also as a conservation factor. It consists of the number of litres of ethanol present in 100 litres of wine, measuring both volumes at 20°C, and is expressed in percentage by volume (% vol.). It will be determined by the OIV method (2015), in which the difference in the boiling point of water and the boiling point of ethanol is used to determine the amount of ethanol present in a wine.

#### g) Reducing Substances

The amount of reducing substances is expressed in g invert sugar/ I of wine, and is usually used to understand if the alcoholic fermentation took occurred normally, resulting in a wine that will not have a second fermentation during its aging processes or after bottling. A wine is considered "dry" when it has less than 2 g invert sugar /I. The values for reducing substances will be determined by the official OIV method (2015).

#### h) Colour Intensity (Absorvance at 420nm)

In relation to colour analysis, the official OIV methods (2015) regarding absorvance at 420nm and by CIElab will be followed. Regarding the Reading of the absorvance at 420nm, the results are expressed in absorvance units.

#### i) Colour parameters – CIElab method

The CIEIab method consists in the chromatic characterization of wines and other beverages by assigning them values in three different axis: the Luminosity axis (L\*) ( $0 < L^* < 100$ , where 0 is black and 100 is the absence of colour), the a\* axis, that evaluates the colour according to its red/green component ( $a^* > 0$  is red and,  $a^* < 0$  is green) and the b\* axis, that evaluates the colour according to its blue/yellow component ( $b^* >$  is yellow and  $b^* < 0$  is blue). With the numeric values for these three coordinates, the colour's Chroma (C\*) and Hue (H\*) are derived. Chroma is obtained with the expression:  $C^* = (a^{*2} + b^{*2})^{1/2}$  and hue, being expressed in angle degrees, is calculated using the following expression: H\*: tan<sup>-1</sup> ( $b^*/a^*$ ). After obtaining all of the values described above, it is possible

to compare two different colours through  $\Delta E$ , which is obtained with the expression:  $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta H^*)^2}$ ; and also through  $\Delta H$ , which is obtained through the expression:  $\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2}$ . According to Spagna et al. (1996), the human eye can distinguish two colours when the value for  $\Delta E$  is larger than two units, while Mokrzycki & Tatol (2011) affirm that for 2 < $\Delta E$  <1 an experienced observer can distinguish two colours while for unexperienced observers being able to distinguish two colours is only possible when  $\Delta E$ > 2.

Hue (H\*), expressed in angle degrees (<sup>o</sup>) is typically considered to be the colour itself, translating the contributions of red, green, blue and yellow into a single colour. Chroma (C\*) determines the intensity observed for a specific value of hue, being the difference between the colour in question and a grey colour with exactly the same luminosity (L\*) value (Mokrzycki & Tatol, 2011).

The practical application of the method consists in centrifuging the wine for 10 minutes at 3500 rpm, to then read it's absorvance at several different wave lengths in between 380 nm and 780 nm (OIV, 2015).

#### j) Phenolic Composition

The Phenolic Compounds present in wines can be broken down into two groups: flavonoids, which include the anthocyanins, flavonols and flavanonols; and non-flavonoids, such as phenolic acids and stilbens. Kramling & Singleton (1969) describe a method for measuring the concentration of total phenolic compounds by measuring the absorbance at 280 nm as well as non-flavonoid compounds consisting essentially of causing the precipitation of flavonoid compounds by making a reaction with formaldehyde occur. After the addition of formaldehyde, the sample is placed in the dark and is incubated for 24 hours. The next day, the absorbance of the sample at 280 nm is read, and the value obtained reflects only the concentration of non-flavonoid compounds. Thus, after collecting the concentration of total phenolic compounds and non-flavonoid phenolic compounds, it is possible to deduce the concentration of flavonoid phenolic compounds.

## 3.4. Quantitative descriptive sensory analysis

The wines were tasted by a panel of experienced tasters in the Ferreira da Lapa building inside the Instituto Superior de Agronomia. The wines were tasted individually by the tasters and were presented in a random order with the coding presented in Annex II. The methodology employed is best described as a quantitative descriptive analysis (Coutinho, 2016), since the tasters were asked to rate several wine characteristics, and then the data collected was processed by analytical tools.

The tasting of all 72 wines (8 from year 2018, 32 from year 2019 and 32 from year 2020) was divided into two separate days – on the first day (May 28<sup>th</sup>) the wines of years 2018 and 2019 were tasted and on the second day (June 18<sup>th</sup>) the wines of year 2020 were tasted. The wines were separated into three different flights on each day, on the first day the first flight was made up of all wines from 2018, the second consisted of blocks 1 and 2 and the third flight consisted of blocks 3 and 4. On the second day one of the wines (specifically the wine from block 2, manual pruning and 10 tons/ha of organic amendment) was tasted twice, so that all three flights were made up of 11 wines each, and therefore the first flight was made up of wines from blocks 1 and 2, the second was made up of wines from blocks 2 and 3 and the third flight was made up of wines from blocks 3 and 4.

On the first tasting day 11 tasters were present, while on the second day only 9 - in order to maintain the integrity of the statistical treatments, only 9 taster's data from the first day were considered, so that all wines were tasted by the same number of tasters

The tasting sheet that was used is also in Annex II. The tasters evaluated from 1 to 5 (where 1 corresponds to non-existent, 2 little intense, 3 moderately intense, 4 intense and 5 very intense) parameters related to the colour, aroma and taste of the wines. The aromas and tastes that are in evaluation are those that are typically associated to the Sauvignon Blanc variety.

# 4. Results and Discussion

IntenSusVITI is a four year projected that started in 2018, the data for years 2018 and 2019 was analysed in a previous master's dissertation that was written by Liggio (2020). That dissertation contains the laboratorial analysis results for the wines of years 2018 and 2019, however it does not contain any data regarding the sensory parameters of the wines, since it was not possible to organize a panel tasting due to the restrictions that were in place in 2020 due to the COVID pandemic. The present dissertation continues the work made by Liggio (2020), since all of the wines from 2018, 2019 and 2020 were tasted.

This dissertation will include the discussion of the laboratory results of years 2018 and 2019 – their addition will contribute towards achieving a more holistic approach towards the data in question, thus allowing better conclusions to be reached.

The complete data collected in the scope of the physicochemical and chromatic analysis are presented in the annex chapter (annexes IV, V and VI).

## 4.1. Physicochemical parameters

This sub chapter will discuss the physicochemical parameters (volatile acidity, total acidity, pH, alcohol by volume, reducing substances) that were evaluated during the dissertation.

Table 6 – Effect of pruning system upon the physicochemical characteristics of the wines from the harvest of 2018. (*Adapted from Liggio, 2020*).

Factor	VA (g acetic acid/ I)	TA (g Tartaric acid/ I)	рН	ABV (% vol.)	RS (g invert sugar /I)
MAN	0.35	8.87	3.15	13.0	0.4
MEC	0.49	8.47	3.15	13.5	0.4
Sig	*	n.s.	n.s.	*	n.s.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. MAN –manual pruning; MEC – mechanical pruning; VA –Volatile Acidity; ABV- Alcohol by Volume; TA –Total Acidity; RS – Reducing Substances.

Table 6 shows the average values (according to the pruning system used) of the physicochemical parameters of the wines of 2018, as well as the statistical significance of the difference between the two averages, evaluated by the F test. These results were collected by Liggio (2020). Volatile acidity and alcohol content both showed significant differences between the two pruning systems, and for

both parameters the wines referent to mechanized hedge pruning are associated with higher values. For total acidity, there is a tendency for manual pruning to result in wines with greater total acidity, although not statistically significant.

Factor	VA (g acetic acid/ I)	TA (g Tartaric acid/ I)	рН	ABV (% vol.)	RS (g invert sugar /I)
MAN	0,46	8,98	3,27	14,4	0,5
MEC	0,49	8,52	3,25	14,6	0,4
Sig	n.s.	n.s.	n.s.	n.s.	n.s.
Test	0,47	8,92	3,25	14,4	0,4
M1	0,51	8,43	3,28	14,7	0,4
M2	0,48	8,77	3,26	14,5	0,5
M3	0,44	8,88	3,26	14,6	0,4
Sig	n.s.	n.s.	n.s.	n.s.	n.s.
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.

Table 7 – Effect of pruning system and Municipal Solid Waste compost upon the physicochemical characteristics of the wines from the harvest of 2019 (adapted from Liggio, 2020).

Sig. – Significance level: n.s. – insignificant at p < 0, 05 level by F test; (\*) - significant at p < 0, 05, (\*\*) - significant at p < 0, 01, (\*\*\*) - significant at p < 0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. MAN – manual pruning; MEC – mechanical pruning; VA –Volatile Acidity; ABV-Alcohol by Volume; TA –Total Acidity; RS – Reducing Substances; Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

For the wines of the year 2019, the results of the physicochemical analysis, as well as their statistical significance, are shown in table 7. The results presented were collected by Liggio (2020). There were no significant changes due either to the pruning system used or to the quantity of municipal solid waste compost applied, yet it is possible to again identify a tendency for wines from the vines subject to mechanical hedge pruning to have higher alcohol content and lower total acidity.
Factor	VA (g acetic acid/ I)	TA (g Tartaric acid/ I)	рН	ABV (% vol.)	RS (g invert sugar /I)
MAN	0,45	8,96	3,04	13,1	0,8
MEC	0,57	8,23	3,05	3,05 13,5	
Sig	**	*	n.s.	**	n.s.
Test	0,46	8,87	3,02	13,3	1,1
M1	0,53	8,34	3,06	13,3	0,6
M2	0,51	8,76	3,05	13,2	0,8
M3	0,53	8,42	3,06	13,4	0,8
Sig	n.s.	n.s.	n.s.	n.s.	n.s.
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.

# Table 8 – Effect of pruning system and Municipal Solid Waste compost upon the physicochemical characteristics of the wines from the harvest of 2020.

Sig. – Significance level: n.s. – insignificant at p < 0, 05 level by F test; (\*) - significant at p < 0, 05, (\*\*) - significant at p < 0, 01, (\*\*\*) - significant at p < 0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. MAN – manual pruning; MEC – mechanical pruning; VA –Volatile Acidity; ABV-Alcohol by Volume; TA –Total Acidity; RS – Reducing Substances; Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

Table 8 presents the results of the analysis of the physicochemical parameters, as well as their statistical significance, for the wines of year 2020. The volatile acidity, the alcoholic content and the total acidity showed significant differences between the two types of pruning studied, while for pH and quantity of reducing substances no significant differences were verified. The differences in the level of volatile acidity and alcohol content had already been verified in the year 2018, meaning that mechanical hedge pruning is related to higher alcohol content and higher volatile acidity in two years and there being a tendency for this in 2019, although not statistically significant.

Regarding volatile acidity, significant differences were noted between the two types of pruning contemplated both in 2018 and 2020, and in 2019 a trend similar to that observed in the remaining years was noted, as shown in figure 6.



Figure 6 – Average values for volatile acidity, according to the adopted pruning system, for years 2018, 2019 and 2020

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 001, (\*\*\*) - significant at p <0,001. MAN – manual pruning; MEC – mechanical pruning.

The higher volatile acidity detected in the mechanized pruning wines is probably due to other factors that are not being studied, since it is not plausible to attribute this difference to a viticulture-related parameter that takes place many months before the vinification process and should not interfere with the sanitary state of the grapes. The volatile acidity increase is not necessarily problematic, since the values are not outside of the average concentrations of 0.2 to 0.5 g Acetic acid /I that are typically verified in white wines (Bely et al., 2003), and are still much lower than the legal limit of 1.08 g acetic acid / I (IVV, 2016) for white wines in Portugal.

The results for total acidity and alcohol content over the three years, according to the adopted pruning system, are illustrated in figures 7 and 8, respectively. These results, which generally reflect the maturation that was possible to obtain within the considered time frame, seem to contradict the results of Botelho et al. (2021), Castro et al. (2010) and Clingeleffer (1988), where all researchers found an association between mechanical pruning and lower accumulations of sugar, and as a result of lower berry sugar content, lower alcohol content in wine. The authors attribute this delay in maturation to the increase in production due to the increase in bud load. In this particular case, there was no noticeable difference in production between manual pruning and mechanical pruning, the better maturation observed in mechanical pruning wines is probably due to the factors responsible for improving the efficiency of canopy from hedge pruned vines, such as for example a larger leaf surface area and more leafs directly exposed to the sun. In the study performed by Poni et al. (2004), similar

productions were obtained between mechanical hedge pruning and manual pruning. In this study, no significant qualitative differences were observed between the two modalities of pruning.



# Figure 7 – Average values of total acidity, according to the adopted pruning system, for years 2018, 2019 and 2020



Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 001, (\*\*\*) - significant at p <0,001. MAN – manual pruning; MEC – mechanical pruning.

Figure 8 – Average values for Alcohol by Volume (%vol.), according to the adopted pruning system, for years 2018, 2019 and 2020.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0,001. MAN –manual pruning; MEC – mechanical pruning.

Regarding pH and reducing substances, whose average values according to the adopted pruning system are represented in figures 9 and 10 respectively, no significant differences were observed in any of the three years.



Figure 9 – Average values for pH, according to the adopted pruning system, for years 2018, 2019 and 2020.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 001, (\*\*\*) - significant at p <0,001. MAN – manual pruning; MEC – mechanical pruning.



# Figure 10 – Average values for reducing substances, according to the adopted pruning system, for years 2018, 2019 and 2020

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 001, (\*\*\*) - significant at p <0,001. MAN –manual pruning; MEC – mechanical pruning.

### 4.2. Phenolic Composition and colour analysis of wines

This sub chapter will discuss the phenolic composition of the wines, measured by the Kramling and Singleton (1969) method. In addition, this sub chapter also includes the results and discussion of the colour parmeters, which include Absorvance at 420nm and the CIElab method.

Table 9 – Effect of pruning system over phenolic composition and chromatic characteristics of the wines from year 2018 (adapted from Liggio, 2020)

Factor	Total Phenolics (mg/l Gallic acid)	Non Flavonoids (mg/l Gallic acid)	Flavonoids (mg/l Gallic acid)	ABS 420nm	L*	a*	b*	С*	Н*
MAN	166,3	87,1	79,2	0,084	99,2	-0,43	2,99	3,02	81,8
MEC	173,1	89,6	83,5	0,085	99,3	-0,27	2,76	2,77	84,3
Sig	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	***

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0,001. MAN –manual pruning; MEC – mechanical pruning; L\* - Luminosity; a\* - red-green contribution towards wine colour; b\* - yellow-blue contribution towards wine colour; C\* - Chroma; H\* - Hue

Table 9 shows the average values obtained for the analysis of phenolic composition, colour intensity measured through absorbance at 420 nm and for the CIElab method, for the wines of year 2018. The values were collected by Liggio (2020). In terms of phenolic composition, in both pruning modalities the values for total phenolics were low, around 170 mg Gallic acid/ I. Jackson (2008) states that the typical value for a young white wine, made by classical wine making procedures, is 215 mg eq. Gallic acid/ I. This low concentration of phenolic compounds is possibly due to the fact that Sauvignon Blanc is a grape variety that typically presents low levels of phenolic compounds (Jackson, 2008), and the fact that during winemaking all forms of skin contact were avoided. Total phenols are made up of non-flavonoid phenols, which have concentrations of around 88 mg / I, and flavonoid phenols with concentrations of around 81 mg / I. Typically, non-flavonoid phenols are found in greater quantities than flavonoid phenols because a significant portion is stored in the berry pulp (Jackson, 2008), and in this study they are present in similar concentrations. No significant differences were observed through the pruning system adopted for any of the three parameters related to phenolic composition.

Regarding the absorbance values at 420 nm, the wines of the years 2018 present an average value of 0,084 for manual pruning, and 0,086 for mechanical hedge pruning. Kanavouras et al. (2020) and Lopes et al. (2009) got slightly lower values closer to 0, 06, for Sauvignon Blanc wines that were evaluated

immediately after bottling. This slightly higher value than what is observed in the studies mentioned above could be interpreted as being a sign of oxidation, however, Waterhouse & Elias (2010) state that the absorvance value at 420nm only indicates deterioration after 0,2. The values observed therefore are not out of the ordinary, and are in between the values observed for the other years of this project.

The values observed in the CIElab method indicate that, in general, wines have high luminosity values that are close to the maximum value of 100, values on the a \* axis (where positive values mean that the substance being evaluated is closer to the colour green and negative values mean that the substance has a higher tendency for being red in colour) very close to zero and positive values on the b \* axis (where negative values equate to blue and positive values to yellow). The hue, which is calculated from the values of axis a \* and b \*, places all values between 81º and 85º, which corresponds to the yellow colour in the CIElab space. The relatively low values observed for Chroma indicate that the wines do not have a very high colour intensity. Taking into account the tonality values close to 90° and the luminosity values very close to the maximum value, it is possible to characterize the wines, in relation to their colour, as being pale white wines. The significant differences observed for the a\* coordinate and the Hue, depending on the pruning system adopted, indicate that a more greenish colour corresponds to the manual pruning wines, although the overall difference is very small. This difference is in line with the lower total acidity and higher alcohol by volume that is identified in the mechanical pruning wines of 2018. The value of  $\Delta E$  between the average values of the two pruning modalities is  $\Delta E = 0.35$  lower than the threshold of  $\Delta E = 1$  from which an experienced observer can distinguish between two colours (Mokrzycki & Tatol, 2011).

# Table 10 – Effect of pruning system and Municipal Solid Waste Compost over phenolic composition and chromatic characteristics of the wines from year 2019 (adapted from Liggio, 2020)

Factor	Total Phenolics (mg/I Gallic acid)	Non Flavonoids (mg/l Gallic acid)	Flavonoids (mg/l Gallic acid)	ABS 420nm	L*	a*	b*	С*	H*
MAN	180,3	65,5	114,8	0,100	98,5	-0,34	3,56	3,69	84,3
MEC	188,6	66,1	122,5	0,093	98,8	-0,26	3,43	3,44	85,7
Sig	n.s.	n.s.	n.s.	**	**	**	n.s.	n.s.	*
Test	185,9	64,9	120,9	0,094	98,7	-0,33	3,41	3,43	84,3
M1	180	65,3	114,6	0,100	98,6	-0,25	3,48	3,49	85,8
M2	187,2	65,7	121,4	0,100	98,8	-0,31	3,49	3,5	85,0
M3	184,8	67,2	117,6	0,100	98,6	-0,32	3,60	3,64	84,9
Sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test; (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC; L\* - Luminosity; a\* - red-green contribution towards wine colour; b\* - yellow-blue contribution towards wine colour; C\* - Chroma; H\* - Hue.

Table 10 shows the average values obtained for the analysis of the phenolic composition, the colour intensity measured through the absorbance at 420 nm and the CIElab method for the wines of the year 2019. The values were collected by Liggio (2020). The wines of the year 2019 have slightly higher values for the concentration of total phenols, slightly lower for non-flavonoid phenols and higher values for flavonoid phenols compared to 2018. For the year 2020, all figures are relatively similar. In 2019, there were no significant differences between the two pruning modalities adopted, or through the different doses of application of organic amendment.

For the absorbance at 420 nm, the wines from year 2019 have values higher than what is verified for years 2018 and 2020. There also significant differences between the two pruning systems being studied, where for manual pruning there is an average value of 0,100 and for mechanical pruning there is an average value of 0,003. Regarding the inter annual difference, 2019 was the year in which it was possible to obtain wines with higher alcohol content and higher pH, regardless of the adopted pruning system (figures 5 and 6), which may indicate that it was the year where the climatic conditions for wines with higher alcohol content, less acidity, and greater colour intensity were verified. As for the

difference between the two pruning systems, it is on par with the other colour parameters of the CIElab method (manual pruning has lower luminosity and higher Chroma values).

The values observed for the CIElab system for the wines from year 2019 are very similar to the values observed for the wines of 2018. The values for luminosity are slightly lower than those verified for year 2018, but still very high, very similar values on the a\* axis and slightly higher on the b\* axis. As the calculation required to arrive at the Chroma values (C \*) include the values of axes a\* and b\*, the observed values for Chroma are slightly higher. With regard to hue, the values are distributed between 82 and 87°. Thus, the colour of 2019 wines can be considered as being pale yellow, like the wines of year 2018. There are statistically significant differences between the two pruning systems for coordinates L\*, a\* and for hue, the wines of manual pruning have lower values on all three parameters, therefore, they are considered to be darker wines and of a more greenish tonality. It should be noted that despite there being significant differences, the average values are very similar – the differences are significant because the differences are consistent across the different experimental units, not because the differences are of great magnitude.

There are also significant differences derived from the interaction between the two factors for the a\* coordinate and for hue. Figure 11 shows the average values obtained in the a\* coordinate for each individual combination of MSWC modality with pruning system – from this figure it can be seen that in the case of mechanical pruning there is a decrease in the a\* coordinate once there is the addition of the organic amendment, and this difference becomes larger as the organic amendment dose increases. Figure 12, referring to the hue, shows that for this parameter the difference between pruning modalities is not as evident as what is observed in the a\* coordinate, making it impossible to understand which pruning modality for which the MSWC dose applied is significant.



Figure 11 – Average values of a\* coordenate (CIElab method) for 2019 wines.



Pruning system: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): Test – No MSWC application; M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

#### Figure 12 – Average values of Hue (H\*) for 2019 wines

Pruning system: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): Test – No MSWC application; M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

The values observed for absorvance at 420nm and the values observed in the CIElab method both point to wines from manual pruning as having more colour intensity, however, it is necessary to contextualize this difference, and to this effect the value of  $\Delta E$  is shown in Table 11. Table 12 shows the  $\Delta E$  values for the various organic matter doses, while fixing the pruning system According to Mokrzycki & Tatol (2011) and their scale of values for  $\Delta E$ , it should not be possible to differentiate two wines of different pruning but same MSWC modality through their colour, since all values of  $\Delta E$  are lower than one unit. Thus, even if there are statistically significant differences, the difference is not sufficient to say that the manual pruning wines are different (in terms of colour) from the mechanical pruning wines, and also the same for wines from different organic matter doses. Table  $11 - \Delta E$  values for the wines from year 2019, comparing average values of wines from mechanical pruning to manual pruning (*adapted from Liggio, 2020*)

MSWC	MAN/MEC	ΔΕ
Test	MAN/MEC	0,82
M1	MAN/MEC	0,53
M2	MAN/MEC	0,41
M3	MAN/MEC	0,54

Pruning system: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): Test – No MSWC application, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

Table  $12 - \Delta E$  values verified for the wines of year 2019, comparing average values of different MSWC doses of application (with the same pruning system)

Pruning	MSWC	ΔΕ*
MAN	TEST/M1	1,08
MAN	TEST/M2	0,58
MAN	TEST/M3	0,57
MAN	M1/M2	0,53
MAN	M1/M3	0,82
MAN	M2/M3	0,42
MEC	TEST/M1	0,40
MEC	TEST/M2	0,29
MEC	TEST/M3	0,52
MEC	M1/M2	0,23
MEC	M1/M3	0,36
MEC	M2/M3	0,35

Pruning system: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): Test – No MSWC application, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

Table 13 – Effect of pruning system	and Municipal	Solid Waste	Compost on	phenolic
composition and chromatic	characteristics	s of wines fro	m year 2020	).

Factor	Total Phenolics (mg/l Gallic acid)	Non Flavonoids (mg/l Gallic acid)	Flavonoids (mg/l Gallic acid)	ABS 420nm	L*	а*	b*	C*	H*
MAN	176,4	68,5	108,0	0,06	99,6	-0,12	4,92	4,93	88,3
MEC	177,6	68,1	109,6	0,06	99,0	-0,12	4,30	4,30	88,0
Sig	n.s.	n.s.	n.s.	n.s.	***	n.s.	***	***	n.s.
Test	177,8	69,0	108,8	0,06	99,3	-0,06	4,66	4,66	89,0
M1	178,1	68,1	110,1	0,06	99,3	-0,16	4,66	4,66	87,5
M2	172,2	66,0	106,2	0,06	99,5	-0,14	4,47	4,47	88,1
M3	180,0	70,0	110,0	0,06	99,2	-0,12	4,66	4,66	87,8
Sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Sig. – Significance level: n.s. – insignificant at p <0,05 level by F test, (\*) - significant at p <0,05, (\*\*) - significant at p <0,01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC; L\* - Luminosity; a\* - red-green contribution towards wine colour; b\* - yellow-blue contribution towards wine colour; C\* - Chroma; H\* - Hue.

Table 13 shows the values of phenolic composition, absorvance at 420nm and values referring to the CIElab method for the wines of 2020. The observed values referring to phenolic composition are similar to what had been verified in 2019. Moreover, there are no significant differences between either the two pruning systems or between the different MSWC modalities for the phenolic composition of wines.

The observed values for absorbance at 420nm are lower than those observed in the previous two years, and show no significant differences due to either the pruning system or the organic amendment dosage.

The results obtained in the CIEIab method are similar to those observed in previous years, with the luminosity values (L \*) being closer to 100 (maximum value of the axis), on the a\* axis there are lower values and on the b\* axis there are higher values. The observed differences in axes a\* and b\* result in higher Chroma values (C\*) and hue(H\*) values that are dispersed between 87° and 90°. The wines are again characterized as being pale white wines, however the differences between the wines from 2020 and the wines from 2019 and 2018 is larger than the ones observed between the wines from 2018 and

2019. The wines from 2020 have higher Chroma, lower luminosity and higher hue values, which means, respectively, that they are darker, have higher colour intensity and are more yellow in tone.

There are significant differences due to the pruning system for luminosity (L\*), the b \* coordinate and Chroma (C\*), and due to the MSWC modality significant differences can be identified between control and the application of 5 t/ha of MSWC. The differences found between pruning systems indicate that the wines from manual pruning tend to have higher values in Luminosity, tend to be more yellowish and tend to have higher colour intensity (due to higher Chroma values). Regarding MSWC, wines where there was no MSWC application correspond to a higher hue value when compared to wines where MSWC was applied, meaning that the wines from vines where there was no application of MSWC tend to be more yellowish. Table 15 shows the average values of  $\Delta E$  for the pruning system (where wines with the same MSWC modality are compared) and table 16 shows the average values of  $\Delta E$  for the amount of MSWC used (where wines with the same pruning system are compared).

#### Table 14 – $\Delta E$ values verified for the wines of year 2020, comparing average values of wines from manual pruning to average values of wines from mechanical pruning (with the same dose of MSWC application)

MSWC	Pruning	ΔΕ*
Test	MAN/MEC	1,43
M1	MAN/MEC	1,27
M2	MAN/MEC	1,76
M3	MAN/MEC	1,77

Pruning system: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): Test – No MSWC application, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

### Table $15 - \Delta E$ values verified for the wines of year 2020, comparing average values of different MSWC doses of application (with the same pruning system)

Pruning	MSWC	ΔΕ*
MAN	TEST/M1	1,79
MAN	TEST/M2	1,48
MAN	TEST/M3	1,16
MAN	M1/M2	1,09
MAN	M1/M3	1,66
MAN	M2/M3	1,17
MEC	TEST/M1	1,84
MEC	TEST/M2	0,80
MEC	TEST/M3	2,15
MEC	M1/M2	1,32
MEC	M1/M3	0,73
MEC	M2/M3	1,50

Pruning system: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): Test – No MSWC application, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

According to Mokrzycki & Tatol (2011), from  $\Delta E>1$ , it should be possible for an experienced observer to be able to differentiate two colours, and from  $\Delta E>2$  it becomes possible for most observers. According to Spagna et al. (1996) from  $\Delta E>2$  the human eye distinguishes two colours. The values observed in Table 14 indicate that, for an experienced observer, it is possible to distinguish wines of mechanical pruning from wines of manual pruning by their colour. For the different doses of application of MSWC, the values of  $\Delta E$  (table 15) suggest that it would be possible to distinguish almost all wines, which contradicts the results of the statistical tests, since according to the Tukey test it is only possible to differentiate the hue of the wines in which 5t/ha of MSWC was applied to wines in which no organic concealer was applied. Although this comparison (no MSWC against 5 t MSWC/ha) corresponds to relatively high values ( $\Delta E=1$ , 79 for manual pruning and  $\Delta E=1$ , 84 for mechanical pruning), close to 2 units, the comparison does not correspond to the highest values observed for  $\Delta E$  (that would be  $\Delta E=2$ , 15 for mechanically pruned vineyards, comparing no use of MSWC to 20 t MSWC/ha).

### 4.3. Sensory Analysis

This subchapter presents the results and discussion of the wine tasting panels that were carried out on two separate days (May 28<sup>th</sup> and June 18<sup>th</sup>).

Table 16 – Colour intensity evaluation values obtained in sensory analysis, in a scale of 1 to 5, of the wines from year 2018

Colour	Yellow	Green
MAN	1,86	2,57
MEC	1,95	2,59
sig	n.s.	n.s.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning.

The tasters evaluated the colour of the wines of year 2018 as being of low colour intensity in general, and being more "green" than "yellow" (table 16). No statistically significant differences were found between the two pruning modalities.

Table 17 – Aroma descriptors evaluation values, in a scale of 1 to 5, of the wines from year 2018

Aroma	Intensity	Fruity	Floral	Grapefruit	Passion Fruit	Cat's Pee	Herbal	Equilibrium
MAN	2,98	2,45	1,75	1,89	1,7	1,64	2,66	2,91
MEC	2,72	2,36	1,86	1,84	1,61	1,3	2,11	2,65
Sig	n.s.	n.s.	n.s.	n.s.	n.s.	*	**	n.s.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning.

The scores in terms of aroma of the wines from 2018, shown in table 17, show that, in general, the wines are of average intensity, scored higher in fruity aroma, and relatively lower in the more typical Sauvignon Blanc aromas (except for the "green" aroma). In this case, significant differences were found in cat's pee and green aromas, with the wines from manual pruning being more aromatically intense in both parameters than the wines of mechanical pruning.

# Table 18 – Taste descriptors evaluation values and global appreciation values, in a scale of 1 to 5, of the wines from year 2018

Taste	Intensity	Acidity	Volume	Persistence	Equilibrium	<b>Global Appreciation</b>
MAN	3,09	3,34	2,63	2,93	2,95	3,04
MEC	2,95	3,34	2,46	2,95	2,86	2,9
Sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning.

Table 18 reports the results obtained in the sensory analysis for the descriptors related to taste for the wines of year 2018. There are no significant differences to report between the two different pruning modalities. The wines, in general, can be considered as being fairly average in all evaluated parameters.

# Table 19 – Colour intensity evaluation values obtained in sensory analysis, in a scale of 1 to 5, of the wines from year 2019

Colour	Yellow	Green
MAN	2,03	2,56
MEC	2,01	2,58
sig	n.s.	n.s.
Test	2,04	2,57
M1	2,01	2,55
M2	1,96	2,57
M3	2,06	2,59
sig	n.s.	n.s.
P*MSWC	n.s.	n.s.

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.

Table 19 shows the scores obtained in the two parameters related to colour for the wines of year 2019. No significant differences can be attributed to either the pruning modality or to the organic amendment dose of application chosen. The wines were considered as being "greener" in tonality by the tasters, but for both colours evaluated (green and yellow) the wines were considered as being of relatively low colour intensity.

Aroma	Intensity	Fruity	Floral	Grapefruit	Passion Fruit	Cat's Pee	Herbal	Equilibrium
MAN	2,86	2,29	1,96	1,73	1,77	1,58	2,57	2,86
MEC	2,72	2,27	1,92	1,69	1,61	1,48	2,57	2,70
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Test	2,74	2,07	1,96	1,64	1,67	1,56	2,53	2,71
M1	2,79	2,32	1,97	1,63	1,59	1,54	2,58	2,70
M2	2,81	2,26	1,94	1,74	1,69	1,58	2,64	2,72
M3	2,83	2,48	1,87	1,85	1,8	1,44	2,54	2,99
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 20 – Aroma descriptors evaluation values, in a scale of 1 to 5, of the wines from year 2019

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.

Table 20 shows how the wines of 2019 were evaluated in regards to aromatic descriptors by the tasting panel. In general, the wines were considered as being of average aromatic intensity, and somewhat lacking in the typical Sauvignon Blanc aromas (except for the green/herbaceous aroma). There were no significant differences between the two different pruning modalities but in the "equilibrium" parameter significant differences due to different organic amendment modalities were observed at the ANOVA level, but not at the Tukey test level – this is due to the fact that the Tukey test is "stricter" than the ANOVA test, meaning that the Tukey test needs the two results being compared to have a higher degree of distinction in order to be considered as being "statistically different" than the ANOVA f-test does. The difference detected is probably established between the highest amount of organic amendment and one of the other three doses, since there is a large difference between this result and the others observed for "equilibrium".

Taste	Intensity	Acidity	Volume	Persistence	Equilibrium	<b>Global Appreciation</b>
MAN	3,06	3,39	2,66	2,97	2,87	2,93
MEC	2,98	3,33	2,67	2,90	2,81	2,77
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Test	2,92	3,38	2,54	2,81	2,76	2,76
M1	3,00	3,36	2,72	3,00	2,87	2,84
M2	3,01	3,31	2,69	3,01	2,90	2,92
M3	3,15	3,38	2,70	2,9	2,82	2,87
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 21 – Taste descriptors evaluation values and global appreciation values, in a scale of 1 to 5, of the wines from year 2019

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

Table 21 shows the panel's evaluations for the wines of year 2019 in terms of taste related descriptors. No significant differences were established between the different pruning systems and organic amendment doses. The scores attributed by the tasters qualify this wine as being fairly average, and with scores similar to the wines from the year 2018.

Colour	Yellow	Green
MAN	2,51	2,17
MEC	2,46	2,16
sig	n.s.	n.s.
Test	2,52	2,16
M1	2,5	2,16
M2	2,43	2,18
M3	2,5	2,16
sig	n.s.	n.s.
P*MSWC	n.s.	n.s.

Table 22 – Colour intensity evaluation values obtained in sensory analysis, in a scale of 1 to 5, of the wines from year 2020

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 001, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.

Table 22 shows the colour intensity scores obtained by the wines from year 2020, attributed by the tasting panel. The wines were considered as being of low colour intensity, with higher scores for "yellow" than for "green", and with very small (to non-existent) differences due to either pruning or organic amendment dose. Curiously the wines from 2020 oppose the wines from previous years in terms of colour, since they are more yellow instead of greener – this may be due to them being younger than the other two wines or due to the fact that the wines from 2020 were evaluated by a different tasting panel than the one that evaluated the wines from 2018 and 2019.

Aroma	Intensity	Fruity	Floral	Grapefruit	Passion Fruit	Cat's Pee	Herbal	Equilibrium
MAN	3,24	3,05	1,90	2,44	2,07	1,63	2,28	2,99
MEC	3,04	2,99	1,91	2,52	2,03	1,49	2,17	3,03
sig	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Test	3,21	3,07	1,97	2,43	2,14	1,59	2,23	3,11
M1	3,14	2,94	1,89	2,56	2,05	1,52	2,32	2,94
M2	3,16	3,10	1,97	2,46	1,99	1,51	2,18	3,01
M3	3,06	2,97	1,78	2,48	2,04	1,63	2,17	2,98
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 23 – Aroma descriptors evaluation, in a scale of 1 to 5, of the wines from year 2020

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0, 01, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.

Table 23 shows the scores attributed by the tasting panel to the wines from year 2020 in regards to their aroma descriptors. The wines in general show scores that classify them as being in between low and moderate aromatic intensity for the various aromatic descriptors. When compared to the wines from previous years, it is clear that the wines from 2020 were classified as being of higher aromatic intensity for most aromatic descriptors, except for the "green" descriptor. The comparison between different organic amendment doses shows no significant differences, while in the pruning system case, there is a statistically significant difference in between manual and mechanical pruning for the overall aromatic intensity that favours the wines from manual pruning as opposed to those of mechanical pruning.

Taste	Intensity	Acidity	Volume	Persistence	Equilibrium	Global Appreciation
MAN	3,35	3,50	2,75	2,91	3,01	3,07
MEC	3,26	3,46	2,78	2,86	2,99	3,05
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Test	3,37	3,39	2,79	2,92	3,05	3,12
M1	3,27	3,52	2,79	2,86	3,02	2,99
M2	3,31	3,51	2,68	2,88	2,97	3,04
M3	3,29	3,48	2,82	2,87	2,98	3,08
sig	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P*MSWC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 24 – Taste descriptors evaluation values and global appreciation values, in a scale of 1 to 5, of the wines from year 2020

Sig. – Significance level: n.s. – insignificant at p <0, 05 level by F test, (\*) - significant at p <0, 05, (\*\*) - significant at p <0,001, (\*\*\*) - significant at p <0,001. In each column values followed by the same letter do not significantly differ by Tukey HSD test at  $\alpha$ =0.05. Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.

Table 24 shows the evaluation of the 2020 wines by the tasting panel. According to the evaluation, there is very little difference between the manual pruning wines and the mechanical pruning wines, and there is also very little to no difference between the different amounts of organic amendment. When comparing the scores given by the panel for the wines of 2020 with the wines of previous years, there are only small differences in terms of acidity and intensity, in general the panel scored the wines very similarly to the wines of years 2019 and 2018.

### 5. Conclusions

The aim of the present work was to evaluate the effects of mechanical pruning and the addition of organic amendments (specifically Municipal Solid Waste Compost) on Sauvignon Blanc wine quality. The evaluation was based upon the classic physicochemical parameters, phenolic composition (total phenolic, flavonoids and non-flavonoids) colour analysis (absorbance at 420nm and CIELAB space coordinates) and sensory analysis by panel test. The wines were made from grapes cultivated in Quinta do Gradil, which were then transported to ISA's winery so that the vinification could be carried out. The wines were produced in years 2018, 2019 and 2020, following the classical white wine winemaking technologies.

In terms of the physicochemical parameters, the wines from all three years seem to follow the same pattern, where mechanical pruning leads to wines that have higher alcohol content, and lower total acidity, while the organic amendment dose seems to have little effect upon grape composition. For their phenolic composition all wines have similar total phenolic composition (slightly lower than 200 mg galic acid /l total phenolics) as well as the concentrations of flavonoids and non-flavonoids.

For the colour analysis, the absorbance at 420nm showed that there were very small differences regarding pruning system and organic amendment (with exception for year 2019, where manual pruning wines had superior results), the CIEIab space coordinates indicate that all wines can be classified as pale white wines and also that there are significant differences attributable to the pruning modality for all years, where in 2018 and 2019 the wines from manual pruning were found to be darker and greener in colour, and for 2020 the manual pruning wines were classified as being of higher luminosity, more yellow in tone and of higher colour intensity. The tasting panel was not able to differentiate the wines according to pruning modality or organic amendment dose, and classified the wines from years 2018 and 2019 as being greener in tone, while for year 2020 the panel classified the wines as being more yellow in tone.

Regarding the results obtained in the tasting panel, all wines followed the pattern of being classified as average colour intensity, average aromatic descriptors' intensity (while both the "fruity" and "green" aroma descriptors are consistently described as being more intense than the other descriptors) and slightly above average for the "taste" related descriptors, specially acidity, intensity and volume. There were statistically significant difference regarding the pruning modality for the "cat's pee" and "green" aroma descriptors for the wines of 2018, with the manual pruning wines being classified as more intense for these descriptors, and also for the wines of 2020, where the manual pruning wines were classified as being more aromatically intense. Regarding the organic amendment there were statistically significant differences for the "equilibrium" aromatic descriptor for the wines of year 2019 at the ANOVA level, although not at the tukey test level, therefore it is not possible to understand what pair (or pairs) of organic amendment doses are different from each other. The fact that there are more statistically significant differences in the laboratory assays when compared to the statistical differences that can be observed in the wine tastings may mean that the differences are not significant enough to be noticeable in a more practical setting.

The decision to adopt mechanical pruning must naturally fall onto the viticulturist, the differences reported in the laboratory assays and by the tasting panel are not exceedingly high, while the savings that are involved in mechanical pruning are very significant. Interestingly, the aroma descriptors most responsible for the Sauvignon Blanc typicity, namely "grape fruit" and "passion fruit" were never affected by the pruning system, meaning that the adoption of mechanical pruning does not compromise the aromatic typicity of Sauvignon Blanc wines. Manual pruning can have its place in the contemporary wine sector, since it allows the farmer to tailor the number of buds to the vine capacity of the vineyard, thus being more suited to vineyards that typically make the best wine, however the increase in cost must be reflected in the final price of the wine, which may not always be possible due to factors that are not easily controllable.

Soil analysis and plant nutrient analysis assays must be carried out in order to take full advantage of the use of chemical fertilizers or organic amendments, since no single fertilizer can assure perfect nutrition status. The use of organic amendments, such as municipal solid waste compost, may complement fertilization protocols, since they contribute significantly to the organic matter content of the soil and can be used to supply some organic minerals. Their effect upon wine quality is not clear, since there were small to insignificant differences between the different municipal solid waste compost doses that were used.

### 6. Bibliographical references

- Allen T., Herbst-Johnstone M., Girault M., Butler P., Logan G., Jouanneau S., Nicolau L., Kilmartin P. A., 2011. Influence of Grape-Harvesting Steps on Varietal Thiol Aromas in Sauvignon blanc Wines. J. Agric. Food Chem., 59, 19, 10641-10650
- Andrea-Silva J., Cosme F., Ribeiro L. F., Moreira A. S. P., Malheiro A. C., Coimbra M. A., Domingues M. R. M., Nunes F. M., 2014. Origin of the Pinking Phenomenon of White Wines. *J. Agric. Food Chem.*, 62, 24, 5651-5659
- Anfang N., Brajkovich M., Goddard M. R., 2009. Co-fermentation with Pichia kluyveri increases varietal thiol concentrations in Sauvignon Blanc. *Aust. J. Grape Wine Res.*, **15**, 1, 1-8
- Baiano A., Scrocco C., Sepielli G., Del Nobile M., 2016. Wine Processing: A Critical Review of Physical, Chemical, and Sensory Implications of Innovative Vinification Procedures. *Crit Rev Food Sci Nutr.*, 56, 14, 2391-2407
- Bely M., Rinaldi A., Dubourdieu, D., 2013. Influence of assimilable nitrogen on volatile acidity production by Saccharomyces cerevisiae during high sugar fermentation. *J. Biosci. Bioeng.*, 96, 6, 507-512.
- Botelho M., Cruz A., Silva E.B., Mexia A., Ricardo-da-Silva J., Castro R., Ribeiro H., 2020. Mechanical pruning in non-irrigated vineyards: effects on yield and grape composition of cultivar 'Syrah' (Vitis vinifera L.). Acta Hortic., **1276**, 125-130
- Botelho M., Cruz A., Silva E.B., Mexia A., Ricardo-da-Silva J., Castro R., Ribeiro H., 2020. Organic amendments application to soil of mechanically pruned vineyards: effects on yield and grape composition of cultivar 'Syrah' (Vitis vinifera L.). *Acta Hortic.*, **1276**, 119-123
- Botelho M., Cruz A., Duarte D. F., Faria D. L., Ricardo-da-Silva J., Castro R., Ribeiro H., 2021. Mechanical pruning and soil organic amendments in vineyards of Syrah: effects on grape composition. *Oeno One*, **55**, 1, 267-277.
- Carvalho M., 2020. Quinta do Gradil ganha uma "nova vida". Available at: <u>https://marketingvinhos.com/2020/10/28/quinta-do-gradil-ganha-uma-nova-vida/</u> (accessed on: 25.01. 2021)
- Castelluci F., 2008. OIV Sustainable Vitiviniculture Guide: Environmental Issues. Available at: <u>http://www.oiv.int/public/medias/2089/cst-1-2008-en.pdf</u> (accessed on: 02.01. 2021)
- Castro R., Claro A., Rodrigues A., Teixeira A., Machado J., Piovene C., Cruz A., 2010. Poda mecânica na vinha efeitos no rendimento e na qualidade. *Actas do 8º Simpósio de Vitivinicultura do Alentejo.*, 1, 167-176, Évora

- Cavaco A., Silva H., Canhoto P., Neves S., Neto J., Pereira M. C., 2016. Radiação Solar Global em Portugal e a sua variabilidade, mensal e annual. Available at: <u>http://www.ipes.pt/ipes/wpcontent/uploads/2017/10/Radia%C3%A7%C3%A3o-Solar-Global-em-Portugal-e-a-suavariabilidade.pdf</u> (accessed on: 23.12.2021)
- Chan K. Y., Fahey D. J., 2011. Effect of composted mulch application on soil and wine grape potassium status. *Soil Res.*, **49**, 5, 455-461
- Chapman D. M., Matthews M. A., Guinard J. X., 2004. Sensory attributes of cabernet sauvignon wines made from vines with different crop yields. *Am. J. Enol Vitic.*, **55**, 4, 325-334
- Chen L., Capone D. L., Nicholson E. L., Jeffery D. W., 2019. Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for Vitis vinifera Sauvignon blanc. *Food Chem.*, **295**, 637-645
- Clingeleffer P.R., 2013. Mechanization in Australian vineyards. Acta Hortic. 978, 169-177.
- Clingeleffer P. R., 1988. Response of Riesling clones to mechanical hedging and minimal pruning of cordon trained vines (MPCT) implications for clonal selection. *Vitis*, **27**, 87-93
- Coetzee C., du Toit W. J., 2012. A comprehensive review on Sauvignon Blanc aroma with a focus on certain positive volatile thiols. *Food Res. Int.*, **45**, 287-298.
- Cojocaru G. A., Antonce A. O., 2016. Oxygen intake and colour evolution in sauvignon blanc and muscat ottonel wines treated with ascorbic acid and glutathione. *Agrolife Sci. J.*, **5**, 1, 44-50
- Coutinho A., 2016. Perfis sensoriais dos vinhos jovens tranquilos brancos e tintos com indicação geográfica protegida. Potenciais marcadores da tipicidade num estudo à escala nacional. Tese de Doutoramento em Engenharia Alimentar, Instituto Superior de Agronomia – Universidade de Lisboa, Lisboa.
- Darriet P., Tominaga T., Demole E., Dubourdieu D., 1993. Evidence of the presence of a 4mercapto-4-methylpentan-2-one precursor in vitis-vinifera sauvignon blanc grape variety. *Comptes Rendus De L Academie Des Sciences Serie lii-Sciences De La Vie-Life Sciences.*, **316**, 11, 1332-1335
- Des Gachons C. P., Tominaga T., Dubourdieu D., 2002. Sulfur aroma precursor present in Sglutathione conjugate form: Identification of S-3-(hexan-1-ol)-glutathione in must from Vitis vinifera L. cv. Sauvignon blanc. J. Agric. Food Chem., 50, 14, 4076-4079
- Des Gachons C., Van Leewin C., Tominaga T., Soyer J. P., Gaudillere J.-P., Dubourdieu D., 2005. Influence of Water and Nitrogen Deficit on Fruit Ripening and Aroma Potential of Vitis Vinifera L. Cv. Sauvignon Blanc in Field Conditions. *J. Agric. Food Chem.*, **85**, 73–85.

- Dubourdieu D., Tominaga T., Masneuf L., des Gachons C. P., Murat M. L., 2006. The Role of Yeasts in Grape Flavor Development during Fermentation: The Example of Sauvignon blanc. *Am. J. Enol. Vitic.*, 57, 81-88
- Fraga H., Santos J. A., Malheiro A. C., Moutinho-Pereira J., 2012. Climate change projections for the Portuguese viticulture using a multi-model ensemble. *Cienc. Tec. Vitivinic.*, **27**, 39–48.
- Gaiotti F., Marcuzzo P., Belfiore N., Lovat L., Fornasier F., Tomasi D., 2017. Influence of compost addition on soil properties, root growth and vine performances of Vitis vinifera cv Cabernet sauvignon, *Sci. Hortic.*, 225, 88-95.
- García-Gil J.C., Ceppi S.B., Velasco M. I., Polo A., Senesi N., 2004. Long-term effects of amendment with municipal solid waste compost on the elemental and acidic functional group composition and pH-buffer capacity of soil humic acids. *Geoderma*, **121**, 1–2, 135-142.
- García-Gil J.C., Plaza C., Soler-Rovira P., Polo A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass, *Soil Biol. Biochem.*, **32**, 13, 1907-1913.
- Gatti M., Civardi S., Bernizzoni F., Poni S., 2011. Long-Term Effects of Mechanical Winter Pruning on Growth, Yield, and Grape Composition of Barbera Grapevines. *Am. J. Enol. Vitic.*, **62**, 19
- Gutiérrez-Gamboa G., Díaz-Galvéz L., Verdugo-Vásquez N, Moreno-Simunovic Y., 2019. Leaf-to-Fruit Ratios in Vitis vinifera L. cv. "Sauvignon Blanc", "Carmenère", "Cabernet Sauvignon", and "Syrah" Growing in Maule Valley (Chile): Influence on Yield and Fruit Composition. *Agri.*, 9, 176.
- Gutiérrez-Gamboa G., Pérez-Donoso A.G., Pou-Mir A., Acevedo-Opazo C., Valdés-Góme H., 2019.
  Hydric behaviour and gas exchange in different grapevine varieties (Vitis vinifera L.) from the Maule Valley (Chile). S. Afr. J. Enol. Vitic., 40, 2.
- Hargreaves J. C., Adl M. S., Warman P.R., 2008. A review of the use of composted municipal solid waste in agriculture. Agric. Ecossyst. Environ. **123**, 1-14.
- Huglin P., 1986. Biologie et écologie de la vigne. Lavoisier, Paris. ISBN 2-60103-019-4.
- IPMA, 2021. Caracterização das regiões vitivinícolas com os índices biotérmicos IW, IH e IF. Available
   at:

https://www.ipma.pt/pt/enciclopedia/agrometeorologia/index.html?page=bio.vitivi.index.xml& print=true (accessed on: 24.01. 2021)

- IPMA, 2021. Clima de Portugal Continental. Available at: <u>https://www.ipma.pt/pt/educativa/tempo.clima/</u> (accessed on: 25.01.2021)
- IVV, 2016. Limites analíticos e limites de emprego de certas substâncias em vinhos, bebidas espirituosas e vinagre de vinho. Available at: <u>https://www.ivv.gov.pt/np4/89/</u> (accessed on: 26.01.2021)

- IVV, 2019. Vinhos e Aguardentes de Portugal. Anuário 2018
- Jackson R. S., 2008. Wine Science: Principles and Applications. 3<sup>rd</sup> edition. Elsevier.
- Kanavouras A., Coutelieris F., Karanika E., Kotseridis Y., Kallithraka S., 2020. Colour change of bottled white wines as a quality indicator. *OENO One*, **54**, 3, 543–551.
- Kramling T. E., Singleton V. L., 1969. An Estimate of the Nonflavonoid Phenols in Wines. Am. J. Enol. Vitic., 20, 86-92.
- Lacey M. J., Allen M. S., Harris R., Brown W. V., 1991. Methoxypyrazines in sauvignon blanc grapes and wines. *Am. J. Enol. Vitic.*, **42**, 2, 103-108
- Liggio A., 2020. 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). 57p. Master's Dissertation in Viticulture and Oenology engineering, Universidade de Lisboa – Instituto Superior de Agronomia
- Lopes C., Melicias J., Aleixo A., Laureano O., Castro R., 2000. Effect of mechanical hedge pruning on growth, yield and quality of Cabernet Sauvignon grapevines. *Acta Hortic.*, **526**, 261–268.
- Lopes P., Silva M.A., Pons A., Tominaga T., Lavigne V., Saucier C., Darriet P., Teissedre P.L. and Dubourdieu D., 2009. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. *J. Agri. Food Chem.*, **57**, 10261-10270.
- Magalhães N., 2015. Tratado de Viticultura A videira, A vinha e o "Terroir". 2ª edição, Esfera Poética. Lisboa.
- Marais J., 1994. Sauvignon blanc Cultivar Aroma A Review. S. Afr. J. Enol. Vitic., 15, 41-45
- Mokrzycki W., Tatol M., 2011. Colour difference Delta E A survey. Mach. Graph. Vis., 20, 383-411
- Mugnai S., Masi E., Azzarello E., Mancuso S., 2012. Influence of Long-Term Application of Green Waste Compost on Soil Characteristics and Growth, Yield and Quality of Grape (Vitisvinifera L.). *Compost Sci. Util.*, 20, 1, 29-33
- Nel A.P., du Toit W.J., van Jaarsveld F.P., 2020. Pinking in White Wines A Review. S. Afr. J. Enol. Vitic., 41, 2, 151-157
- OIV, 2015. Compendium Of International Methods Of Analysis of Wines and Musts. Organisation Internationale de la Vigne et du Vin. Paris.
- OIV, 2017. Distribution of the world's grapevine varieties. Available at: <u>http://www.oiv.int/public/medias/5888/en-distribution-of-the-worlds-grapevine-varieties.pdf</u> (accessed on: 26.01.2021)
- Olejar K. J., Fedrizzi B., Kilmartin P. A., 2015. Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. *Food Chem.*, **183**, 181-189

- Patel P., Herbst-Johnstone M., Lee S. A., Gardener R. C., Weaver R., Nicolau L., Kilmartin P. A., 2010. Influence of Juice Pressing Conditions on Polyphenols, Antioxidants, and Varietal Aroma of Sauvignon blanc Microferments. J. Agric. Food Chem., 58, 12, 7280-7288
- Perucci P., 1990. Effect of the addition of municipal solid-waste compost on microbial biomass and enzyme activities in soil. *Biol. Fertil. Soils*, **10**, 221-226
- Pezzi F., Balducci G., Barca E., Caprara C., 2013. Effects of winter pruning on physical and mechanical properties of grapes. *Acta Hortic.*, **978**, 347-352.
- Pinamonti F., 1998. Compost mulch effects on soil fertility, nutritional status and performance of grapevine. *Nutr. Cycl. Agroecosyst.*, **51**, 3, 239-248.
- Poni S., Bernizzoni F., Presutto P., Rebucci B., 2004. Performance of Croatina under Short-Cane Mechanical Hedging: A Successful Case of Adaptation. *Am. J. Enol. Vitic.* 55. 4. 379-388
- Poni S., Tombesi S., Palliotti A., Ughini V., Gatti M., 2016. Mechanical winter pruning of grapevine: Physiological bases and applications. *Sci. Hortic.* 204. 2. 88-98
- Pordata, 2021. População empregada: total e por grandes sectores de actividade económica. Available
   at:

https://www.pordata.pt/Portugal/Popula%c3%a7%c3%a3o+empregada+total+e+por+grandes+s ectores+de+actividade+econ%c3%b3mica-32-2743 (accessed on: 22.08.2021)

- Pordata, 2021. Produto Interno Bruto na óptica da produção. Available at: <u>https://www.pordata.pt/Portugal/Produto+Interno+Bruto+na+%c3%b3ptica+da+produ%c3%a7%</u> <u>c3%a3o+(base+2016)-2280-179826</u> (accessed on: 22.08.2021)
- Robinson J., Harding J., Vouillamoz J., 2012. Wine Grapes. 3ª edição. Penguin Books. Londres.
- Sadoudi M., Tourdot-Marechal R., Rousseaux S., Steyer D., Gallardo-Chacon J. J., Ballester J., Vichi S., Guerin-Schneider R., Caixach J., Alexandre H., 2012. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-Saccharomyces and Saccharomyces yeasts. *Food Microbiol.*, **32**, 2, 243-253
- Santos J. R., 2012. Fertilização Fundamentos da Utilização dos Adubos e Corretivos. Publicações Europa-América. Mem Martins
- Skoutelas D., Ricardo-da-Silva J. M., Laureano O., 2011. Validation and Comparison of Formol and FT-IR Methods for Assimilable Nitrogen in Vine Grapes. *S. Afr. J. Enol. Vitic.*, **32**, 2, 262-266
- Smith S. R., 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.*, **31**, 1, 142-156.
- Spagna G., Pifferi P. G., Rangoni C., Mattivi F., Nicolini G., Palmonari, R., 1996. The stabilization of white wines by adsorption of phenolic compounds on chitin and chitosan. *Food Res. Int.*, 29, 241-248

- Tangolar S., Tangolar S., Torun, A. A., Ada M., Göçmez S., 2020. Influence of supplementation of vineyard soil with organic substances on nutritional status, yield and quality of 'Black Magic' grape (*Vitis vinifera L.*) and soil microbiological and biochemical characteristics. *OENO One*, 54, 4, 1143–1157.
- Tominaga T., Baltenweck-Guyot R, Des Gachons C. P., Dubourdieu D., 2000. Contribution of volatile thiols to the aromas of white wines made from several Vitis vinifera grape varieties. *Am. J. Enol. Vitic.*, **51**, 2, 178-181
- Tominaga T., Darriet P., Dubourdieu D., 1996. Identification of 3-mercaptohexyl acetate in Sauvignon wine, a powerful aromatic compound exhibiting box-tree odor. *Vitis*, **35**, 4, 207-210
- Tominaga T., des Gachons C.P., Dubourdieu D., 1998. A new type of flavor precursors in Vitis vinifera L cv Sauvignon blanc: S-cysteine conjugates. *J. Agric. Food Chem.*, **46**, 12, 5215-5219
- Van Leeuwen C., Destrac-Irvine A., Dubernet M., Duchêne E., Gowdy M., Marguerit E., Pieri P., Parker A., de Rességuier L., Ollat N., 2019. An Update on the Impact of Climate Change in Viticulture and Potential Adaptations. *Agronomy*, 9, 514.
- Waterhouse A. L., Elias R. J., 2010. Chemical and physical deterioration of wine. In: Chemical Deterioration and Physical Instability of Food and Beverages. 466-482. Elsevier Inc.

### 7. Annexes

	B1			<b>B1</b>			<b>B1</b>			<b>B1</b>			<b>B2</b>			<b>B2</b>			<b>B2</b>			<b>B2</b>	
I	MAN TEST	Г		MAN M1			MAN M2			МАМ МЗ		ſ	VAN TES	Г		MAN M1			MAN M2			MAN M3	
Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т
е	(mg/d m³)	(ºC )	е	(mg/d m³)	(ºC )	е	(mg/d m³)	(ºC )	е	(mg/d m³)	(ºC )	е	(mg/d m³)	(ºC )	е	(mg/d m³)	(ºC )	е	(mg/d m³)	(ºC )	е	(mg/d m <sup>3</sup> )	(ºC )
04/	1088	24	04/	1090	24	04/	1091	24	04/	1093	24	04/	1091	24	04/	1089	24	04/	1090	24	04/	1095	24
set			set			set			set			set			set			set			set		
06/	1085	24	06/	1089	24	06/	1090	24	06/	1092	24	06/	1088	24	06/	1088	24	06/	1089	24	06/	1092	24
set			set			set			set			set			set			set			set		
07/	1061	26	07/	1062	26	07/	1064	26	07/	1069	26	07/	1064	26	07/	1060	26	07/	1064	26	07/	1065	26
set			set			set			set			set			set			set			set		
08/	1038	26	08/	1034	26	08/	1035	26	08/	1047	26	08/	1038	26	08/	1035	26	08/	1040	26	08/	1033	26
set			set			set			set			set			set			set			set		
09/	1018	26	09/	1013	26	09/	1015	26	09/	1028	26	09/	1016	26	09/	1016	26	09/	1024	26	09/	1013	26
set			set			set			set			set			set			set			set		
10/	1002	26	10/	1000	26	10/	1003	26	10/	1024	28	10/	1003	25	10/	1003	25	10/	1010	25	10/	999	25
set			set			set			set			set			set			set			set		
11/	994	25	11/	995	25	11/	996	25	11/	1001	25	11/	995	25	11/	995	25	11/	999	25	11/	994	25
set			set			set			set			set			set			set			set		
12/	993	24	12/	993	23	12/	993	25	12/	994	24	12/	993	24	12/	993	24	12/	993	24	12/	993	24
set			set			set			set			set			set			set			set		
13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24
set			set			set			set			set			set			set			set		
									14/ set	993	24												

#### ANNEX I – FERMENTATION CONTROL OF MUSTS FROM YEAR 2020

	<b>B3</b>			<b>B3</b>			<b>B3</b>			<b>B3</b>			B4			B4			<b>B4</b>			B4	
P	VAN TEST	Г		MAN M1			MAN M2			MAN M3		ſ	VAN TEST	Г		MAN M1			MAN M2			MAN M3	
Dat	Р	Т																					
е	(mg/d m <sup>3</sup> )	(ºC )																					
04/	1093	24	04/	1090	24	04/	1089	24	04/	1091	24	04/	1092	24	04/	1093	24	04/	1088	24	04/	1090	24
06/	1090	24	06/	1089	24	06/	1088	24	06/	1089	24	06/	1090	24	06/	1090	24	06/	1086	24	06/	1089	24
set	1070	26	set	1068	26	set	1063	26	set	1066	26	set	1067	26	set	1064	26	set	1057	26	set	1062	26
set	1070	20	set	1000	20	set	1005	20	set	1000	20	set	1007	20	set	1004	20	set	1057	20	set	1002	20
08/ set	1051	26	08/ set	1047	26	08/ set	1039	26	08/ set	1046	26	08/ set	1042	26	08/ set	1037	26	08/ set	1033	26	08/ set	1040	26
09/ set	1031	26	09/ set	1030	26	09/ set	1018	26	09/ set	1018	26	09/ set	1043	26	09/ set	1015	26	09/ set	1011	26	09/ set	1018	26
10/	1017	25	10/ set	1016	25	10/ set	1004	25	10/ set	1002	25	10/ set	1007	25	10/ set	1002	25	10/	1000	25	10/ set	1005	26
11/	1006	25	11/	1009	23	11/	997	25	11/	995	25	11/	999	25	11/	995	25	11/	994	25	11/	998	25
set			set	0.00		set																	
12/ set	998	24	12/ set	996	24	12/ set	993	24	12/ set	994	24	12/ set	994	24	12/ set	993	24	12/ set	993	24	12/ set	994	24
13/	994	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24	13/	993	24
set			set			set			set			set			set			set			set		
14/	993	24	14/	993	24				14/	993	24	14/	993	24							14/	993	24
15/ set	993	24	Set						Set			Set									Set		

	B1 B1			B1			B1			B2			<b>B2</b>		B2			B2					
I	MEC TEST			MEC M1			MEC M2			MEC M3		I	MEC TEST			MEC M1			MEC M2			MEC M3	
Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т	Dat	Р	Т
е	(mg/d	(ºC	е	(mg/d	(ºC	е	(mg/d	(ºC	е	(mg/d	(ºC	е	(mg/d	(ºC	е	(mg/d	(ºC	е	(mg/d	(ºC	е	(mg/d	(ºC
	m <sup>3</sup> )	)		m <sup>3</sup> )	)		m <sup>3</sup> )	)		m³)	)		m³)	)		m³)	)		m³)	)		m³)	)
04/	1093	24	04/	1095	24	04/	1091	24	04/	1093	24	04/	1094	24	04/	1094	24	04/	1093	24	04/	1094	24
set			set			set			set			set			set			set			set		
06/	1089	24	06/	1091	24	06/	1089	24	06/	1089	24	06/	1092	24	06/	1089	24	06/	1090	24	06/	1091	24
set			set			set			set			set			set			set			set		
07/	1071	26	07/	1070	26	07/	1070	26	07/	1066	26	07/	1078	26	07/	1069	26	07/	1072	26	07/	1068	26
set			set			set			set			set			set			set			set		
08/	1050	26	08/	1046	26	08/	1051	26	08/	1048	26	08/	1060	26	08/	1054	26	08/	1056	26	08/	1046	26
set			set			set			set			set			set			set			set		
09/	1030	26	09/	1021	26	09/	1035	26	09/	1030	26	09/	1047	26	09/	1041	26	09/	1041	26	09/	1029	26
set			set			set			set			set			set			set			set		
10/	1017	25	10/	1009	25	10/	1019	26	10/	1018	26	10/	1035	26	10/	1025	25	10/	1028	26	10/	1019	25
set			set			set			set			set			set			set			set		
11/	1005	25	11/	998	25	11/	1008	25	11/	1005	25	11/	1023	25	11/	1014	25	11/	1015	25	11/	1005	25
set			set			set			set			set			set			set			set		
12/	997	24	12/	993	24	12/	998	24	12/	996	24	12/	1011	25	12/	1003	24	12/	1009	25	12/	996	24
set			set			set			set			set			set			set			set		
13/	994	24	13/	992	24	13/	994	24	13/	993	24	13/	1002	24	13/	998	24	13/	1002	24	13/	993	24
set	002	24	set	002	24	set	002	24	set	002	24	set	007	24	set	004	24	set	007	24	set	002	24
14/	993	24	14/	992	24	14/	993	24	14/	992	24	14/	997	24	14/	994	24	14/	997	24	14/	992	24
15/	002	24	set			15/	002	24	1E/	002	24		004	24		002	24		004	24	1E/	002	24
15/	992	24				15/	992	24	LD/	992	24	LD/	994	24	15/	395	24	LD/	994	24	LD/	992	24
16/	992	2/				16/	992	24	301			16/	003	24	16/	992	2/	16/	003	2/	301		
set	552	24				set	552	24				set	555	24	sot	552	24	set	555	24			
500						500						17/	992	24	17/	992	24	17/	992	24			
												set	552	24	set	552	2-7	set	552	2-7			
												18/	992	24				18/	992	24			
												set						set					

	B3		B3		B3			B3			B4			B4		B4			B4				
ſ	MEC TEST			MEC M1			MEC M2			MEC M3		I	MEC TEST			MEC M1			MEC M2			MEC M3	
Dat	Р	Т																					
е	(mg/d m <sup>3</sup> )	(ºC )																					
04/ set	1096	24	04/ set	1093	24	04/ set	1092	24	04/ set	1090	27	04/ set	1088	24	04/ set	1094	24	04/ set	1094	24	04/ set	1094	24
06/ set	1092	24	06/ set	1091	24	06/ set	1089	24	06/ set	1087	27	06/ set	1086	24	06/ set	1090	24	06/ set	1090	24	06/ set	1091	24
07/ set	1078	26	07/ set	1074	26	07/ set	1070	26	07/ set	1070	26	07/ set	1073	26	07/ set	1073	26	07/ set	1076	26	07/ set	1075	26
08/ set	1065	26	08/ set	1058	26	08/ set	1053	26	08/ set	1055	26	08/ set	1056	26	08/ set	1057	26	08/ set	1060	26	08/ set	1053	26
09/ set	1049	26	09/ set	1046	26	09/ set	1038	26	09/ set	1039	26	09/ set	1046	26	09/ set	1040	26	09/ set	1048	26	09/ set	1039	26
10/ set	1040	25	10/ set	1032	25	10/ set	1025	25	10/ set	1025	25	10/ set	1035	25	10/ set	1030	25	10/ set	1034	25	10/ set	1025	25
11/ set	1028	25	11/ set	1021	25	11/ set	1015	25	11/ set	1013	24	11/ set	1024	25	11/ set	1018	25	11/ set	1021	25	11/ set	1011	25
12/ set	1018	24	12/ set	1009	24	12/ set	1004	24	12/ set	1003	24	12/ set	1012	24	12/ set	1008	24	12/ set	1011	24	12/ set	1002	24
13/ set	1011	24	13/ set	1002	24	13/ set	997	24	13/ set	997	24	13/ set	1005	24	13/ set	1000	24	13/ set	1004	24	13/ set	996	24
14/ set	1002	24	14/ set	997	24	14/ set	994	24	14/ set	994	24	14/ set	999	24	14/ set	996	24	14/ set	998	24	14/ set	993	24
15/ set	998	24	15/ set	994	24	15/ set	993	24	15/ set	993	24	15/ set	995	24	15/ set	993	24	15/ set	994	24	15/ set	992	24
16/ set	995	24	16/ set	993	24	16/ set	992	24	16/ set	992	24	16/ set	994	24	16/ set	992	24	16/ set	993	24	16/ set	992	24
17/ set	993	24	17/ set	992	24	17/ set	992	24	17/ set	992	24	17/ set	993	24	17/ set	992	24	17/ set	992	24			
18/ set	992	24	18/ set	992	24							18/ set	992	24				18/ set	992	24			
19/ set	992	24										19/ set	992	24									

#### ANNEX II - WINE CODING AND TASTING SHEET

Block	Pruning	Reference				
B1	MAN	442				
B1	MEC	367				
B2	MAN	639				
<b>B2</b>	MEC	148				
B3	MAN	251				
B3	MEC	577				
B4	MAN	854				
B4	MEC	378				

Table 25 – Coding used during the panel tasting for the wines of year 2018.

Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.

Block	Pruning	Organic Matter	Reference					
B1	MAN	TEST	507					
B1	MAN	M1	200					
B1	MAN	M2	795					
B1	MAN	M3	714					
B1	MEC	TEST	336					
B1	MEC	M1	902					
B1	MEC	M2	935					
B1	MEC	M3	383					
B2	MAN	TEST	781					
B2	MAN	M1	899					
B2	MAN	M2	536					
B2	MAN	M3	642					
B2	MEC	TEST	923					
B2	MEC	M1	234					
<b>B2</b>	MEC	M2	514					
B2	MEC	M3	446					
B3	MAN	TEST	407					
B3	MAN	M1	727					
B3	MAN	M2	117					
B3	MAN	M3	839					
B3	MEC	TEST	184					
B3	MEC	M1	800					
B3	MEC	M2	608					
B3	MEC	M3	171					
B4	MAN	TEST	842					
B4	MAN	M1	668					
B4	MAN	M2	629					
B4	MAN	M3	855					
B4	MEC	TEST	772					
B4	MEC	M1	627					
B4	MEC	M2	336					
B4	MEC	M3	649					

#### Table 26 – Coding used during the panel tasting for the wines of year 2019

Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC

Block	Pruning	Organic Matter	Reference
B1	MAN	TEST	866
B1	MAN	M1	409
B1	MAN	M2	784
B1	MAN	M3	281
B1	MEC	TEST	748
B1	MEC	M1	580
B1	MEC	M2	823
B1	MEC	M3	481
B2	MAN	TEST	586
B2	MAN	M1	511
B2	MAN	M2	590
B2	MAN	M2	591
B2	MAN	M3	522
B2	MEC	TEST	807
B2	MEC	M1	650
<b>B2</b>	MEC	M2	209
B2	MEC	M3	975
B3	MAN	TEST	327
B3	MAN	M1	695
B3	MAN	M2	586
B3	MAN	M3	649
B3	MEC	TEST	164
B3	MEC	M1	851
B3	MEC	M2	236
B3	MEC	M3	183
B4	MAN	TEST	526
B4	MAN	M1	381
B4	MAN	M2	966
B4	MAN	M3	904
B4	MEC	TEST	771
B4	MEC	M1	827
B4	MEC	M2	401
B4	MEC	M3	174

#### Table 27 – Coding used during the panel tasting for the wines of year 2020

Pruning System: MAN – manual pruning; MEC – mechanical pruning. Municipal Solid Waste Compost (MSWC) modality: Test – No application of MSWC, M1 – 5000kg/ha MSWC; M2 – 10000 kg/ha; M3 – 20000 kg/ha MSWC.
LISB	UNING Scrupo Operacional IntenSusVITI   Intensificação Sustentável da Vitivinicultura Aravés da Poda Mecânica   Image: Comparison of the Prova de Vinho Brancos Instituto Superior de Agronomia   Ficha de Prova de Vinho Brancos Projeto IntenSusVITI (PDR 2020-101-032001)																	
Nom Prove os	e: vinhos na ordem aj	presen	ıtada <del>(</del>	e classif	ique os	diferen	tes atrib	utos uti	lizando	as segui	Data ntes esc	/ alas:	/		Sessi	ăo:		
Para Cor, Aroma e Gosto:1. Inexistente2. Pouco Intenso(a)3. Medianamente Intenso(a)4. Intenso(a)5. Muito Intenso(a)																		
Para Eq	uilíbrio (Aroma e G	osto) e	e Apre	ciação	Global:		1. Medí	ocre	2.	Satisfató	ório	<b>3.</b> Be	om	4. Mu	ito Bom		5. Excel	ente
		Г							V			200						
									V1	NHOS /	CODI	305						
-	AMARELO		<u>. · . · . · . · . · . · . ·</u>			. <mark>*.*.*.*.*.*.*</mark>	<u></u>		• <u>•</u> •••••••••••••••••						. <mark>*.*.*.*.*.*.*.</mark>	• • • • • • • • • • • • • • •		
CÖR	VERDE																+	
	INTENSIDADE																	
	FRUTADO																	
	FLORAL																	
AROMA	Toranja																	
AKOMA	Maracujá																	
	CHICHI DE GATO																	
	VEGETAL																	
	Equilíbrio																	
	INTENSIDADE																	
	ACIDEZ																	
GOSTO	VOLUME																<u> </u>	
	Persistência																<u> </u>	
	EQUILÍBRIO																	
APRECIA	ÇÃO GLOBAL																	

Observações:

Figure 13 – Tasting Sheet used in the wine tasting

#### ANNEX III – FREE AND TOTAL SULPHUR DIOXIDE OF 2020 WINES, MEASURED BEFORE BOTTLING

Block	Pruning	MSWC	Free SO2 (mg SO2/L)	Total SO2 (mg SO2/L)
1	MAN	TEST	29	100
1	MAN	M1	33	104
1	MAN	M2	29	105
1	MAN	M3	20	90
2	MAN	TEST	26	95
2	MAN	M1	30	98
2	MAN	M2	26	98
2	MAN	M3	34	113
3	MAN	TEST	22	103
3	MAN	M1	25	93
3	MAN	M2	24	108
3	MAN	M3	33	123
4	MAN	TEST	27	141
4	MAN	M1	37	133
4	MAN	M2	30	88
4	MAN	M3	26	128
1	MEC	TEST	28	148
1	MEC	M1	31	108
1	MEC	M2	22	125
1	MEC	M3	20	110
2	MEC	TEST	16	115
2	MEC	M1	20	114
2	MEC	M2	17	125
2	MEC	M3	23	123
3	MEC	TEST	13	123
3	MEC	M1	13	88
3	MEC	M2	15	109
3	MEC	M3	17	95
4	MEC	TEST	14	70
4	MEC	M1	14	113
4	MEC	M2	14	93
4	MEC	M3	23	100

Table 28 – Free and total sulphur dioxide of 2020 wines, measured before bottling.

### ANNEX IV – COMPLETE RESULTS OF LABORATORIAL ANALYSES OF PHISICAL-CHEMICAL PARAMETERS AND CHROMATIC PARAMETERS OF 2018 WINES

Block	Pruning	ABV (% vol.)	TA (g tartaric acid/l)	VA (g Acetic acid/ I)	рН	RS (g invert sugar /l)
1	MAN	12,6	8,7	0,35	3,17	0,3
2	MAN	13.0	9,0	0,40	3,14	0,4
3	MAN	13,2	8,7	0,33	3,15	0,3
4	MAN	12,8	8,9	0,32	3,16	0,5
1	MEC	13,5	8,3	0,50	3,14	0,4
2	MEC	13,4	8,4	0,54	3,15	0,5
3	MEC	13,5	8,7	0,49	3,16	0,3
4	MEC	13,7	8,3	0,44	3,13	0,4

# Table 29 – Results of Physicochemical analyses of the wines from 2018 (adapted from Liggio,2020)

Pruning System: MAN –manual pruning, MEC – Mechanical pruning; ABV – Alcohol by Volume; TA – Total Acidity; VA – Volatile Acidity; RS – Reducing Substances.

# Table 30 – Colour intensity, expressed in absorvance at 420nm, of wines form year 2018 (adapted from Liggio, 2020)

Block	Pruning	ABS 420nm
1	MAN	0,068
2	MAN	0,086
3	MAN	0,092
4	MAN	0,090
1	MEC	0,090
2	MEC	0,084
3	MEC	0,085
4	MEC	0,083

Pruning System: MAN – manual pruning, MEC – Mechanical pruning

Block	Pruning	Total Phenols (mg Gallic acid/l)	Non Flavonoids(mg Gallic acid/l)	Flavonoids (mg Gallic acid/l)
1	MAN	165,9	86,6	79,4
2	MAN	162,5	88,5	73,9
3	MAN	179,3	90,1	89,2
4	MAN	157,4	83,3	74,1
1	MEC	178.0	87,2	90,7
2	MEC	177,6	94,7	89,9
3	MEC	166,5	87,2	79,2
4	MEC	170,3	89,3	81

Table 31 – Phenolic Composition of wines from 2018 (adapted from Liggio, 2020)

Pruning system: MAN – manual pruning; MEC – mechanical pruning

#### Table 32 – CIElab method results for 2018 wines (adapted from Liggio, 2020)

Block	Pruning	L*	a*	b*	С*	Н*
1	MAN	99,3	-0,43	3,05	3,08	0,12
2	MAN	99,1	-0,40	2,79	2,82	0,13
3	MAN	99,2	-0,41	3,17	3,19	0,13
4	MAN	99,2	-0,47	2,96	2,99	0,11
1	MEC	99,2	-0,30	3,12	3,13	0,18
2	MEC	99,3	-0,23	2,62	2,63	0,20
3	MEC	99,3	-0,28	2,66	2,67	0,17
4	MEC	99,3	-0,28	2,62	2,63	0,20

Pruning System: MAN – manual pruning; MEC – mechanical pruning; L\* - luminosity; a\* - red-green colour' contribution; b\* - yellow-blue colour' contribution; C\* - Chroma; H\* - Hue.

### Table 33 – Chromatic Difference between mechanical pruning and manual pruning of 2018 wines (adapted from Liggio, 2020).

Samples	ΔΕ*
MAN B1 VS MEC B1	0,20
MAN B2 VS MEC B2	0,27
MAN B3 VS MEC B3	0,53
MAN B4 VS MEC B4	0,41

MAN – manual pruning; MEC – mechanical pruning;  $\Delta E^*$  - Chromatic Difference

### ANNEX V – COMPLETE RESULTS OF LABORATORIAL ANALYSES OF PHISICAL-CHEMICAL PARAMETERS AND CHROMATIC PARAMETERS OF 2019 WINES

Block	Pruning	MSWC	VA (g acetic	рН	ABV (%	TA (g tartaric	RS (g invert
			acid/L)		voi.)	acid /L)	sugar /I)
1	MAN	M1	0,34	3,36	14,6	8,3	1,7
1	MAN	M2	0,41	3,33	14,5	8,7	1,8
1	MAN	M3	0,33	3,31	14,4	9,3	1,7
1	MAN	TEST	0,35	3,20	14,1	9,3	2,0
1	MEC	M1	0,27	3,21	15,1	8,6	0,6
1	MEC	M2	0,28	3,20	14,6	8,7	0,9
1	MEC	M3	0,32	3,33	14,6	8,4	1,1
1	MEC	TEST	0,20	3,25	14,1	8,7	0,3
2	MAN	M2	0,29	3,23	13,7	9,2	1,5
2	MAN	M3	0,37	3,21	14,5	9.0	0,8
2	MAN	M1	0,33	3,29	14,3	8,9	0,7
2	MAN	TEST	0,11	3,26	14,8	8.0	0,4
2	MEC	M1	0,38	3,30	14,8	8,4	0,7
2	MEC	TEST	0,37	3,25	14,5	8,4	0,4
2	MEC	M2	0,34	3,25	14,9	8,4	1,6
2	MEC	M3	0,30	3,34	15,1	8,3	0,7
3	MAN	M1	0,28	3,26	14,8	8,9	0,7
3	MAN	M2	0,40	3,30	14,3	9,3	0,4
3	MAN	M3	0,56	3,27	14,3	8,9	0,6
3	MAN	TEST	0,40	3,28	14,8	8,4	0,7
3	MEC	M1	0,34	3,31	15,7	6,3	0,8
3	MEC	M2	0,35	3,26	14,6	9,3	0,8
3	MEC	M3	0,33	3,27	14,4	8,7	0,5
3	MEC	TEST	0,31	3,23	14,9	8,9	0,5
4	MAN	M1	0,27	3,24	14,2	9,5	0,4
4	MAN	M2	0,38	3,28	14,6	9,2	0,6
4	MAN	M3	0,40	3,30	14,6	10,0	0,4
4	MAN	TEST	0,38	3,30	14,0	8,7	0,5
4	MEC	M1	0,35	3,27	14,4	8,6	0,4
4	MEC	M2	0,41	3,26	14,9	9,0	0,7
4	MEC	M3	0,41	3,29	14,4	8,7	0,4
4	MEC	TEST	0,62	3,11	14,0	9,6	0,4

Table 34 – Results of Physicochemical analyses of the wines from 2019 (adapted from Liggio, 2020).

Pruning system: MAN –manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): TEST – No MSWC application, M1 –5000kg/ha MSWC, M2 – application of 10000kg/ha MSWC, M3 –20000kg/ha MSWC; VA – Volatile Acidity; ABV – Alcohol by Volume; TA – Total Acidity; RS –Reducing Substances.

Block	Pruning	MSWC	ABS 420nm
B1	MAN	TEST	0,101
B1	MAN	M1	0,094
B1	MAN	M2	0,096
B1	MAN	M3	0,113
B2	MAN	TEST	0,100
B2	MAN	M1	0,099
B2	MAN	M2	0,098
B2	MAN	M3	0,111
B3	MAN	TEST	0,084
B3	MAN	M1	0,105
B3	MAN	M2	0,099
B3	MAN	M3	0,088
B4	MAN	TEST	0,106
B4	MAN	M1	0,088
B4	MAN	M2	0,105
B4	MAN	M3	0,113
B1	MEC	TEST	0,084
B1	MEC	M1	0,097
B1	MEC	M2	0,093
B1	MEC	M3	0,098
B2	MEC	TEST	0,087
B2	MEC	M1	0,100
B2	MEC	M2	0,094
B2	MEC	M3	0,097
B3	MEC	TEST	0,094
B3	MEC	M1	0,095
B3	MEC	M2	0,092
B3	MEC	M3	0,092
B4	MEC	TEST	0,092
B4	MEC	M1	0,089
B4	MEC	M2	0,090
B4	MEC	M3	0,091

Table 35 – Colour intensity, expressed in absorvance at 420nm, of the wines of year 2019 (adapted from Liggio, 2020).

			Total Phenols (mg	Non Flavonoids (mg	Flavonoids (mg
BIOCK	Pruning	WSWC	Gallic acid/l)	Gallic acid/l)	Gallic acid/l)
1	MAN	TEST	168,3	61,4	106,9
1	MAN	M1	168,8	68,8	100,0
1	MAN	M2	185,1	67,2	117,9
1	MAN	M3	164,0	61,4	102,6
2	MAN	TEST	186,4	63,9	112,6
2	MAN	M1	175,1	60,5	114,6
2	MAN	M2	189,6	69,5	120,1
2	MAN	M3	199,0	72,5	126,5
3	MAN	TEST	189,9	67,3	122,5
3	MAN	M1	188,5	64,9	123,6
3	MAN	M2	177,1	64,9	112,2
3	MAN	M3	182,1	64,3	117,7
4	MAN	TEST	185,1	68,5	116,6
4	MAN	M1	167,5	58,9	108,5
4	MAN	M2	179,1	66,5	112,6
4	MAN	M3	179,1	67,2	111,8
1	MEC	TEST	224,6	66,6	158,0
1	MEC	M1	182,0	70,2	111,7
1	MEC	M2	211,4	55,6	155,8
1	MEC	M3	188,8	62,9	125,9
2	MEC	TEST	173,3	63,5	109,8
2	MEC	M1	181,9	61,9	120,0
2	MEC	M2	188,5	67,8	120,8
2	MEC	M3	202,2	73,7	128,5
3	MEC	TEST	188,5	65,6	122,9
3	MEC	M1	194,0	67,8	126,2
3	MEC	M2	184,3	85,9	98,4
3	MEC	M3	174,9	64,9	109,9
4	MEC	TEST	170,9	62,7	108,2
4	MEC	M1	181,9	69,5	112,4
4	MEC	M2	182,2	68,5	113,7
4	MEC	M3	188,5	70,7	117,7

Table 36 – Phenolic Composition of wines from year 2019 (adapted from Liggio, 2020).

Block	Pruning	MSWC	L*	a*	b*	С*	H*
1	MAN	TEST	98,8	-0,49	3,41	3,45	0,12
1	MAN	M1	98,4	-0,21	3,67	3,67	0,31
1	MAN	M2	98,7	-0,31	3,44	3,44	0,19
1	MAN	M3	98,8	-0,39	3,28	3,43	0,15
2	MAN	TEST	97,6	-0,59	2,70	2,76	0,08
2	MAN	M1	98,9	-0,36	3,58	3,59	0,17
2	MAN	M2	98,9	-0,38	3,47	3,49	0,16
2	MAN	M3	98,2	-0,20	4,00	4,00	0,36
3	MAN	TEST	99,0	-0,25	3,16	3,16	0,22
3	MAN	M1	98,3	-0,20	3,92	3,92	0,35
3	MAN	M2	98,8	-0,27	3,52	3,52	0,23
3	MAN	M3	98,7	-0,29	3,42	3,43	0,21
4	MAN	TEST	98,5	-0,41	4,16	4,20	0,18
4	MAN	M1	99,0	-0,29	3,16	3,17	0,19
4	MAN	M2	98,6	-0,52	4,00	4,03	0,13
4	MAN	M3	98,1	-0,35	4,10	4,11	0,21
1	MEC	TEST	99,2	-0,19	2,81	2,83	0,26
1	MEC	M1	99,1	-0,26	3,40	3,40	0,23
1	MEC	M2	99,1	-0,2	3,15	3,18	0,28
1	MEC	M3	98,9	-0,32	3,63	3,73	0,2
2	MEC	TEST	98,7	-0,24	3,63	3,63	0,27
2	MEC	M1	98,7	-0,17	3,34	3,35	0,35
2	MEC	M2	98,8	-0,26	3,46	3,46	0,23
2	MEC	M3	98,5	-0,37	3,76	3,77	0,18
3	MEC	TEST	98,8	-0,24	3,69	3,69	0,27
3	MEC	M1	98,8	-0,26	3,39	3,39	0,23
3	MEC	M2	99,0	-0,25	3,44	3,44	0,24
3	MEC	M3	99,0	-0,33	3,33	3,34	0,17
4	MEC	TEST	98,7	-0,23	3,75	3,74	0,29
4	MEC	M1	98,4	-0,26	3,40	3,40	0,23
4	MEC	M2	98,7	-0,26	3,47	3,47	0,24
4	MEC	M3	98,8	-0,27	3,26	3,28	0,21

Table 37 – CIElab coordinates for 2019 wines (adapted from Liggio, 2020).

Pruning System: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): TEST – No MSWC application, M1 –5000kg/ha MSWC, M2 – application of 10000kg/ha MSWC, M3 –20000kg/ha MSWC; L\* - luminosity; a\* - red-green colour' contribution; b\* - yellow-blue colour' contribution; C\* - Chroma; H\* - Hue.

Table 38 – Chromatic differences verified in 2019 wines, between different pruning systems but from the same block and Municipal Solid Waste Compost modality (adapted from Liggio, 2020).

Block	MSWC	Pruning	ΔΕ
B1	Test	MAN/MEC	0,72
B1	M1	MAN/MEC	0,42
B1	M2	MAN/MEC	0,71
B1	M3	MAN/MEC	0,38
B2	Test	MAN/MEC	1,48
B2	M1	MAN/MEC	0,36
B2	M2	MAN/MEC	0,13
B2	M3	MAN/MEC	0,41
B3	Test	MAN/MEC	0,56
B3	M1	MAN/MEC	0,74
B3	M2	MAN/MEC	0,2
B3	M3	MAN/MEC	0,33
B4	Test	MAN/MEC	0,52
B4	M1	MAN/MEC	0,60
B4	M2	MAN/MEC	0,59
B4	M3	MAN/MEC	1,05

# ANNEX VI – GLOBAL RESULTS OF PHYSICHOCHEMICAL AND CHROMATIC PARAMETERS OF 2020 WINES

Block	Pruning	MSWC	VA (g acetic acid/L)	TA (g tartaric acid /L)	рН	ABV (% vol.)	RS (g invert sugar /I)
1	MAN	TEST	0,39	12,8	2,96	12,2	0,4
1	MAN	M1	0,55	8,3	3,07	13,0	0,4
1	MAN	M2	0,40	9,0	3,06	13,4	0,3
1	MAN	M3	0,56	8,4	3,03	13,6	0,2
2	MAN	TEST	0,53	7,5	2,99	13,2	1,3
2	MAN	M1	0,53	9,0	3,05	12,8	0,7
2	MAN	M2	0,51	8,7	3,01	13,2	1,1
2	MAN	M3	0,54	8,7	3,08	13,6	0,6
3	MAN	TEST	0,06	10,1	3,00	13,4	0,9
3	MAN	M1	0,48	8,6	2,98	13,0	0,6
3	MAN	M2	0,49	9,6	3,02	12,8	0,5
3	MAN	M3	0,46	9,3	3,00	12,8	0,7
4	MAN	TEST	0,56	8,0	3,10	13,6	1,1
4	MAN	M1	0,62	8,3	3,13	13,4	1,0
4	MAN	M2	0,53	8,9	3,13	12,4	1,2
4	MAN	M3	0,55	8,6	3,05	13,4	1,0
1	MEC	TEST	0,49	8,1	3,06	13,6	0,4
1	MEC	M1	0,40	8,4	3,10	13,9	0,3
1	MEC	M2	0,53	8,6	3,04	13,2	0,8
1	MEC	M3	0,39	8,6	3,04	13,4	0,9
2	MEC	TEST	0,63	8,3	3,03	13,6	0,9
2	MEC	M1	0,63	8,0	3,07	13,6	0,7
2	MEC	M2	0,57	8,7	3,07	13,6	0,7
2	MEC	M3	0,62	8,1	3,07	13,6	1,1
3	MEC	TEST	0,54	7,7	3,03	14,0	3,0
3	MEC	M1	0,50	8,4	3,01	13,6	0,5
3	MEC	M2	0,46	8,1	3,03	13,2	0,5
3	MEC	M3	0,54	7,7	3,09	13,0	0,7
4	MEC	TEST	0,51	8,7	3,01	13,0	0,8
4	MEC	M1	0,55	8,0	3,06	13,4	0,9
4	MEC	M2	0,61	8,6	3,06	13,6	1,2
4	MEC	M3	0,56	8,1	3,10	14,0	1,1

Table 39 – Results of the physicochemical analyses of 2020 wines

Pruning system: MAN –manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): TEST – No MSWC application, M1 –5000kg/ha MSWC, M2 – application of 10000kg/ha MSWC, M3 –20000kg/ha MSWC; VA – Volatile Acidity; TA – Total Acidity; ABV – Alcohol by Volume; RS –Reducing Substances.

Block	Pruning	MSWC	Abs. 420 nm
B1	MAN	TEST	0,059
B1	MAN	M1	0,068
B1	MAN	M2	0,065
B1	MAN	M3	0,062
B2	MAN	TEST	0,071
B2	MAN	M1	0,062
B2	MAN	M2	0,061
B2	MAN	M3	0,057
<b>B3</b>	MAN	TEST	0,066
B3	MAN	M1	0,059
<b>B3</b>	MAN	M2	0,059
<b>B3</b>	MAN	M3	0,061
<b>B4</b>	MAN	TEST	0,064
B4	MAN	M1	0,060
<b>B4</b>	MAN	M2	0,062
B4	MAN	M3	0,068
B1	MEC	TEST	0,059
B1	MEC	M1	0,069
B1	MEC	M2	0,053
B1	MEC	M3	0,061
<b>B2</b>	MEC	TEST	0,055
B2	MEC	M1	0,060
B2	MEC	M2	0,054
B2	MEC	M3	0,067
B3	MEC	TEST	0,064
B3	MEC	M1	0,065
B3	MEC	M2	0,062
B3	MEC	M3	0,068
B4	MEC	TEST	0,066
B4	MEC	M1	0,065
B4	MEC	M2	0,071
<b>B4</b>	MEC	M3	0,075

Table 40 – Colour intensity, expressed in absorvance at 420 nm, of the wines from 2020

Diash		DACIN/C	Total Phenols (mg	Non Flavonoids (mg	Flavonoids (mg
ыоск Ргиліпд		IVISVVC	Gallic acid/l)	Gallic acid/l)	Gallic acid/l)
B1	MAN	TEST	166,0	71,3	94,7
B1	MAN	M1	186,8	72,4	114,4
B1	MAN	M2	166,9	61,6	105,2
B1	MAN	M3	179,2	65,9	113,3
B2	MAN	TEST	177,7	68,9	108,8
B2	MAN	M1	170,6	66,5	104,1
B2	MAN	M2	174,8	68,2	106,6
B2	MAN	M3	173,5	66,4	107,1
B3	MAN	TEST	190,6	70,8	119,8
B3	MAN	M1	171,2	66,8	104,5
B3	MAN	M2	175,3	67,8	107,4
B3	MAN	M3	177,1	68,9	108,2
B4	MAN	TEST	184,5	69,0	115,5
B4	MAN	M1	184,5	70,1	114,4
B4	MAN	M2	161,9	64,9	97,1
B4	MAN	M3	182,4	75,8	106,6
B1	MEC	TEST	177,1	68,9	108,1
B1	MEC	M1	181,8	70,8	111,0
B1	MEC	M2	180,4	67,8	112,7
B1	MEC	M3	186,0	71,9	114,0
<b>B2</b>	MEC	TEST	179,1	64,9	114,3
B2	MEC	M1	174,3	66,9	107,3
B2	MEC	M2	169,7	64,9	104,8
B2	MEC	M3	185,1	73,0	112,1
<b>B3</b>	MEC	TEST	180,7	69,7	111,0
B3	MEC	M1	184,4	69,3	115,0
B3	MEC	M2	175,8	67,1	108,7
B3	MEC	M3	167,8	64,9	103,0
B4	MEC	TEST	166,8	68,8	98,0
B4	MEC	M1	171,6	61,9	109,7
B4	MEC	M2	172,6	65,3	107,3
B4	MEC	M3	189,2	73,1	116,1

#### Table 41 – Phenolic Composition of 2020 wines

Block	Pruning	MSWC	L*	a*	b*	С*	Н*
B1	MAN	TEST	99,9	-0,15	4,80	4,80	88,21
B1	MAN	M1	99,9	-0,24	5,33	5,33	87,46
B1	MAN	M2	99,8	-0,18	5,00	5,01	87,98
B1	MAN	M3	99,6	0,14	4,77	4,77	88,28
B2	MAN	TEST	99,0	-0,13	5,35	5,36	88,60
B2	MAN	M1	99,9	-0,34	4,89	4,90	86,06
B2	MAN	M2	100,0	-0,17	4,72	4,73	87,88
B2	MAN	M3	99,7	0,04	4,50	4,50	89,51
B3	MAN	TEST	99,8	-0,09	5,61	5,61	89,06
B3	MAN	M1	99,4	0,02	4,65	4,65	89,71
B3	MAN	M2	99,9	-0,08	4,66	4,66	89,08
B3	MAN	M3	99,5	-0,10	5,21	5,21	88,93
B4	MAN	TEST	99,9	-0,01	4,85	4,85	89,88
B4	MAN	M1	99,7	-0,19	4,75	4,75	87,67
B4	MAN	M2	99,8	-0,28	4,79	4,80	86,64
B4	MAN	M3	98,6	-0,17	4,90	4,91	88,02
B1	MEC	TEST	98,8	-0,09	4,39	4,40	88,84
B1	MEC	M1	98,9	-0,30	4,84	4,85	86,50
B1	MEC	M2	99,0	-0,13	3,99	3,99	88,18
B1	MEC	M3	99,0	-0,26	4,27	4,27	86,45
<b>B2</b>	MEC	TEST	99,1	-0,07	3,91	3,91	89,00
B2	MEC	M1	99,0	-0,27	4,22	4,23	86,29
<b>B2</b>	MEC	M2	99,3	-0,15	3,90	3,91	87,76
B2	MEC	M3	99,0	-0,26	4,61	4,62	86,80
B3	MEC	TEST	99,0	0,07	4,14	4,14	88,97
B3	MEC	M1	99,1	-0,11	4,18	4,19	88,43
B3	MEC	M2	99,1	-0,14	4,22	4,22	88,14
B3	MEC	M3	99,1	-0,14	4,42	4,42	88,16
B4	MEC	TEST	98,8	0,00	4,24	4,24	89,93
B4	MEC	M1	98,8	0,14	4,40	4,41	88,23
B4	MEC	M2	98,9	-0,02	4,48	4,48	89,78
B4	MEC	M3	98,8	-0,24	4,59	4,59	86,95

Table 42 – CIElab coordinates for 2020 wines

Pruning System: MAN – manual pruning, MEC – mechanical pruning; Municipal Solid Waste Compost (MSWC): TEST – No MSWC application, M1 –5000kg/ha MSWC, M2 – application of 10000kg/ha MSWC, M3 –20000kg/ha MSWC; L\* - luminosity; a\* - red-green colour' contribution; b\* - yellow-blue colour' contribution; C\* - Chroma; H\* - Hue.

Table 43 – Chromatic differences verified in 2020 wines, between different pruning systems but from the same block and Municipal Solid Waste Compost modality.

BLOCK	MSWC	Pruning	<b>ΔE</b> *
B1	Test	MAN/MEC	1,31
B1	M1	MAN/MEC	1,49
B1	M2	MAN/MEC	1,32
B1	M3	MAN/MEC	2,00
B2	Test	MAN/MEC	1,51
B2	M1	MAN/MEC	1,12
B2	M2	MAN/MEC	1,11
B2	M3	MAN/MEC	2,80
B3	Test	MAN/MEC	1,69
B3	M1	MAN/MEC	1,39
B3	M2	MAN/MEC	1,32
B3	M3	MAN/MEC	1,15
B4	Test	MAN/MEC	1,20
B4	M1	MAN/MEC	1,07
B4	M2	MAN/MEC	3,28
B4	M3	MAN/MEC	1.13

Block and pruning	MSWC	ΔΕ*
	TEST/M1	0.92
	TEST/M2	0,32
B1 MAN	TEST/M3	0.35
	M1/M2	0,62
	M1/M3	1,04
	M2/M3	0,45
	TEST/M1	2.74
	TEST/M2	1.40
Β2 ΜΔΝ	TEST/M3	1.48
DE NUM	M1/M2	1.83
	M1/M3	3.47
	M2/M3	1.66
	TFST/M1	1 26
	TEST/M2	0.96
	TEST/M3	0.55
DS WAN	M1/M2	0,55
	N11/N12	0,87
	N12/N13	0,37
		2.22
		2,25
		3,25
B4 MAN		2,25
		1,04
		1,15
		1,83
	IESI/M1	2,39
	TEST/M2	0,79
B1 MEC	TEST/M3	2,40
	M1/M2	1,90
	M1/M3	0,59
	M2/M3	1,76
	TEST/M1	2,74
	TEST/M2	1,25
B2 MEC	TEST/M3	2,31
	M1/M2	1,53
	M1/M3	0,65
	M2/M3	1,22
	TEST/M1	0,54
	TEST/M2	0,84
B3 MEC	TEST/M3	0,87
	M1/M2	0,30
	M1/M3	0,37
	M2/M3	0,20
	TEST/M1	1,71
	TEST/M2	0,31
B4 MEC	TEST/M3	3,01
	M1/M2	1,55
	M1/M3	1,30
	M2/M3	2.84

Table 44 - Chromatic differences verified in 2020 wines, between different Municipal Solid Waste Compost modalities, but from the same block and same pruning systems.