Effect of low and high cordon training system and organic amendment with municipal solid waste on the chemical composition of Marselan grapes and wines in Qª da Aroeira, Lisbon wine region

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Abstract

In this study the experimental vineyards are located in Quinta do Aroeira (Lisboa, Portugal) and a randomized complete block design was established. Samples derived from two different training systems “Low Cordon” (LC) and “High Cordon” (HC) and four different amounts of municipal solid organic waste were analyzed in terms of yield parameters, grapes and must qualitative characteristics and basic analytical parameters, phenolic composition, and chromatic characteristics of the wines.

The results showed that samples treated with mechanical pruning on LC training system had lower alcohol content and pH values. Furthermore, it negatively influenced the non-flavonoids content, while promoted the interaction between anthocyanins and flavonols. The color was decreased in blue tonalities and this was obvious by human eyes.

The organic treatment with the most significant effect in wine quality was M3 (highest supply of municipal solid waste), The treatment with M3 resulted in the decrease of alcohol and color intensity of the wines.

Keywords: Wine, Marselan, training system, municipal solid organic waste, phenolic composition, chromatic characteristics.

Resumo

O ensaio deste estudo foi delineado em blocos casualisados na Quinta da Aroeeira (Lisboa, Portugal). Foram analisadas amostras dos dois sistemas de formação "Cordão Baixo" (LC) e "Cordão Alto" (HC) e das quatro doses diferentes de resíduos sólidos urbanos compostados em termos de rendimento, qualidade das uvas e dos mostos, parâmetros analíticos básicos, composição fenólica e características cromáticas dos vinhos.

Os resultados mostraram que a poda mecânica aliada ao sistema de formação LC origina uvas e vinhos com teores alcoólicos e valores de pH mais baixos. Além disso influenciou negativamente o teor de fenois não-flavonóides, tal como promoveu a interacção entre as antocianinas e os flavonóis. No que respeita às tonalidades azuis, a cor foi diminuída, algo que era notório visualmente.

A dose mais elevada (M3) do tratamento orgânico obteve o efeito mais significativo na qualidade do vinho. O tratamento com M3 resultou na diminuição do teor alcoólico e das características cromáticas dos vinhos.

Palavras-chave: Vinho, Marselan, sistemas de condução, resíduos sólidos urbanos compostados, composição fenólica, características cromáticas.

Resumo Alargado

O ensaio deste estudo foi instalado na Quinta da Aroeeira (Lisboa, Portugal). Na vinha, com 2,6 metros de distância entre linhas e 1 metro de distância entre plantas, foram casualisados 3 blocos, de modo a permitir padronizar o efeito dos blocos não tratados. Cada bloco foi dividido em 4 secções, cada uma composta por 14 plantas (num total de 56 plantas por linha e 168 plantas por bloco). Em cada bloco foram distribuídos dois diferentes sistemas de condução: "Cordão Alto" e "Cordão Baixo", ambos podados mecanicamente, e diferentes doses de resíduos sólidos orgânicos compostados (MSW). As correcções orgânicas usadas (MSW) foram aplicadas em 4 diferentes concentrações: 0, 5000, 10000, 20000 Kg/ ha, correspondendo respetivamente a "testemunha", "M1", "M2", "M3".

As uvas foram colhidas manualmente a 15 de Setembro de 2020 e devido a um imprevisto, não foi possível obter uvas do terceiro bloco. Neste estudo apenas os dois primeiros blocos foram analisados, tendo-se obtido 16 vinhos diferentes. As amostras provenientes dos dois sistemas de condução "Cordão Baixo" (LC) e "Cordão Alto" (HC) e das diferentes correções orgânicas, foram analisadas em termos de parâmetros de produção, de características qualitativas das uvas e dos mostos, de parâmetros analíticos básicos, da composição fenólica e das características cromáticas dos vinhos.

Os resultados obtidos na análise laboratorial foram submetidos a análise estatística (ANOVA e teste de significância Tuckey) com o programa Rstudio.

Os resultados mostraram que as amostras tratadas com poda mecânica no sistema de condução LC obtiveram menor teor alcoólico e de pH, influenciaram negativamente o conteúdo em fenóis não-flavonóides e promoveu a interacção entre as antocianinas e os flavonóis, aumentando o poder tanante, a co-pigmentaçao e a polimerização entre antocianinas e flavonóis.

Em termos de características cromáticas, o sistema de condução LC afectou negativamente as tonalidades azuis do vinho, apresentando um aumento dos valores de b e H, o que foi notório visualmente.

O tratamento orgânico com efeito mais significativo na qualidade do vinho foi o M3 (maior fornecimento de resíduos sólidos urbanos compostados). Este tratamento resultou num decréscimo de teor alcoólico e das características cromáticas dos vinhos. Em particular estes obtiveram a menor quantidade de antocianinas, afectando a intensidade da cor, a quantidade de antocianinas ionizadas, o grau de polimerização das antocianinas, os pigmentos totais, o índice de polimerização, os pigmentos polimerizados e a co-pigmentação.

De acordo com os resultados do método CIELab, os vinhos provenientes da correção M3 mostraram um decréscimo na intensidade geral, demonstrado pelo valor de C e na tonalidade da cor com baixos valores de vermelhos (a*) e azuis (b*; H).

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1 Introduction and objectives

Agriculture is one of the most ancient and important activity in all the world, which is always developing, meeting human development goals without compromising the ability of natural systems to provide natural resources and ecosystem service upon which the economy and society depend.
Nowadays it is used to talk about “sustainability” which follow the principle of integration of environmental, social, and economic concerns into all aspects of decision.
Regarding the viticulture, OIV created resolutions and defined “Sustainable vitiviniculture” 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.” (OIV, 2008).
Winter pruning of grapevines is a time-consuming operation that requires specialized workers, whom are decreasing in the time, depending on many factors as variety, trellis system and equipment (Gatti, et al., 2011). In the 1990’s, mechanical pruning techniques extended rapidly to different winegrowing regions because, in many countries, hand pruning represented 30% of yearly wine grape production costs (Pérez-Bermudez, et al., 2015).
The introduction of the mechanical pruning has resulted in significant benefits such as overall increased productivity, an increase in efficiency and timeless of agronomic interventions (Tomasi, et al., 2013) and a reduction of the labor costs, saving to 80% of the costs comparing with hand pruning (Gatti, et al., 2011) without compromising the balance of the vines, the canopy architecture and microclimate, and the quality and the composition of the grapes (Kurtural, et al., 2012; Pérez-Bermudez, et al., 2015).
Training is the physical manipulation of a plant’s form. Vine training systems were performed since the antiquity in the Middle East, Greece, and Rome and nowadays many different training systems can be encountered, several of which are indigenous to the viticultural regions in which they were found (Reynolds & Vanden Heuvel, 2009). Is very important to understand the characteristics of the training systems in order to adapt them to the terroir and to the grape variety behaviour in order to optimize the plant growth, the sunlight interception, the productivity and to promote efficient and sustainable vineyard management practices, both for the environmental and for economical sustainability. However, the traditional viticultural system has as most common training system the vertical shoot positioning (VSP), where the processes of winter pruning and canopy management require a substantial amount of manual labour. The introduction of low-input training system such as minimal pruning (MP) and semi-minimal pruned hedge (SMPH) (Intrieri, et al., 2011) thus reduce the demand for labour and permit a higher degree of mechanization, reducing the cost of manual labour in viticultural processes (Strub, et al., 2021b).

Nowadays, lacks in soil fertility are increasing in Europe, so the preservation and improvement of soil organic matter content in vineyard are key goals of agroecosystem resilience against climate change and its negative effects on grapevine water status and vine physiology (Tarricone, et al., 2018). A way to follow sustainability could be the utilization of municipal solid organic waste (MSW) which is a low-cost resource and improve the soil in organic matter, humic substances, and other nutrients for the vines (Montemurro, et al., 2006; Hargreaves, et al., 2008; Botelho, et al., 2020b; Botelho, et al., 2021b).

The present work takes part of a research project, called *IntenSusVITI*. The main aim of the study was to analyze the effect of mechanical pruning applied to two different training systems "Low Cordon" and "High Cordon" and the supply of different quantities of municipal solid waste organic compost on the soil on the wine quality. In this study the experimental vineyards used were located in Quinta do Aroeira (Lisboa, Portugal) and a randomized block design was established. The main oenological parameters under study were physicochemical analysis, phenolic composition and color analysis.

2 Bibliography Review

2.1 Marselan Grapevine variety

Marselan is a French red grapevine variety as results from the cross between Cabernet Sauvignon with Grenache by Paul Truel in 1961 at INRA (Institut National de la Recherche Agronomique) and ENSAM (École Nationale Supérieure Agronomique de Montpellier) in Montpellier, southern France, in order to obtain a productive large-berried variety. Furthermore, Marselan variety presented small berries with low yield of must (approximately 160kg/hl) so was initially neglected. In 1990, with the demands of high-quality grapes with good resistance to disease, Marselan grapevine variety was included in the official register of varieties (Robinson, et al., 2012).

Marselan is a mid-late ripening variety which present a good resistance to powdery mildew, mites and mainly to Botrytis bunch rot (Robinson, et al., 2012). Due to the characterization of a strong adaptability to the environment, Marselan grapevine variety has been gradually accepted and included in the winemaking of other countries of Europe like Portugal, Spain, Bulgaria, Croatia, Italy, (INRAE, 2009-2020) and also in the 'new world' wine countries as Brazil and China (Lyu, et al., 2019; El dos Santos, et al., 2019).

The wines obtained from Marselan present a high degree of phenolic compounds, intense colour and good tannic structure (Pozzatti, et al., 2020). The aroma profile of Marselan wines is characterized by notes of blackberry, green pepper, honey, caramel, raspberry, smoky aromas (Lyu, et al., 2019) and vegetal aroma, according with the winemaking process (Pozzatti, et al., 2020).

2.2 Grapevine Winter Pruning

Winter pruning is a cultural practice performed during winter dormancy that consist in the suppression, up to 95%, of the vegetative growth from the preceding year with the aim of optimizing the sunlight interception, photosynthetic capacity, the airflow inside the canopy and fruit microclimate to improve yield and wine quality particularly in vigorous and shaded vineyards. This operation is designed to maintain vine shape and size, to control number of buds, for maintaining better distribution of potentially productive buds, controlling number of shoots, which controls number of bunches and their size (Intrieri, et al., 2011; Gatti, et al., 2011).

According to Tomasi, et al. (2020), winter pruning contributes to determine the development of wider subterrean parts of the vines leading to a better ratio between the above and below ground structures of the plants, with the advantage of having a good vegetative-productive balance capable of achieving yield and quality purposes.

2.2.1 Manual Pruning

It is well known that winter pruning is one of the operations with more time-consuming activity in wine industry (Clingeleffer & Krake, 1992; Intrieri & Poni, 1995; Zabadal, et al., 2002; Cruz, et al., 2011; Intrieri, et al., 2011; Gatti, et al., 2011; Gambella & Sartori, 2014). In most cases the cost factor of this operation it is considered significantly big as the harvest (Morris & Main, 2008). According to Intrieri et al. (2011) this process may require from 70 to 100 hr/ha of human labor, depending on training system. This labor estimate depends on the structural parameters of the vineyard, such as the training form, the planting pattern, the arrangement of the vegetation, and the type of pruning (Gambella & Sartori, 2014).

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

In the winter pruning the choose of the number of buds to let on the vine plays a key role, as if this value is too low the vegetative and the reproductive growth compete for resources, leading to an increase of vigour and decrease of yield. On the other hand, the higher bud load leads to an increase in the shoot number per vine and to a reduction of budbreak, reduced shoot individual weight, development of smaller clusters and berries, and self-pruning because of abscission of immature wood (Keller, et al., 2004; Poni, et al., 2004; Botelho, et al., 2020a;).

In grapevines, vegetative growth and reproductive growth compete for available resources, consequently, will be necessary to achieve a balance between shoot and fruit growth that there is sufficient (but not excessive) photosynthetic leaf area to ripen the grapes to desired specifications and restore the reserves in the permanent structure of the vine (Keller, et al., 2004; Poni, et al., 2004).

Manual pruning results to a lower yield than mechanical pruning (Keller, et al., 2004) consequently the grapes' and wines' characteristics are affected. According to Pérez-Bermudez et al. (2015) a traditional manual pruning, in comparison with a mechanical light pruning, leads to a significatively higher values of Brix, total polyphenol index (TPI) and potassium (K) accumulation (Table 1.). However, pH and assimilable nitrogen tended to lower, hence the delay of fruit ripeness.

Table 1. Influence of mechanical light pruning (MP) and traditional manual pruning (TP) on Bobal and Tempranillo grapes' composition (extracted from Pérez-Bermudez, et al., 2015).

Parameter	**Bobal**		**Tempranillo**	
	MP	TP	MP	TP
Brix	18.2 a	20.1 b	21.4 a	23.2 b
Sugar(g)/vine	760 b	614 a	836 b	662 a
pH	3.4 a	3.5 a	3.6 a	3.7 a
Titratable acidity (g/L)	6.74 a	6.55 a	5.45 a	5.34 a
Total polyphenol index	60 a	69 b	61 a	73 b
K (mg/L)	1624 a	1798 b	1951 a	2185 b
Assimilable N (mg/L)	250 a	258 a	240 a	257 a

2.2.2 Mechanical Pruning

2.2.2.1 General characterization

In the last decades, the lack of manual labour and the increasing of labour costs brought to the introduction of mechanization of the vineyard (Intrieri, et al., 2011; Gatti, et al., 2011; Dokoozlian, 2013; Poni, et al., 2016; Dobrei, et al., 2020). The adoption of mechanical pruning led to a possible reduction by 50% to 90% of the labor demand, depending on what technique of mechanical pruning is used and on the conditions of soil and vines (Gatti, et al., 2011).

According to Gambella and Sartori (2014), to reach the economic sustainability, mechanized pruning can only be used in vineyards with vigorous variety where the buds are highly productive, so the vegetation is more extensive and the quantity of shoots is high. In addition, an adequate row length (more than 100m) (Figure 1.) is necessary for the mechanical pruner to be practical and economical (Gambella & Sartori, 2014). Furthermore, Winter mechanical pruning appears to be facilitated when performed on single, high-wire trellises with free-growing vegetation concentrated in the upper 180° hemisphere of the cordon (Gatti, et al., 2011).

Vines trained in low-input systems are also trained in a trellis, but they are grown in hedges and are cut using a mechanical trimmer so the cane tying, shoot thinning, wire lowering, and shoot positioning are usually not required (Strub, et al., 2021b).

According to Clingeleffer (1988), the higher yields and the associated lower leaf area to fruit ratios tend to delay maturation in vines subjected to mechanical pruning. The latter could be a potential advantage due to the climate change: the shift in phenological development is the most conspicuous biological effect of recent warming with advanced maturity of grapevines. Shifts in phenology and advanced maturity have implications for wine style and generate logistic problems for the wine industry like the compression of the harvest period, the reach of maturity of different varieties at similar dates, and the overripe of grapes which lead to inferior quality wines (Petrie, et al., 2017). According to Schäfer, et al. (2021), an earlier ripening period will expose the ripening grapes to higher temperature leading to a higher degree of alcohol, a lower concentration of organic acids, especially malic acid, and to changes in the aroma composition of wines.

Nevertheless, in some wine producer country, a delay of harvest, could be a disadvantage due to the rainfall where *Botrytis cinerea* infections could occur (Botelho, et al., 2021b).

Adaptation to mechanical pruning is achieved when the rise in production, due to the higher bud load, is compensated by canopy efficiency such as earlier canopy formation and larger leaf areas allowed by the increased shoot number (Poni, et al., 2004).

According to Poni (2004; 2016), varieties with low-to-medium fruitfulness of the basal nodes (positions 1-4) is a condition favoring yield compensation under mechanical. On the other hand, cultivars of highly fruitful basal nodes have more difficulty reaching the same balance in that they tend to overcrop easily, with a consequent loss of fruit quality. If this balance is achieved, then mechanical winter pruning turns out to be a very attractive option since labor demand is reduced by at least 50% as compared with manual management (Intrieri and Poni,1995; Poni, et al., 2004) without alteration of grape composition (Keller, et al., 2004; Zabadal, et al., 2002).

However, this balance is not always achieved (Zabadal, et al., 2002) and suggests that genotype is probably a more sensitive factor than environment in determining the long-term response to mechanical pruning (Poni, et al., 2004).

Regarding physical and mechanical proprieties of the berries obtained under pruning regime were evaluated by Pezzi et al. (2013). The authors show that mechanical pruning with hand follow up had a higher detachment force of the pedicel, that can hinder the work of grape harvesters, and higher skin hardness, that might better preserve berry integrity during mechanical harvest shaking, than those of hand pruning vines. Those might suggest that under mechanical pruning, mechanical harvest is more favorable.

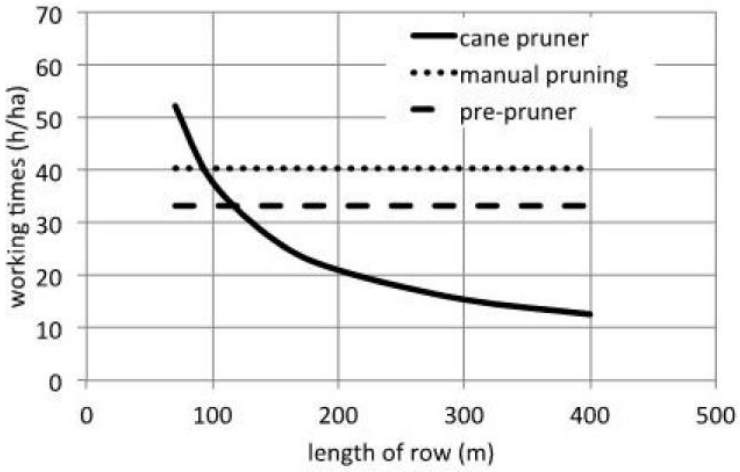

Figure 1 Working times for manual pruning, the operation with cane pruner and with pre-pruner on Cabernet (extracted from Gambella & Sartori, 2014).

2.2.2.2 Assisted Pruning – Partial mechanization

Partial mechanization includes the utilization of power-assisted shears either by pneumatic, hydraulic, or electric systems; the use of a mechanical pre-pruning with a subsequent manual finish; or the combination of mechanical pre-pruning with the use of assisted scissors.

The manual finishing after pre-pruning consists of removing branches of the previous season's fruit and the shoots that formed the excess spurs and suckers; the clean of the remaining brunches, which will host the following season's production; positioning of the removed branches in the interrow which will be taken away or mulched (Gambella & Sartori, 2014).

This pre-pruning generally works best for vineyards that are vertically positioned or lyre type of trellising system, where the canes can be arranged in vertical plane and can be easily cut down by the machines. Then later manual follow up is done in order to control number of buds to be maintained on the retained spurs and some other clean up can be done.

2.2.2.3 Types of mechanical pruning

2.2.2.3.1 Hedge Pruning

Hedge pruning, or box pruning, is a machine pruned system, trained to a vertical shoot positioning type trellis that, according to a research by Lopes et al. (2000), consists in hedging and topping the vines' canopy, in order to create a box shaped canopy and, for this reason this technique takes the name of hedge or box pruning. As result, the vine is hedged to relatively short bearing units, one year old canes reduced in length to two to three bud spurs, without preferential selection of spur positions (Dokoozlian, 2013).

Mechanical hedging allows a higher crop load which influences the balance between shoot and fruit growth by the self-regulating mechanism of the vine. The results of the adaptive processes are a diminution of the budbreak rates, reduced shoot fruitfulness and growth, development of smaller bunches and berries, and self-pruning because of abscission of immature wood (Poni, et al., 2004). Moreover, an increase of bunch number and of yield per vine, in comparison with manual pruning, was underlined, without strong negative effects on cordon trained vines longevity (Lopes, et al., 2000) and in wine quality in terms of total phenols and anthocyanins (Cruz, et al., 2011; Wessner & Kurtural, 2012; Pérez-Bermudez, et al., 2015; Botelho, et al., 2021b).

In general, a cultivar adapts well to mechanical pruning if grape quality is not compromised significantly and when the labor demand is reduced by at least 50% as compared to manual pruning (Poni, et al., 2004). According to the same author, increasing node number per vine can lead to an increase in total effective leaf area which can counteract the yield increase, allowing to maintain similar quality.

On the other hand, however, in a study by Gatti et al. (2011), it is emphasized that some grape varieties are not able to adapt to mechanical pruning, and this translates into a grape overproduction with consequent loss of quality.

In an economic point of view, Kurtural, et al. (2012), underlined that mechanica box-pruning with mechanical shoot thinning reduced the labor operation time and the labor costs which provides a viable labor cost savings of 80% compared with conventional hand pruning without any adverse effects on canopy architecture and microclimate, fruit composition, berry phenolics, and balanced vines for sustained production in a warm climate condition.

Ideally, from an economic perspective, growers should try to maximize both the quantity and quality of the harvest at sustainable levels at the lowest possible cost (Keller et al. 2004).

Nowadays, mechanical pruning is a system that blindly cuts caned so the bud load is independent of vine capacity. In the last years, the development of more sophisticated pruning machines, that can maintain a defined distance from the cutting area to the cordon, allows to regulate the pruning level to the vine vigor as hand pruning usually does (Botelho, et al., 2021a). These new generation machines, like variable-rate mechanical pruning (VRMP), are equipped of GPS and prescription maps created referring to several index. The most used is the normalized difference vegetation index (NDVI), assessed with satellites or with hyperspectral camera from drones, that is directly correlated with dormant pruning weight and has a good correlation with the leaf area development (Dobrowski, et al., 2003; Drissi, et al., 2009). Thus, NDVI is a powerful tool to evaluate vine vigor and can be used to define the pruning level in the following year (Botelho, et al., 2021a).

The effects of VRMP are an increase of the yield in the most vigorous areas and a reduction of it in the least vigorous ones, increasing the discrepancy between different vigor areas and balancing the vines by adapting the crop load to their capacity in comparison with uniform-rate mechanical pruning (URMP). Thus, the grape quality tends to be more uniform (Botelho, et al., 2021a).

2.2.2.3.2 Minimal Pruning

Minimal pruning of cordon-trained vines (MPCT) is a low-input system replacing tradition spur and cane pruning (Clingeleffer & Krake, 1992) in order to decrease pruning costs and optimize the production. According to Strub, et al. (2021a), minimal pruning can significantly reduce costs, up to 59%, in flat (decreased from 0.52 €/L to 0.29 €/L in Germany) and steep terrain sites wherever mechanical harvesting is possible.

MPCT system consist of leaving the vines unpruned and only skirted to stop shoots and fruit contacting the ground. It is being used commercially for vigorous vines in both warmer irrigated vineyards and in cooler premium Australian wine areas (Clingeleffer & Krake, 1992).

Minimal pruning system require grape picking by a mechanical harvester, since the grapes grow throughout the whole canopy. Therefore, MP systems are generally recommended for the production of basic quality wines, for which no manual selection of grapes or other quality improving measurements are required (Morris and Main 2010, Gatti et al. 2011; Strub, et al., 2021b).

Minimal pruning allows to carry out a low severity pruning with rapid speed and low cost. In fact, minimal pruning only needs between 5 and 10 working hr/ha and removes more or less 10% of old wood from the vine (Clingeleffer, 1988).

According to Poni, et al. (2016), the effects of minimal pruning were smaller berries with higher concentrations of anthocyanins, tannins and total phenolics than manual pruning. However, wines obtained from MPCT had the lowest quality scores despite the higher skin-to-flesh ratio.

Minimal pruning showed typical behavior of vine with high bud load: increase in number of shoots and clusters numbers, and reduction in shoot length, leaf size, cluster and berry weight, and number of berries per cluster, hence increase of bunch rot prevention (Rousseau, et al., 2013; Poni, et al., 2016). In addition, the increased yield, by 71% in comparison with hand pruning, is not balanced by the increase of leaf area per vine (19%) (Poni, et al., 2016) thus, can explain a delayed ripening (Rousseau, et al., 2013).

With minimal pruning, yield tended to increase during the first years following the change of pruning system, then decrease after 3 to 5 years. In the longer term (10 years), yields of minimal pruned vines were more stable, than for pruned vines (Rousseau, et al., 2013).

The compensation mechanism is more difficult to achieve for medium-to-late ripening and highly fruitful cultivars such as Sangiovese at a site allowing prolonged shoot growth well beyond bloom (Poni, et al., 2016). In late-ripening cultivars optimum sugar maturity was rarely achieved for a French research (Rousseau, et al., 2013). Likewise, in Tempranillo and Bobal varieties, minimal pruning vines showed an increase over time of Ravaz index for yield-to-pruning weight ratio, suggesting that increased yield was not assisted by a corresponding increase in vine capacity (Gil, et al., 2012).

According to Rousseau, et al., (2013) minimal pruning influenced significantly grape composition and wine quality. Grapes presented lower sugar content (1-2 °Brix), that could be an advantage to obtain low alcohol wines, but at least, negatively affecting the malic acid content, anthocyanins and total phenols content. Wines, having the same level of acohol, compared with hand pruning, showed lower pH, higher total acidity with higher concentration of tartaric acid, and lower levels of potassium, colour intensity and total polyphenols.

Sensory analyses showed that wines produced from minimal pruning compared to hand pruning were a little less intense and fruity, with a less concentrated and more acidic (fresh) palate. The overall style of wines from minimal pruning was balanced and adapted to consumers requirements for light fresh wines (Rousseau, et al., 2013).

The conversion of a VSP to a MP system requires about 50 labour hours per hectare and should be consider in advance because reverting back to a manual pruning system is difficult if not impossible (Strub, et al., 2021a). This includes the fastening of canes and the reinforcement of the trellis system to withstand the pressure of the large canopy. Particularly for minimal pruning training systems every second row has to be removed, because the space between rows usually is too narrow (Strub, et al., 2021a). According to the same authors, the total cost could be reduced by about 46 % in steep slope terrain in comparison with VSP.

In conclusion, the cost advantages of low-input systems cannot be assessed without considering their potential drawbacks, including the prospect of lower wine quality resulting from higher yields (Deloire, et al., 2016) the increased demand for water and compulsory machine harvesting (Strub, et al., 2021a).

2.2.2.3.3 Semi-minimal-pruned Hedge

The grapevine training system, known as Semi-Minimal-Pruned Hedge (SMPH) (Figure 2.) has first been described in Italy by Intrieri et al. (2011) to overcome the limitation of minimal pruning concerning over-cropping, delayed ripening, and alternating yield levels. SMPH blends features of traditional vertical shoot positioning trellising systems with the concept of minimal pruning. Vines are mechanically pruned in winter to a hedge shape of the trellis system using a normal grapevine hedger saving approximately 60 to 70 labor hours per hectare (Intrieri, et al., 2011).

Experiment of Intrieri, et al. (2011) showed that the yield for SMPH was 35-40 % higher than spur pruned cordon. Clusters appeared less compact and less susceptible to botrytis attack.

Besides the lower susceptibility towards bunch rot (Intrieri, et al., 2011) the investigations of Kraus et al. (2018) indicated a higher susceptibility of SMPH to downy and powdery mildew, as well as to Drosophila suzukii due to the micro-climatic conditions inside the extended canopy.

Even though higher cropping was achieved, must titratable acidity, brix and pH did not differ in case of SMPH compared to spur-pruned cordon vines, while total anthocyanin concentration showed higher values.

These results were confirmed by Poni, et al. (2016) concluding that SMPH is a good alternative to minimal pruning because it quickly adjusts cluster number and potential yield supported by the higher leaf area. Furthermore, when SMPH is trained to 120 cm from the cordon, improves vine's physiological functions in comparison with minimal pruning.

More recent studies (Molitor, et al., 2019; Schäfer, et al., 2021) focus the attention to climate change problematic. The ripening delay observed in SMPH represents a risk for full grape maturity in the case of late maturation, such as in late vintages and/or late-ripening cultivars and/or late-ripening vineyards or regions. Consequently, SMPH systems should be avoided where the heat transfer is a limiting factor to reach the full maturity (Molitor, et al., 2019).

In cool climate grapegrowing regions with sufficient water availability, such as Luxembourg (Molitor, et al., 2019) the dalayed maturation and the shift of maturation period towards later, observed for white wine from grapes in SMPH, might contribute to conserving the freshnes and lightness while still mantaining analytical grape parameters on a comparable level as in vertical shoot positioning (VSP). The same authors analyzed also the mechanical cluster thinning effect on SMPH which provided to achieve lower total soluble solids with a higher degree of organic acids, or to postpone the date of harvest by maintaining grape quality compared to VSP. Furthermore, damages from extreme weather events like hail or sunburn were found to be lower in SMPH, than in VSP, due to the higher yield potential and the specific distribution of clusters in canopy (Molitor, et al., 2019). Schäfer, et al. (2021) confirmed the results obtain by Molitor, et al. (2019) studying Riesling grapevine varieties in SMPH trellis system in Germany, i.e. moderate climate region, with sufficient water availability.

To conclude, a research conducted by Strub, et al. (2021b) underlined that SMPH low-input training system improve the economic sustainability, by increasing the degree of mechanization in viticulture and reduce production costs, in cold to moderate climates wineregions, either in flat terrain and steep slope vineyards. For flat terrain sites, low-input SMPH training can result in an average cost savings of 2,161 €/ha or 46% of the total cost. For steep slope terrain the total cost could be reduced by about 34%.

Figure 2 Mechanical pruning on a semi-minimal pruned hedge (SMPH) system (extracted from Intrieri et al., 2011).

2.2.2.4 Effects on vegetative and reproductive growth

It is well known that mechanical pruning strongly increases the bud load per vine (Keller, et al., 2004; Gatti, et al., 2011) consequently, reduce the percentages of budbreak (to 60% in some years), shoot fruitfulness and cluster weight (Keller, et al., 2004; Gatti, et al., 2011; Botelho, et al., 2020a; Botelho, et al., 2020b). On the other hand, it increases the number of clusters per vine and, consequently, the yield (Botelho, et al., 2020a; Botelho, et al., 2020b; Botelho, et al., 2021b).

When the yield is increased until 40% in comparison with manual pruning, no differences in terms of grape composition were observed, over this value, a delay in the maturation occurs (Botelho, et al., 2020a). The effect of higher yields is strongest in the first years after the conversion if no counter measures, such as thinning, are taken. Furthermore, in the same period, yield regulation is essential in low-input systems to achieve a satisfactory leaf area to fruit ratio and to enhance phenolic maturation (Strub, et al., 2021b).

Since bud load drastically increases, changes in carbohydrates production and partitioning occur when mechanical pruning is established (Botelho, et al., 2020a). As a result, the vine invests less energy in cane formation obtaining an increase in shoot number and a reduction of shoot individual weight (Clingeleffer & Krake, 2002) and, in the other side, a higher available carbohydrate redirected to reproductive growth and to the formation of reserves (Botelho, et al., 2020a; Botelho, et al., 2020b).

Mechanically pruned grapevines lead to a higher leaf area in the beginning of the season that intensifies the water consumption, but at least the traspiration rate per leaf unit area have some reduction (Schmid & Schultz, 2000; Botelho, et al., 2020a; Botelho, et al., 2020b).

Although mechanical pruning showed a tendency for lower transpiration and photosynthetic rates, in an overall analysis it increased total water consumption as well as total carbohydrate production (Botelho, et al., 2020a).

2.2.2.5 Effects on grape and wine composition

Nowadays, the market is giving more attention to human's health, so the production of wines with lower alcohol concentration is a becoming a new goal for wineries.

A strategy for achieving this goal could be the use of mechanical pruning that shows a decrease of alcohol content in the wines, without affecting other berry composition variables, (Cruz, et al., 2011; Wessner & Kurtural, 2012; Pérez-Bermudez, et al., 2015; Botelho, et al., 2021b) and without difference in the sensory analysis (Santos, et al., 2015) or with distinct sensory properties which can be differently tasted and accepted by experts or consumers (Pérez-Bermudez, et al., 2015).

From the qualitative point of view, in musts obtained from grapes under mechanical winter pruning, even if they are increased in yield, they were never significantly affected in terms of total acidity, anthocyanins and total phenols (Kurtural, et al., 2012; Kurtural, et al., 2019; Botelho, et al., 2020a) while a decrease of grape total soluble solids and pH was observed (Botelho, et al., 2020a). Such pH reduction is particularly important in warm Mediterranean conditions, especially in a climate change scenario, and, in addition, low pH favors wine stability (Botelho, et al., 2021b).

According to Tomasi et al. (2013), analyzing healthy compounds content, such as resveratrol and other stilbenes, in 'Cabernet Sauvignon' variety subjected to mechanical pruning, underlined the double amount of trans resveratrol in mechanical pruning in comparison with manual pruning. The authors suggested that mechanical pruning can affect the synthesis of this compounds and that a less invasive pruning methods can lead to a higher content of resveratrol.

2.3 Organic Amendment: Municipal Solid Organic Waste (MSW)

Soil organic matter decline and soil acidity are global problems for crop production that can be accentuated by climate change. Almost half of the European soils have low organic matter content: In Portugal, most soils have low organic matter content due to climatic conditions, poor agricultural practices, and low soil pH. In these soils, plants grow poorly because of low water availability. In addition, the combination of H3O+, Al, and Mn toxicities lead to a lack of essential nutrients (Machado, et al., 2021).

Preservation and improvement of soil organic matter content in vineyard are key goals of agroecosystem resilience against climate change and its negative effects on grapevine water status and vine physiology (Tarricone, et al., 2018).

The addition of organic amendments to crop soils can improve soil quality by affecting soil aeration, structure, drainage, soil water holding capacity, nutrient availability and microbial biomass. Furthermore, organic amendments increase disease 'suppressiveness' i.e., the ability of soil to suppress plant nematode and fungal pathogenes and induce plant defense, in relation to presence of more antagonistic, competitive bacteria and fungi that contribute to the activation of induced systemic resistance (Tarricone, et al., 2018).

With rising interest in organic agriculture, the production of organic-grade MSW compost for agriculture is also gaining popularity because of its positive effect on biological, physical, and chemical soil properties (Hargreaves, et al., 2008).

Agricultural utilization of municipal solid organic waste (MSW) is become one of the most promising, cost-effective and sustainable options for disposal of MSW thanks to its effect on soil and its huge availability and low price (Tessfaw, et al., 2021).

A primary benefit of MSW compost is the high organic matter content, low bulk density and humic substances as humic acid (Hargreaves, et al., 2008) in fact repeated application of this compost increased soil organic matter content and soil C/N ratio to levels greater than those of unamended soil (Montemurro, et al., 2006; Walter, et al., 2006). Due to the high organic matter content, MSW have a high water holding capacity which improve the water holding capacity of the soil (Hargreaves, et al., 2008).

Chemical properties revealed are increased of soil pH, electrical conductivity and salt effects, nitrogen, phosphorus, potassium and other plant essential nutrients as calcium, sulfur, magnesium, copper, zinc (Hargreaves, et al., 2008).

According to Tarricone, et al. (2018), SMW at moderate and high rate provided to vineyard soil, increased vine growth, yield and affect water status correlated to the effect of organic matter on soil. The latter, could be an advantage on contrasting summer drought effect on vines. Furthermore, while the grape's composition showed an increment of titratable acidity, the wines had similar chemical parameters (alcohol content, titrable acidity and pH).

On the contrary, high yearly amounts of organic amendments decreased vine pruning weight and yield and increased vine mortality (Morlat, 2008).

According with Botelho, et al. (2020b), MSW influence the vegetative growth of the vine increasing the shoot number / vine, thus, cluster number / vine than vines without MSW. Furthermore was observed also increases on berry weight (g), berry number / cluster and cluster weight (g) and finally, the yield (kg/vine) (Botelho, et al., 2021b) in comparison with a soil without organic amendment. Anyway, the increase in shoot number/vine was significant only in interaction with mechanical pruning. Regarding the grape quality parameters the MSW amendment show a trend on the decrease of total soluble solids, anthocyanins and total phenols, while a light increase of pH was observed (Botelho, et al., 2021b).

In the same studies of Botelho, et al. (2020b; 2021b), the effect of the interaction between mechanical pruning and differents soil organic amendments, in different quantities, was analyzed. The results of this combination are an increase of yield, without a decrease of vegetative growth, obtaining grapes with lower total soluble solids without alterations in total anthocyanins and total phenols. On the other hand, due to the increase in productivity that some organic amendments promote, a negative relationship between yield and total anthocyanins was observed. The decrease of anthocyanins and phenols could be explain by the N content in the soil: high rate of N

supplied during bloom delay the accumulation of phenolic compound, particularly flavonols, in the grape skin at veraison. Thus N fertilization can decrease wine quality, particularly on poor light conditions during the ripening. On the other hand, reduced N availability at bloom time has been correlated with enhanced polyphenol accumulation in grape skin (Mulasà, et al., 2004; Keller & Hrazdina, 1998).

2.4 Training systems

Training a vine can be defined as “the physical arrangement of plant parts spatially” (Reynolds & Vanden Heuvel, 2009). The goal of the training is to find a trellis system that, in a specific “terroir”, will adapts the characteristic of a specific grape cultivar optimizing the utilization of sunlight, promote the productivity and to promote efficient and sustainable vineyard management practices, both for the environmental and for economical sustainability.

The choose of the appropriate training system involves several decisions including choice of the site, cultivar and associated cultivar practices. Furthermore, an appropriate training system can favorably modify growth, yield and fruit composition by altering the vine physiology and microclimate (Reynolds, 1988). More specifically, the disposition of the perennial wood and canes, the trellis height, and the associated type of pruning to manipulate the exposure of leaf area and thus influences interception of sunlight. Sunlight penetration into a canopy affects flower bud initiation and subsequent fruitfulness. The management of the canopy allows also to change the microclimate of the internal microclimate of the canopy in order to control the disease. Training system influences vine size, as measured by the weight of cane pruning, and, through the application of balanced pruning practice, also the yield (Howell, et al., 1987).

The training system can facilitate the mechanization of the vineyard operations through the distribution of the bearing units. Organize the disposition of the trunks and canes in order to avoid competition for light between vines. The training allows the formation of a renewal zone ensuring that the vine form is perpetuated and yield is maintained. Lastly, promote the amount of perennial wood that reduce the risk of winter injury (Reynolds & Vanden Heuvel, 2009).

To achieve the optimum vine performance, relationship between vegetative growth and reproductive growth, is also necessary to implement an appropriate pruning strategy following the concept based on the balanced pruning (Reynolds, 1988; Poni, et al., 2004). Poni et al. (2004) showed that in a high cordon training system, the compensation mechanism short-cane mechanical pruning, brought the vines with low-fruitful to a remunerative grape yield and quality balance.

2.4.1 Low and High Cordon

Low and High cordon are short-cane training systems, thus they adapt very well both for mechanical and manual pruning.

Low cordon (LC) is a training system suitable for cultivars with an upright to semi-upright growth habit, and it require a vertical shoot positioning (VSP). Shoot positioning consists of tucking the canes between the set of catch wires as they develop. The cordon is trained on to a middle-level wire set about 0,5 – 1 m above the ground, and additional set of catch wires are required for the shoot positioning. The pruning of LC training system it is a short-cane with renewal spurs pruning selecting the upright growing canes.

High Cordon (HC), or bilateral cordon, training system could be single or double curtain and it is suitable for moderate to vigorous cultivars with a trailing / drooping growth habit. The central trunk with cordon is trained to the top wire set at about 1.80m and the canes growing along or cross the top of the cordon are repositioned so that they flow downward from the cordon. The pruning is the same of LC with the difference that the downward canes are selected. (Nonnecke, 2002)

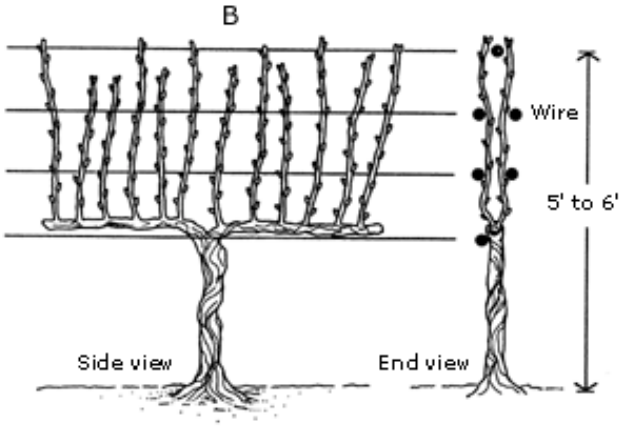

Figure 3 Single curtain bi-lateral low cordon (LC) on vertical shoot positioning with cordon 1m above the ground (extracted from Nonnecke, 2002)

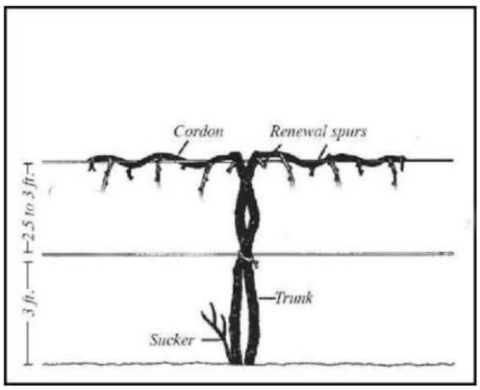

Figure 4 Single curtain bi-lateral high cordon (HC) with cordon 1.80m above the ground (extracted from Nonnecke, 2002)

According with Howell et al. (1987), in Vidal blanc grapevine, the LC pruning system, in comparison with HC, lead to a higher vine size (kg/vine), nodes retained per vine; contrarywise, HC shows a tendency to have a light higher yield (kg/vine) and clusters per vines (Howell, 2001) because of improved node fruitfulness (yield/node retained) (Howell, et al., 1991) than LC.

According with the literature (Howell, et al., 1987; Botelho, et al., 2020a) a higher yield led to a lower level of soluble solids (%) and in some cases also to a decrease in pH while the total acidity is not affected. The lower levels of soluble solids and pH were explained by Howell et al. (1987): count fruit soluble solids and pH are higher than fruit on non-count shoots. HC-trained vines produced up to 23,1% of the total yield from non-count shoots, that is correlated with the higher percentage of two-year-old and older wood in the systems, hence the lower amounts of soluble solids and pH shown in HC. Fruit from count and non-count shoots differ also in cluster weight, berry weight, and number of berries per cluster on both HC and LC. Differences between the two training systems are shown, where HC has more berries per clusters and cluster weight but smaller berry weight in comparison with LC.

Contrarywise, in a more recent research (Coletta, et al., 2014) single spur pruned low cordon (SLC) shown a significant higher yield (kg/vine), pruning weight (kg), bunch weight (g), and light higher levels on berry weight (g), soluble solids (Brix) and pH, and, on the other side, lower Ravaz Index in comparison with single spur pruned high wire cordon (HSLC). By the author (Coletta, et al., 2014) the small canopy development in HSLC with the highest yield/pruning weight ratio could be related to the canopy architecture and to the height of the cluster zone.

In the same research (Coletta, et al., 2014) was conducted the evaluation of polyphenolic compounds of the two different training systems. HSLC shown a higher content in Total polyphenols (TPP), total flavonoids (TF), flavonoids without anthocyanins (FNA), flavans (F) and proanthocyanidins (PrA) than SLC that, on the other hand, had higher levels of anthocyanins (TA).

The results obtained in this trial can be explained also by the mild water stress condition in the late ripening period that can cause a lower canopy density, with a consequent cluster exposition to sunlight, resulting in an improvement of total polyphenols concentration. Furthermore, the HSLC seems increase the antioxidant capacity of polyphenolic compounds in comparison with SLC, but these results should be confirmed by other studies because it is possible that in the trial there was an influence by the variety used.

3 Materials and Methods

3.1 Experimental Design

The present experiment take part of IntenSusVITI research project and, in 2020, started an experimental design based on Marselan grapevine variety in Quinta do Aroeira, Lisboa. The vineyards were organized with 2.6 meters distance interrow and 1 meter distance on the row. For the trial, the vineyards were distributed in three randomized blocks to allow us to standardize non-treatment effects. Each block was divided in 4 parcels, each composed by 14 plants, for a total of 56 plants / row and 168 plants / block. In each block were distributed two different training system "High Cordon" and "Low Cordon", both mechanical pruned, and four amounts of Municipal Solid Organic Waste (MSW). The organic amendment used (MSW) was applied in four different amounts: 0, 5.000, 10.000, 20.000 kg/ha that correspond respectively as "Test", "M1", "M2", "M3". The grapes were manually harvested on 15 September 2020 and, for unpredictable issues, was not possible to obtain the grapes from the third block. In this experiment only the first two block were analyzed, obtaining 16 different wines.

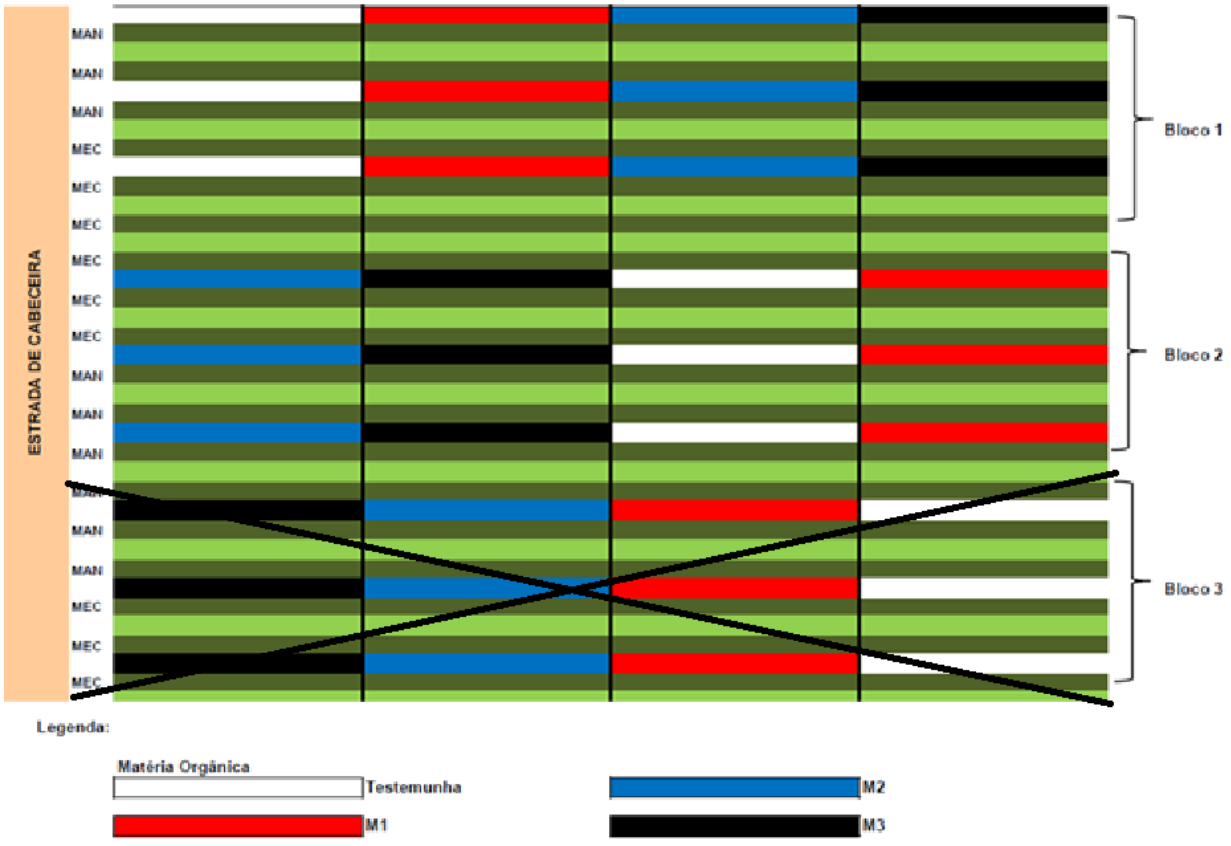

Figure 5 Experimental Design: MAN – Mechanical pruned Low Cordon; MEC – Mechanical pruned High Cordon. Red = M1 – 5.000 kg/ha of MSW; blue = M2 – 10.000 kg/ha of MSW; black = M3 – 20.000 kg/ha of MSW; white = TEST– Without MSW. Light green = interrow; dark green = row; red, blue, black and white = treatments applied in the interrow.

3.2 Winemaking

Before the harvest, the grapes from the vineyard involved in this project were analyzed, in order to access their yield (Table 2.), quality and maturation stage. The parameters controlled (Table 4.) in this phase were weight of a hundred berries (g), volume of must (ml), °Brix, potential alcohol content (%), pH and total acidity (g tartaric acid L^{-1}). Total anthocyanins (mg/L malvidin 3-O-glucoside) and total phenols (mg/L gallic acid) were assessed also at the harvest.

When the grapes were at the ideal stage of maturation the manual harvest was performed and the grapes were transported to the experimental winery at Instituto Superior de Agronomia (Lisbon), where the vinification took place. In the same day of the harvest, grapes were de-stemmed, crushed and sulphur dioxide was added (50 mg/L). The must was analyzed (Table 6.) for °Brix, potential alcohol content (%), pH, total acidity (g tartaric acid L^{-1}) and assimilable nitrogen (mg/L) and inoculated with the yeast Zymasil® Bayanus (AEB®).

The alcoholic fermentation lasted between 7 and 8 days with temperatures between 24 and 27°C (annex 3 to 18) and the maceration time was extended to 13 days for the low cordon wines and 14 days for high cordon wines. After the maceration the skins were from the juice using a vertical press and the pressed juice was added to the free-run juice. After the racking of the wines, 20 liters of wine with 3 liters of lees, the wines were analyzed (Table 8.) for alcohol content (%), pH, total acidity (g tartaric acid L^{-1}), volatile acidity (g acetic acid L^{-1}), total and free sulphur dioxide (mg/L) and reducing substances (g/L).

The malolactic fermentation developed spontaneously after the racking, and it concluded in February and physico-chemical analysis (Table 9.) took place to control the total and free sulphur dioxide, volatile acidity, total acidity, pH and the total dry extract (g/L). Free SO_2 content was then adjusted to 35 mg/L and the wines were bottled.

3.3 Basic chemical characteristics of the wine

3.3.1 Sulfur dioxide

Total sulfur dioxide represents the total of all the various forms of sulfur dioxide present in the wine, either in the Free State or combined with their constituents (OIV, 2009). Free sulfur dioxide is determined by potentiometric titration with iodide/iodate while the total one is determined by potentiometric titration with iodide/iodate after alkaline hydrolysis. Using the semi-automatic apparatus “Sulfilyser”, it’s possible to measure free and total SO_2 in red wine. The beaker containing the solution is put on the plate of the apparatus and the double platinum electrodes are dip into the solution (which is maintained stirring). The titration starts by pressing an opposite bottom (at the rate of one pressure every second) and with the LED indicator, the electrodes detect the electric current as soon as the oxidizing

solution of iodide/iodate is in excess. The measurement continues till the red LEDs remain lighted for 5 sec. The calculation of SO2 is based on “n” ml of the volume of iodine used.

a) *Free sulfur dioxide*: 25 ml of wine measured with a volumetric pipette, placed in a backer of 50ml and covered with Parafilm. After the addition of 5ml of solution 941 to the sample, the solution was titrated by Sulfilyser using as reagent Iodure/Iodate $KIKIO_3$ N/64 (1 liter of distilled water and Iodide Iodate concentrate 38064-1EA). After the titration, the obtained result was multiplied per 20.
b) *Total Sulfur dioxide*: 10 ml of wine measured with a volumetric pipette, placed in a backer of 50ml and covered with Parafilm. After the addition of 2ml of solution 908 to the sample, after 5’ minutes, the solution was titrated by Sulfilyser using as reagent Iodure/Iodate $KIKIO_3$ N/64 (1 liter of distilled water and Iodide Iodate concentrate 38064-1EA). After the titration, the obtained result was multiplied per 50.

3.3.2 Volatile Acidity

The method consists of the separation of Volatile acids by steam distillation and titration using standard sodium hydroxide. The acidity of free and combined sulfur dioxide, of any sorbic acid, which may have been added to the wine, and the acidity of the salicylic acid must be determined and subtracted from the acidity.

About 50 mL of wine were placed in a vacuum flask and, for the elimination of carbon dioxide, was applied vacuum though a vacuum pump. 20 mL of prepared wine were placed into the flask. After the addition of about 0.5 g of tartaric acid, the sample was distillate by steam distillation to separate volatile acids from the wine and at least 250 mL of the distillate were collected. The distillate was titrated with the sodium hydroxide solution, using two drops of phenolphthalein as indicator obtaining “*n”* mL as the volume of sodium hydroxide used.

Afterwards, into the sample were added four drops of the dilute hydrochloric acid, 2 mL starch solution and a few crystals of potassium iodide. Then, the first titration with iodine solution (0.005 M) to obtain the free sulfur dioxide (n’ = mL of the volume used), and after addition of Borax solution, the second titration with iodine solution (0.005 M) to obtain the combined sulfur (n’’ = mL of volume used) (OIV, 2021).

3.3.3 Titratable Acidity

The measure of the amount of acidity in wine is known as the “titratable acidity” or “total acidity”. It refers to the test that measures the total of all acids present, while pH expresses the strength of acidity.

The total acidity of the wine is the sum of its titratable acidities when it’s titrated to pH 7 against a standard alkaline solution. Carbon dioxide is not included in the total acidity. The total acidity for the tested wine was measured by potentiometric titration and titration with bromothymol blue as indicator and comparison with an end-point colour standard.

After the elimination of carbon dioxide from about 50 mL of wine, 10 mL of prepared sample were placed into a volumetric flask of 500 ml with 30 mL of boiled distilled water and 1 mL of bromothymol blue solution. Then, the titration was conducted with sodium hydroxide solution (1M) until the color changed to blue-green (OIV, 2021).

3.3.4 Alcohol Strength

The alcoholic strength by volume is the number of litres of ethanol contained in 100 litres of wine, both volumes being measured at a temperature of 20°C. It is expressed by the symbol % vol. For this determination, the distillation method was used.

About 250 mL of wine were placed in a vacuum flask and, for the elimination of carbon dioxide, was applied vacuum though a vacuum pump. After the distillation of 250 mL of prepared wine, the distillate was collected in a volumetric flask of 250ml. The distilled wine at 20°C was placed in a graduated cylinder of 250 mL and filled with distilled water at 20°C, tapped with parafilm and put in oven at 20°C. When the solution reached 20°C, the alcohol content was read using the alcoholometer % volume of ethanol 10-20 or 0-10 for 20°C (OIV, 2021).

3.3.5 Total Dry Extract

Total dry extract or total dry matter includes all matter that is non-volatile under specified physical condition. By a chemical point of view the total dry extract consists of organic substances (anthocyanins, proteins, phenolics, tannins and glycerol) and mineral compounds that remain dissolved in the wine matrix and are not volatile under specific conditions (OIV, 2021).

The total dry extract in the tested wine was calculated indirectly from the specific gravity of the alcohol-free wine. This dry extract is expressed in terms of the quantity of sucrose which, when dissolved in water and made up to a volume of one litre, gives a solution of the same gravity as the must or the alcohol-free wine (OIV, 2021).

3.3.6 Reducing substances

Reducing substances comprise all the sugars exhibiting kenotic and aldehyde functions and are determined by their reducing action on an alkaline solution of a copper salt. Since the microbiological stability of wine depends also on its sugars content, the determination is usually done after the end of alcoholic fermentation (OIV, 2021).

First of all, the wines were clarified in order to remove interfering compounds and not to overestimate the reducing substances. The sugar content of the liquid in which sugar is to be determined must lie between 0,5 and 5 g/L. Dry wines should not be diluted during clarification. To clarified red wines was used neutral lead acetate solution (250 g of neutral lead acetate, Pb (CH3COO)2·3H2O in 500mL of hot water, stir until dissolved), sodium hydroxide solution, 1M and calcium carbonate. 50 mL of wine were mixed with: a volume of [0,5 (n - 0,5)] mL of sodium hydroxide solution 1 M (“n” is the volume of NaOH 0,1 M used to determine the total acidity), 2,5 mL of saturated lead acetate solution and 0,5 g calcium carbonate. The solution was shaked several times in order to remove phenolic compounds and after 15 minutes was filtered. 1mL of filtered wine corresponds to 0,5 mL of wine (OIV, 2021). After, 10 mL of the clarified solution was mixed with 25 mL of alkaline copper salt solution and 15 mL of water in a 300 mL conical flask, while a blank assay was prepared with 25 mL of water and 25 mL of alkaline copper salt solution.

In an alkaline environment, the carbonyl groups of the reducing sugars are oxidized by Cu^{2+} ions forming adducts with carboxyl groups. Ion Cu^{2+} is reduced in cuprous oxide that has a brick-red colour.

The mixtures were kept boiling for 10 minutes with a reflux condenser and after the flasks were cooled down in cold running water. Then, 10 mL of potassium iodide solution, 30% (m/v), 25 mL of sulfuric acid, 25% (m/v) (Figure on the left) and 2 mL of starch solution (an indicator for redox reactions) were added.

Cupric ions are in excess compared to the quantity of reducing sugars present. Then, using potassium iodide solution was possible to evaluate the excess of cupric ions thanks to the reaction between potassium iodide and Cu^{2+} ions that had not reacted with the reducing substances.

The solutions are titrated with sodium thiosulfate solution (0,1 M) in order to assess the content of iodium that had not reacted with Cu^{2+} ions. Finally, “n” is the volume of sodium thiosulfate used and “ n’ ” is for the blank assay (OIV, 2021). The final solution has a yellow-orange colour while the blank assay has a milky white colour.

The sugar content of the wine, expressed in grams of invert sugar per liter to one decimal place, is obtained as function of the number (n’ – n) of mL of sodium thiosulfate used, taking account of the dilution made during clarification and of the volume of the test sample.

3.3.7 pH

The principle of the method is based on the measurement of the difference in potential between two electrodes immersed in the liquid under test. One of these two electrodes has a potential that is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode (OIV, 2021). After the calibration of the pH meter at 20°C using standard buffer solutions, the electrode is dipped into the sample to be analysed and the temperature should be between 20 and 25°C (as close as possible 20°C). The pH is read directly off the scale and the final result is taken to be the arithmetic mean of two determinations (OIV, 2021).

3.3.8 Assimilable Nitrogen

For the assimilable nitrogen analysis the formol titration procedure described by Gump et al., (2002) was used. Formaldehyde combines with the free amino groups liberating a proton from the NH3 group which could be titrated directly with NaOH at pH 8.0.

The result obtained in this procedure was calculated according to the general equation: mg nitrogen/L = [(vol 0.05N NaOH) x (concentration 0.05N NaOH) x 14 x (dilution factor) x 1000] / (sample vol).

3.4 Chromatic characteristics and phenolic compounds

3.4.1 Intensity and Tonality

The aim of this method is to determine the color intensity and tonality of the wines.

The absorbance at 420, 520 and 620nm of the wine, previously centrifuged, was detected by spectrophotometer with cuvette with 1mm optical thickness and distilled water as the reference liquid (OIV, 2021).

The color intensity (I) is calculated by the following expression:

$$I\ (a.u.) = A420 + A520 + A620$$

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

$$T = A420 / A520$$

3.4.2 Color due to Copigmentation

In a volumetric flask 1 mL of acetaldehyde (12.6% v/v solution) was added to 10 mL of centrifugated wine (4.000 rpm), and, after 45 minutes, the absorbance at 520nm (A^a520) was measured by spectrophotometer, with 1mm optical thickness cell, corresponding to anthocyanins combined with SO_2.

The absorbance at 520nm (A^b520) in 1cm optical thickness cell, was measured after the addition of 2.5 ml of the wine, previously diluted, in 50 ml of hydroalcoholic solution (12% v/v, pH 3.2 adjusted with tartaric acid and filtrated 0.45 µm) in a volumetric flask of 50ml in order to dissociate the anthocyanin-copigment complexes (Boulton, 2001).

3.4.3 Chromatic Characterization by the anthocyanins and pigments evaluation

Chromatic characterization is an important aspect of red wine quality and depend on the phenolic composition of the wine. The red wine colour is an integration of contributions from monomeric anthocyanins and polymeric pigments. The chromatic characterization include colour intensity, colour shade or tonality, total anthocyanins (mg/l malvidin 3-glucoside), degree of ionization of anthocyanins, ionized anthocyanins, total pigments, polymerization index, polymerized pigments, and total phenolics.

The determination of the wine's chromatic characterization will give information that can be used to compare our wine to another one and that will help in reasoning its colour.

The method used to measure chromatic characteristics was the spectrophotometric analysis based on Somers & Evans method (1977).

The wine was clarified by centrifugation at 10.000 rpm for 10 minutes and placed in 1mm optical thickness cell to measure the absorbance at the wavelengths of 420nm, 520nm and 620nm, using distilled water as a reference.

After the addition of 5 µl of sodium metabisulphite solution (20%) to the above sample and mixed by inversion, the absorbance at 520 nm after 1 minute was recorded (A520 SO_2). The addition of SO2 to the wine has an immediate bleaching effect on the monomeric anthocyanins, causing them to lose their colour. On the opposite, the polymerized anthocyanins are able to resist SO2's bleaching effect a lot more. This means that the remaining coloring matter in the wine is the polymerized anthocyanins (Somers & Evans, 1977).

In a test tube was placed 10 ml of Hydrocloric acid solution 1M (83ml/1000ml) and 100 µl of wine which is subsequently placed to a thermal bath (25°C of temperature) for 3-4 hours. The measurement of the absorbance at 520nm in a 1cm cell against distilled water was done to obtain A520 HCl. The addition of HCl aims to acidify the wine to a pH lower than 1.

At this pH, all monomeric anthocyanins are in the flavylium cation form, then it is possible to achieve a very high optical density value.

The results were obtained by the followings formula.

Colour intensity (a.u.) = $A_{420} + A_{520} + A_{620}$

Colour shade or Tonality = (A_{420} / A_{520})

Total anthocyanins (mg/l malvidin 3-glucoside) = 20 ($A_{520}HCl – 5/3\ A_{520}SO_2$)

Degree of ionisation of anthocyanins (α) (%) = $[(A_{520}) - (A_{520}SO_2)] / [(A_{520}HCl) – 5/3\ A_{520}SO_2)] \times 100$

Ionised anthocyanins (mg/l malvidin 3-glucoside) = α/100 × total anthocyanins= 9,968/100 x 301 mg/L

Total pigments (a.u.) = $A_{520}HCl$

Polymerization index (%) = $[A_{520}SO_2 / A_{520}HCl] \times 100$

Polymerized pigments (a.u.) = $A_{520}SO_2$

3.4.4 CIELab method

The purpose of this method is the definition of the measurement process and the calculation of the chromatic characteristics of wines, using the trichromatic components (X, Y and Z) and trying to imitate real observers regarding their feelings towards color.

The wine was centrifugated at 4000 rpm for 10 minutes at temperature of 15°C.

In a spectrophotometer, using cuvettes with 1mm optical thickness, was measured the transmittance of the sample from 380 to 780 nm every 5 nm (illuminant = D45 and observers = 10°) using distilled water as a reference in a cuvette with the same optical thickness. In this way the device gives us back three values called L*, a* and b* for each sample (OIV, 2021).

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

$$\text{Clarity} = L$$

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

$$H = tg^{-1}\ (b/a)$$

3.4.5 Total Phenols

In a volumetric flask of 100ml a dilution 1-100 of centrifuged sample (4.000 rpm) with distilled water was used. The absorbance reading was performed on a spectrophotometer at the wavelength of 280 nm, using quartz cuvette with 1cm of optical thickness (Ribéreau-Gayon, 1970). With the results obtained we can evaluate:

$$\text{Total phenol index} = \text{Abs } 280\text{nm} * 100\ (\text{au})$$

$$\text{Total phenols (mg/L of gallic acid)} = ((\text{Abs } 280\text{nm} * 100) + 0.0344)/0.038$$

3.4.6 Flavonoids and Non-flavonoids

The method implemented by Kramling & Singleton (1969) allows to divide the total phenols of the wine in flavonoid phenols such as flavanols, flavonols, anthocyanins, and non-flavonoid phenols such as phenolic acids (cinamic and benzoic) and volatile phenols.

a) **Non-flavonoids**: In a centrifuge tube was placed 10mL of sample, 10mL HCl (dilution 1:4) and 5mL of formaldehyde (8mg/mL), mixed, and after the addition of nitrogen the samples were stored in a dark place for 72 hours and then centrifuged at 3500 rpm for 10 minutes. In a volumetric flask of 50mL, 5mL of centrifuged sample were diluted in 45ml of distilled water in order to be measured in a spectrophotometer at the absorbance of 280nm in cells with 1cm of optical thickness. The results were expressed as:

$$\text{Non-flavonoids (mg/L gallic acid)} = ((\text{Abs } 280\text{nm} * 10) + 0.0344) / 0.038$$

b) **Flavonoids**: The concentration of flavonoids in the sample was based on the following calculation:

$$\text{Flavonoids} = [\textit{Total phenols}] - [\textit{Non-flavonoids}]$$

3.4.7 Tannin Power

This method uses a subtractive approach: is based on the potential of proteins to complex and precipitate tannins present in the solution. It leverages the ability of proteins present in BSA (bovine serum albumin) to bind with proanthocyanidins and precipitate them in solution in which the composition of resulting protein-tannin aggregate.

The determination of tannin power takes place with the measurement of the turbidity, due to protein-tannin aggregate, by nephelometry before and after the precipitation with bovine serum albumin (De Freitas & Mateus, 2001).

The wine sample was centrifuged (4000rpm) and diluted 1/50 in a volumetric flask 50ml with a wine model solution (hydroalcoholic solution 12% v/v with tartaric acid 5g/L to pH 3.2) previously filtrated (0.45 µm). 4 ml of the diluted solution were placed in a turbidity measuring tube and the turbidity was determined using nephelometer (2100N ISO METHOD 7027) obtaining the value designated as $\mathbf{d_0}$.

In a test tube was placed 25mL of the diluted wine (1/50) with the addition of 940µl of BSA solution (bovine serum albumin 0.8 g/L) using pipette 100:1000µl and mixed with a vortex. The solution was stored in a dark place at room temperature for 45' minutes and then the turbidity was determined by nephelometer obtaining the value designated as $\mathbf{d_1}$.

The tannin power of the wine was obtained by the formula:

$$\text{Tannin power (NTU/mL)} = (d_1 - d_0) / 0.08$$

3.4.8 Proanthocyanidins according with the polymerization degree

According with Sun et al. (1998) it is possible to evaluate proanthocyanidins according with the polymerization degree in order to measure the monomeric, oligomeric and polymeric fraction.

The wine was diluted 1:50 using 1 ml of red wine in a flask of 50ml and filled with distilled water. The absorbance at 280nm of the sample was read in spectrophotometer in order to evaluate the quantity of wine to be dealcoholized by rotary evaporation (V_initial).

Based on the results obtained for the absorbance at 280nm was used:

- 5 – 10 ml of wine if $Abs_{280}nm < 0.800$
- 3 – 5 ml of wine if $1.200 < Abs_{280}nm < 0.800$
- 0.5 – 3 ml of wine if $Abs_{280}nm > 1.200$

The wine was placed in a flasks 50ml and then in a rotary evaporator under vacuum at temperature of 30°C to obtain wine's dry extract. The sample obtained was diluted with 20ml of buffer solution followed by ultrasound bath to homogenize the solution.

Preconditioned neutral Sep-Pack cartridges (tC18 Sep-Pack and C18 Sep-Pack) were washed with 10ml of methanol, 20 ml of distilled water and 15ml of Buffer solution.

After the washing procedure, 20ml of sample previously obtained were passed through the cartridges followed by 10ml of buffer solution. Cartridges, then, were passed through nitrogen flux for 1 hour. The first extraction of the oligomeric and monomeric proanthocyanidins was done passing 25ml of ethyl acetate through the cartridges, followed by the extraction of the polymeric fraction by 15ml of methanol. These fractions were evaporated by rotary evaporator and, while the polymeric proanthocyanidins (F3) was added of 5ml of methanol, the monomeric and oligomeric proanthocyanidins, with the addition of 3ml of buffer solution, were passed through the cartridges after the washing process with methanol, water and buffer solution. After the drying process of the cartridges in the nitrogen flux for 1 hour, the monomeric fraction (F1) was extracted by the addition of 25ml of diethyl ether, while the oligomeric fraction (F2) was extracted with 15ml of methanol. The last drying process with rotary evaporator and the addition of 5ml of methanol allowed to obtain the samples that were read by spectrophotometry. For the preparation of the samples, for the spectrophotometer analysis, was used a solution formed by 2ml of sample (F1; F2; F3), 5ml of sulfuric acid and 5ml of methanol for the white, while for the sample to be analyzed, instead of 5ml of methanol, was used 5ml of vanillin solution. To conclude, was measured the maximum of absorbance at 500nm after 1:20 hour for F3 and F2, while 20 seconds for F1 (Sun, et al., 1998). The concentration of F1, F2 and F3 (expressed in mg/L) was determined using the following formula: [C] (mg/L) = (V_rs * Abs 500nm) / (B * V_initial). Where: V_rs = volume of methanol used to dissolve the three fractions (5 ml); B = inclination of the curve F1 (0.0081), F2 (0.0046) and F3 (0.0037).

3.5 Statistical Analysis

The role of statistical analysis is to understand the statistical significance of the obtained values during all the laboratory analysis. In this case it has the aim to highlight all the differences existing in the various samples and understand how the pruning technique on different training systems, the application of the MSW organic amendment and the interaction between them could influence the final wine's quality.

Results presented in this work are generally the average value of two analytical repetitions. The result from observations recorded after physiochemical analysis are an average obtained by Tukey test in Rstudio program.

In order to check statistical significance of treatments software Rstudio was used. All treatments were analyzed by an ANOVA analysis of variance test and the Tuckey test in case of significance. The assumptions of ANOVA were confirmed by Shapiro-Wilk test or Lillie test, for the normality of the distribution of the data, and with Levene test for the homogeneity of the variances of the samples. Where the homogeneity was not validated with the test of Levene, Bartlett test or Breusch test was used. To determine significant statistic differences between each treatment the p value was analyzed and the symbol * was used to indicate the confidence level: "." for 0,1, * is equal and below 0.05, ** is below 0.01, *** is below 0.001. When no significant differences were found the letters "n.s." were used. In case of significant values, letters "a", "b" and "ab" were used to indicate the significative values: "a" means the higher value, "b" the lower value and "ab" the similarity between values.

4 Results and Discussion

4.1 Yield, Grape and Must analysis

The data of yield (Table 2.), main qualitative characteristics of grape (Table 4.) and must (Table 6.) were conducted on the harvest date 15 September 2020. The grapes were hand harvested and the sample were divided per each block, type of training system and MSW input.

Regarding yield parameters (Table 3.) there were not statistical significative results for the exception of weight / N° bunches, in the MSW treatment, with a p value of 0.1 that usually is not taken in consideration for the validity of the experiments. Anyways, a trend of higher values in LC regarding the yield, bunch weight and berry weight (Table 5.) was detected in comparison with HC, as observed by Coletta, et al., (2014). Furthermore, MSW, as demonstrated by Botelho, et al. (2021b; 2020b), showed in M3 the highest value of yield with the smallest values of berry weight (weight / 100 berries), suggesting that the berries number is higher in comparison with the other amount of MSW. Unfortunately data of vegetative index that could have may let understand better the effects of the MSW on the vegetive growth were not available.

Table 2. Yield components results collected on 15 September 2020

Treatment	N° Bunches	Bunches weight (kg)	Weight / N° Bunches	Yield (tons/ha)
LC x B1 x Test	48	6.47	135	29.1
LC x B1 x M1	48	7.43	155	24.1
LC x B1 x M2	68	7.23	106	24.3
LC x B1 x M3	71	7.50	106	31.7
LC x B2 x Test	63	8.73	139	29.3
LC x B2 x M1	38	5.15	136	24.3
LC x B2 x M2	42	5.47	130	24.5
LC x B2 x M3	52	9.00	173	31.9
HC x B1 x Test	57	7.10	125	23.2
HC x B1 x M1	49	7.33	150	28.0
HC x B1 x M2	44	5.67	129	19.8
HC x B1 x M3	37	6.53	176	24.7
HC x B2 x Test	48	5.00	104	23.4
HC x B2 x M1	50	7.27	145	28.2
HC x B2 x M2	51	4.70	92	20.0
HC x B2 x M3	66	8.37	127	24.9

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 3. Statistical analysis of yield components affected by training system (TS), different input of MSW and their interaction

Treatment	N° bunches	Bunches weight (kg)	Weight / N° bunches	Yield (tons/ha)
LC	53.8	7.13	133	27.4
HC	50.1	6.25	125	24.0
TS effect	n.s	n.s.	n.s.	n.s.
TEST	53.8	6.83	126 ab	26.2
M1	46.6	6.80	146 a	26.1
M2	51.2	5.77	113 b	22.2
M3	56.2	7.36	131 ab	28.3
MSW effect	n.s.	n.s.	.	n.s.
TS * MSW	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant, 5 % level by F test; . , * , ** , *** = significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

Table 4. Main qualitative characteristics of grapes collected on 15 September 2020

Treatment	Weight / 100 berries (g)	Must (ml)	°Brix	pH	Titratable Acidity (g/L)	Total Anthocyanins (mg/L)	Total Phenols (mg/L)
LC x B1 x Test	121	66	21.0	3.08	6.30	648	640
LC x B1 x M1	123.2	64	21.9	3.10	6.75	881	911
LC x B1 x M2	109.8	47	23.8	3.14	7.20	790	873
LC x B1 x M3	107.4	47	20.1	3.11	6.90	605	761
LC x B2 x Test	129	59	24.4	3.22	6.60	667	776
LC x B2 x M1	125.4	59	24.3	3.15	6.30	749	837
LC x B2 x M2	115.4	55	23.3	3.26	6.45	672	763
LC x B2 x M3	128.8	66	23.0	3.24	6.15	634	711
HC x B1 x Test	106	51	18.9	3.06	6.60	461	651
HC x B1 x M1	111.8	49	21.5	3.13	6.60	622	861
HC x B1 x M2	112.2	53	20.3	3.16	6.00	630	890
HC x B1 x M3	104.4	53	19.6	3.13	6.90	522	783
HC x B2 x Test	124.8	59	19.9	3.15	6.75	705	875
HC x B2 x M1	-	-	-	-	-	-	-
HC x B2 x M2	151.8	69	21.1	3.20	6.90	640	736
HC x B2 x M3	110	56	18.7	3.11	7.35	458	589

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number. "-" = data unavailable.

Table 5. Statistical analysis of berries analysis affected by training system (TS), different input of MSW and their interaction

Treatment	Weight / 100 berries (g)	Vol must (ml)	°Brix	pH	Titratable acidity (g/L)	Anthocyanins (mg/L)	Total phenols (mg/L)
LC	119	54.4	22.6 a	3.18 a	6.67	703 a	799
HC	114	52.2	19.9 b	3.13 b	6.75	575 b	761
TS effect	n.s	n.s.	***	*	n.s.	***	n.s.
TEST	116	54.7	21.1	3.14	6.75	637 ab	751
M1	118	53.3	21.7	3.14	6.60	693 a	834
M2	119	52.3	21.8	3.18	6.78	670 ab	820
M3	114	53.0	20.4	3.17	6.72	555 b	714
MSW effect	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
TS * MSW	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant, 5 % level by F test; . , * , ** , *** = significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

Concerning the main qualitative characteristics of the berries (Table 5.), total soluble solids and pH resulted statistically significative lower in HC, confirmed also in the wine analysis (Table 10.). Furthermore, LC resulted statistically higher in anthocyanins than HC. These effects were observed by the research of Coletta, et al. (2014). On the other hand, the difference in anthocyanins between the two training systems was not revealed on the wine's color and phenols analysis (Table 20.). The supply of the highest amount of MSW (M3) resulted with the lowest amount of total soluble solids (°Brix) and, even if it was not statistically significant in the grapes and must analysis, the differences were more accentuate by the alcohol content in the wine analysis (Table 10.). Furthermore, with the increasing of amount of amendment, M2 and M3, the pH tended to decrease. The trend on the decrease of total soluble solids (TSS) and pH was already observed in the application of MSW (Botelho, et al., 2021b). Delgado et al. (2004) attribute the decrease in TSS to the increase in vine vigour caused by high N supply, which changes the balance in carbon partitioning, thus favouring vegetative growth over reproductive growth.
Finally, the phenolic composition of the berries was not affected by the application of MSW, while anthocyanins were statistically lower in M3 in comparison with the other amounts. The decrease of anthocyanins was already explained by Botelho, et al., (2021b) due to the increase in productivity that some organic amendments promote.

Table 6. Main characteristics analysis of the must analyzed on 18 September 2020

Sample	°Brix	pH	Titratable acidity	Assimilable nitrogen (mg/L)
LC x B1 x Test	19.4	2.68	6.75	192.5
LC x B1 x M1	19.9	2.91	5.77	218.8
LC x B1 x M2	19.2	2.89	6.00	183.8
LC x B1 x M3	19.2	2.92	5.32	166.2
LC x B2 x Test	23.4	3.05	4.95	183.8
LC x B2 x M1	21.9	2.99	4.95	183.8
LC x B2 x M2	22.8	3.11	4.57	218.8
LC x B2 x M3	20.0	3.08	5.47	175.0
HC x B1 x Test	18.8	2.91	5.47	166.2
HC x B1 x M1	19.8	2.95	4.95	183.8
HC x B1 x M2	19.3	2.95	5.10	148.8
HC x B1 x M3	19.2	2.93	5.32	148.8
HC x B2 x Test	19.5	2.95	5.47	131.2
HC x B2 x M1	19.6	2.98	5.32	183.8
HC x B2 x M2	20.1	3.06	5.62	218.8
HC x B2 x M3	17.2	2.95	5.70	236.2

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 7. Statistical analysis of musts parameters affected by training system (TS), different input of MSW and their interaction

Treatment	°Brix	pH	Titratable acidity (g/L tartaric acid)	Assimilable nitrogen (mg/L)
LC	20.7	2.98	5.47	190
HC	19.2	2.96	5.37	177
TS effect	n.s.	n.s.	n.s.	n.s.
TEST	20.3	2.94	5.66	168
M1	20.3	2.96	5.25	192
M2	20.4	3.00	5.33	192
M3	18.9	2.97	5.46	182
MSW effect	n.s.	n.s.	n.s.	n.s.
TS * MSW	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant, 5 % level by F test; . , * , ** , *** = significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

4.2 Basic chemical characteristics of the wine

The alcoholic fermentation for all the wines completed with low levels of reducing substances (Table 8.). The wines were analyzed before the malolactic fermentation (Table 8.) and after the malolactic fermentation (Table 9.). The methods of analysis for the alcohol content for the wines before MLF and after MLF were respectively by ebulliometry and by distillation (as described on material and methods). The distillation method is considered more precise, thus was used for the statistical analysis (Table 10.). Furthermore, after the wines analysis (Table 8 and 9) the sulphur dioxide was adjusted to 35 mg/L of free sulphur.

The wines didn't present high concentration of acetic acid (low volatile acidity) and the differences revealed on the training systems (Table 10.) could be attribute to vinification issues.

According to the berry analysis, the wines followed the same results in terms of alcohol content and pH, both for the training systems and the MSW. Contrarywise, any differences were not found on total acidity and total dry extract for the training systems, while M3 showed the lowest value of total dry extract.

Table 8. Physico-chemical analysis of the wines before the malolactic fermentation

Sample	Alcohol (% Vol.)	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	Free sulphur dioxide (mg/L)	Total sulphur dioxide (mg/L)	pH	Reducing substances (g/L)
LC x B1 x Test	11.4	11.40 [1]	0.48	9	12	3.05	0.1
LC x B1 x M1	11.6	8.70	0.59	7	15	3.03	0.3
LC x B1 x M2	10.7	9.30	0.38	6	15	2.96	0.8
LC x B1 x M3	10.9	7.80	0.44	7	12	3.14	0.2
LC x B2 x Test	13.6	5.85	0.44	11	20	3.15	0.8
LC x B2 x M1	13.4	12.15 [1]	0.43	10	18	3.07	0.2
LC x B2 x M2	13.6	7.50	0.44	11	20	3.22	0.7
LC x B2 x M3	11.9	7.20	0.44	9	15	3.23	0.2
HC x B1 x Test	10.4	8.40	0.38	8	15	3.05	0.2
HC x B1 x M1	12.0	8.25	0.45	9	15	3.14	0.6
HC x B1 x M2	11.1	7.50	0.34	10	15	3.22	0.4
HC x B1 x M3	10.5	8.25	0.43	9	15	3.15	0.4
HC x B2 x Test	11.3	8.10	0.33	10	15	3.09	0.6
HC x B2 x M1	11.3	9.90	0.30	10	18	3.07	0.1
HC x B2 x M2	11.7	6.15	0.37	9	18	3.12	0.7
HC x B2 x M3	8.8	8.70	0.32	9	10	2.99	0.1

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

[1] : abnormal values

Table 9. Physico-chemical analysis of the wines after the malolactic fermentation

Sample	Alcohol (% Vol.)	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	Free sulphur dioxide (mg/L)	Total sulphur dioxide (mg/L)	pH	Total Dry Extract (g/L)
LC x B1 x Test	10.9	6.75	0.48	33	66	3.12	27.1
LC x B1 x M1	11.3	7.01	0.59	31	75	3.10	28.9
LC x B1 x M2	10.5	7.76	0.38	26	67	3.01	27.9
LC x B1 x M3	10.6	6.79	0.44	35	85	3.23	25.8
LC x B2 x Test	13.6	6.19	0.44	33	76	3.28	29.4
LC x B2 x M1	12.9	6.71	0.43	32	74	3.22	29.4
LC x B2 x M2	13.3	5.93	0.44	42	81	3.47	29.7
LC x B2 x M3	11.3	5.70	0.44	34	94	3.37	26.1
HC x B1 x Test	10.4	6.94	0.38	34	78	3.04	28.1
HC x B1 x M1	12.0	6.45	0.45	36	79	3.18	30.5
HC x B1 x M2	11.1	6.38	0.34	36	80	3.27	27.9
HC x B1 x M3	10.5	6.30	0.43	33	82	3.18	28.7
HC x B2 x Test	11.1	6.83	0.33	29	75	3.03	27.9
HC x B2 x M1	11.1	6.90	0.30	38	92	3.07	27.9
HC x B2 x M2	11.5	6.60	0.37	42	89	3.16	28.1
HC x B2 x M3	7.9	7.20	0.32	36	85	3.00	24.8

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 10. Statistical Analysis of physico-chemical parameters of Marselan wine after the malolactic fermentation influenced by training system (TS), MSW and their interaction

Treatment	Alcohol (%Vol.)	Total acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	pH	Total Dry Extract (g/L)
LC	11.8 a	6.60	0.46 a	3.22 a	28.0
HC	10.7 b	6.70	0.37 b	3.11 b	28.0
TS effect	**	n.s.	***	*	n.s.
TEST	11.5 a	6.67	0.42	3.12	28.1 a
M1	11.8 a	6.77	0.44	3.14	29.2 a
M2	11.6 a	6.67	0.38	3.22	28.4 a
M3	10.1 b	6.50	0.41	3.19	26.4 b
MSW effect	**	n.s.	n.s.	n.s.	***
TS * MSW	n.s.	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant, 5 % level by F test; . , * , ** , *** = significant at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

4.3 Chromatic characteristics and phenolic compounds of the wines

4.3.1 Phenolic composition

The HC training enhanced the non-flavonoids content, in comparison with LC, without affecting total phenol content (Table 12.). May, a delay on grape maturation could be a possible effect promoted by the HC training system.

The treatment M3 showed the lowest values for total phenols, total phenols index, flavonoids (Table 12.) and anthocyanins (Table 20.). A possible explanation could be that high amount of N enhanced the vegetative growth obtaining a vine exceeding in vigour with lower exposition of the grapes to the sun, thus a lower accumulation of phenolic compounds. Anyway, any vegetative index is not available, therefore more studies should be conducted to confirm the data.

Finally, LC resulted higher in tannin power (Table 16.) than HC. Tannin power is the measurement of the quantity of proanthocyanidins that precipitate with the proteins, and it is correlated with the astringency perception of the wines. This result was confirmed by the higher values of total proanthocyanindins in LC in comparison with HC (Table 14.).

Table 11. Total phenols index, total phenols, flavonoids and non-flavonoids of wines after MLF

Samples	Total phenols index (a.u.)	Total phenols (mg/L gallic acid)	Flavonoids (mg/L gallic acid)	Non-Flavonoids (mg/L gallic acid)
LC x B1 x Test	28.1	741	696	45
LC x B1 x M1	27.7	729	671	58
LC x B1 x M2	27.4	721	651	70
LC x B1 x M3	34.0	895	845	50
LC x B2 x Test	41.4	1089	934	155
LC x B2 x M1	39.4	1037	890	147
LC x B2 x M2	41.2	1085	895	191
LC x B2 x M3	26.9	708	548	160
HC x B1 x Test	27.0	711	559	152
HC x B1 x M1	39.7	1046	871	175
HC x B1 x M2	36.9	971	807	165
HC x B1 x M3	33.9	894	717	176
HC x B2 x Test	36.4	959	793	165
HC x B2 x M1	37.9	998	790	208
HC x B2 x M2	39.6	1044	827	217
HC x B2 x M3	25.6	674	524	150

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 12. Statistical Analysis of phenolic compounds characteristics of wine after MLF influenced by training system (TS). MSW and their interaction

Treatment	Total phenols index	Total phenols (mg/L gallic acid)	Flavonoids (mg/L gallic acid)	Non-Flavonoids (mg/L gallic acid)
LC	35.9	876	766	109 b
HC	37.2	912	736	176 a
TS effect	n.s	n.s.	n.s.	***
TEST	33.2	875	746	129
M1	36.2	953	805	147
M2	36.3	955	795	160
M3	30.1	793	658	134
MSW effect	n.s.	n.s.	n.s.	n.s.
TS * MSW	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at $p < 0.1$. $p < 0.05$. $p < 0.01$ and $p < 0.001$ respectively.

Table 13. Proantocyanidins compositions of wines after MLF (Sun. 1988 method)

Treatment	Polimeric fraction (mg/L)	Oligomeric fraction (mg/L)	Monomeric fraction (mg/L)	Total proanthocyanidins (mg/L)
LC x B1 x Test	78.4	16.2	14.5	109.0
LC x B1 x M1	118.9	38.6	16.6	174.1
LC x B1 x M2	162.2	28.2	14.1	204.4
LC x B1 x M3	260.7	18.2	12.2	291.0
LC x B2 x Test	375.3	53.3	14.0	442.5
LC x B2 x M1	181.8	32.4	10.4	224.6
LC x B2 x M2	170.9	35.4	16.4	222.7
LC x B2 x M3	144.3	20.8	16.3	181.4
HC x B1 x Test	124.9	15.9	12.5	153.3
HC x B1 x M1	250.7	15.2	16.4	282.2
HC x B1 x M2	167.6	41.3	23.2	232.0
HC x B1 x M3	178.5	27.4	8.9	214.8
HC x B2 x Test	181.8	21.6	12.9	216.3
HC x B2 x M1	121.2	22.9	10.4	154.6
HC x B2 x M2	117.6	27.7	18.3	163.5
HC x B2 x M3	128.1	22.9	16.5	167.6

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 14. Influence of training system (TS). MSW and their interaction on proanthocyanidins parameters of wines after MLF

Treatment	Polimeric fraction (mg/L)	Oligomeric fraction (mg/L)	Monomeric fraction (mg/L)	Total proanthocyanidins (mg/L)
LC	193.1	30.4	14.3	237.8
HC	158.7	24.4	14.9	198.0
TS effect	n.s	n.s.	n.s.	n.s.
TEST	190.0	26.8	13.5	230.3
M1	181.2	27.3	13.5	222.0
M2	154.6	33.1	18.0	205.7
M3	177.9	22.3	13.5	213.7
Amend. effect	n.s.	n.s.	n.s.	n.s.
TS * Amend	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at $p < 0.1$. $p < 0.05$. $p < 0.01$ and $p < 0.001$ respectively.

Table 15. Tannin power (De Freitas e Mateus. 2001 method) of wines after MLF

Sample	Tannin Power (NTU/mL)
LC x B1 x Test	184
LC x B1 x M1	218
LC x B1 x M2	315
LC x B1 x M3	266
LC x B2 x Test	359
LC x B2 x M1	347
LC x B2 x M2	334
LC x B2 x M3	240
HC x B1 x Test	181
HC x B1 x M1	269
HC x B1 x M2	278
HC x B1 x M3	218
HC x B2 x Test	258
HC x B2 x M1	293
HC x B2 x M2	283
HC x B2 x M3	82

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 16. Influence of training system (TS). MSW and their interaction on wines after MLF

Treatment	Tannin Power (NTU/mL)
LC	283 a
HC	233 b
TS effect	*
TEST	246
M1	282
M2	303
M3	202
MSW effect	n.s.
TS * MSW	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at $p < 0.1$. $p < 0.05$. $p < 0.01$ and $p < 0.001$ respectively.

4.3.2 Chromatic characteristic and pigments

In the grape analysis (Table 5.) differences in total anthocyanins were observed between the sample deriving from different training systems, while in the wine analysis the total anthocyanin content was not significantly different (Table 20.). Only the polymerization index (%), polymerized pigment (a.u.) (Table 20.), and copigmentation percentage (Table 22.) were influenced by the training system treatment, with higher values in LC. It can be supposed that LC training system enhanced the accumulation of anthocyanins and proanthocyanidins and promoted their interaction. Regarding the colour profile of the wines, change in intensity and tonality were not observed (Table 18. and 20.) among the training systems. Contrarywise, HC training brought to a significative reduction in “b*” and “H” values, in CIELab analysis (Table 24.), indicating an increase of blue tonalities.

The most significative results were obtained for M3 wines. Total anthocyanin content (mg/L), degree of ionization of anthocyanins (%), ionized anthocyanin content (mg/L), total pigment (a.u.), polymerization index (%), and polymerized pigments (a.u.) were negatively influenced by 20.000 kg/ha of MSW (M3).

M3 treatment negatively influenced also colour intensity and showed the highest value of tonality. High values of tonality change the relation between absorbance at 520 nm (red) and at 420 nm (yellow) in favor of the yellow tonality. These resulted were confirmed by the CIELab analysis (Table 24.) were a decrease of “a*” (red) and an increase of “b*” (increase of yellow at the expense

of blue) were recorded. The lower level of blue was also confirmed by the higher H value. Furthermore, a decrease of C value was also detected in M3 as in correlation with the decrease of colour intensity.

Finally, the values obtained for colour difference (ΔE), between each LC and HC wines (Table 25.), all show values higher than 2 CIELab units, indicating that these colour differences can be discriminated visually (Spagna et al., 1996).

Table 17. Chromatic characteristics (OIV. 2021 method) for intensity and tonality of wines after malolactic fermentation (MLF)

Sample	Intensity	Tonality
LC x B1 x Test	3.595	0.623
LC x B1 x M1	3.990	0.591
LC x B1 x M2	3.726	0.598
LC x B1 x M3	4.153	0.670
LC x B2 x Test	8.977	0.535
LC x B2 x M1	7.637	0.539
LC x B2 x M2	7.715	0.602
LC x B2 x M3	3.668	0.650
HC x B1 x Test	2.636	0.641
HC x B1 x M1	5.884	0.565
HC x B1 x M2	5.173	0.608
HC x B1 x M3	3.617	0.612
HC x B2 x Test	5.908	0.555
HC x B2 x M1	5.796	0.563
HC x B2 x M2	6.929	0.576
HC x B2 x M3	1.540	0.785

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 18. Intensity and tonality of wines as affected by training system (TS). MSW and their interaction

Treatment	Intensity	Tonality
LC	5.432	0.601
HC	4.685	0.613
TS effect	n.s	n.s.
TEST	5.279 a	0.589
M1	5.827 a	0.565
M2	5.885 a	0.596
M3	3.244 b	0.680
Amend. effect	**	n.s.
TS * Amend	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at p < 0.1. p < 0.05. p < 0.01 and p < 0.001 respectively.

Table 19. Chromatic characterization. anthocyanins and pigments (Somers and Evans. 1977 method) of wines after MLF

Sample	Color Intensity (a.u.)	Tonality (a.u.)	Total Anthocyanins (mg/L malvidin 3-O-glucoside)	Degree of ionization of anthocyanins (%)	Ionized anthocyanins (mg/L malvidin 3-O-glucoside)	Total Pigments (a.u.)	Polymerization index (%)	Polymerized pigment (a.u.)
LC x B1 x Test	3.444	0.631	148	10.3	15.2	9.3	12.2	1.14
LC x B1 x M1	3.842	0.599	156	11.9	18.5	9.8	12.6	1.24
LC x B1 x M2	3.577	0.605	145	11.1	16.1	9.3	13.1	1.21
LC x B1 x M3	4.022	0.685	152	12.9	19.7	9.5	12.1	1.16
LC x B2 x Test	8.786	0.539	248	20.3	50.2	16.7	15.4	2.56
LC x B2 x M1	7.451	0.541	205	19.0	39.1	14.2	16.6	2.35
LC x B2 x M2	7.567	0.605	269	13.6	36.7	17.3	13.4	2.31
LC x B2 x M3	3.447	0.653	150	11.1	16.6	9.2	11.1	1.03
HC x B1 x Test	4.022	0.685	152	12.9	19.7	9.5	12.1	1.16
HC x B1 x M1	2.610	0.666	139	9.9	13.7	8.2	9.1	0.75
HC x B1 x M2	5.563	0.578	260	14.1	36.8	15.2	8.7	1.33
HC x B1 x M3	4.887	0.621	240	11.6	27.8	14.2	9.3	1.31
HC x B2 x Test	5.828	0.551	152	25.8	39.2	10.0	14.4	1.44
HC x B2 x M1	5.775	0.566	163	20.8	33.9	10.9	15.0	1.63
HC x B2 x M2	6.892	0.577	223	17.0	38.0	14.5	13.8	2.01
HC x B2 x M3	1.527	0.784	67	9.8	6.6	4.1	11.3	0.46

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 20. Color intensity. tonality. total anthocyanins. degree of ionization of anthocyanins. ionized anthocyanins. total pigments. polymerization index and polymerized pigments in wines after MLF.

Treatment	Color Intensity (a.u.)	Tonality (a.u.)	Total Anthocyanins (mg/L malvidin 3-O-glucoside)	Degree of ionization of anthocyanins (%)	Ionized anthocyanins (mg/L malvidin 3-O-glucoside)	Total Pigments (a.u.)	Polymerization index (%)	Polymerized pigment (a.u.)
LC	5.267	0.607	184	13.8	26.5	11.9	13.3 a	1.62 a
HC	4.566	0.619	181	14.9	27.0	11.1	11.2 b	1.23 b
TS effect	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	**
TEST	5.167 a	0.597	172 ab	16.7 a	29.6 a	11.0 ab	12.8 ab	1.47 a
M1	5.658 a	0.571	196 ab	16.4 a	32.1 a	12.5 ab	13.2 a	1.64 a
M2	5.731 a	0.602	219 a	13.4 ab	29.6 a	13.8 a	12.4 ab	1.71 a
M3	3.110 b	0.682	143 b	11.0 b	15.7 b	8.6 b	10.7 b	0.90 b
MSW effect	**	n.s.	*	*	**	*	*	***
TS * MSW	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at $p < 0.1$. $p < 0.05$. $p < 0.01$ and $p < 0.001$ respectively.

Table 21. Color due to copigmentation (Boulton, 2001 method) parameters of wines after MLF

Sample	CC (a.u.)	CC %
LC x B1 x Test	0.26	38.1
LC x B1 x M1	0.31	39.4
LC x B1 x M2	0.30	38.6
LC x B1 x M3	0.28	37.6
LC x B2 x Test	0.46	29.4
LC x B2 x M1	0.43	31.1
LC x B2 x M2	0.44	31.9
LC x B2 x M3	0.23	32.9
HC x B1 x Test	0.23	38.3
HC x B1 x M1	0.45	41.4
HC x B1 x M2	0.38	38.3
HC x B1 x M3	0.30	38.8
HC x B2 x Test	0.39	39.9
HC x B2 x M1	0.44	40.0
HC x B2 x M2	0.50	40.0
HC x B2 x M3	0.13	40.5

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 22. Influence of training system (TS). MSW and their interaction on copigmentation proprieties of wines after MLF

Treatment	CC (a.u.)	CC%
LC	0.34	39.6 a
HC	0.35	34.9 b
TS effect	n.s.	***
TEST	0.33 a	36.4
M1	0.41 a	38.0
M2	0.40 a	37.2
M3	0.24 b	37.4
Amend. effect	***	n.s.
TS * Amend	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at $p < 0.1$. $p < 0.05$. $p < 0.01$ and $p < 0.001$ respectively.

Table 23. Color characterization of the wines after MLF with CIELab (OIV. 2021) method.

Sample	a*	b*	L*	C	H
LC x B1 x Test	13.51	-1.28	88.58	92.03	-0.09
LC x B1 x M1	15.48	-1.67	87.13	121.16	-0.11
LC x B1 x M2	14.65	-1.49	88.05	108.38	-0.10
LC x B1 x M3	14.81	-0.52	86.87	109.85	-0.03
LC x B2 x Test	31.13	-4.45	73.47	494.49	-0.14
LC x B2 x M1	27.77	-3.89	76.71	393.19	-0.14
LC x B2 x M2	25.39	-2.58	76.99	325.68	-0.10
LC x B2 x M3	12.68	-1.01	88.91	80.85	-0.08
HC x B1 x Test	12.05	-1.56	90.71	73.87	-0.13
HC x B1 x M1	24.92	-3.73	79.85	317.48	-0.15
HC x B1 x M2	21.48	-3.07	81.01	235.47	-0.14
HC x B1 x M3	16.45	-3.16	86.97	140.26	-0.19
HC x B2 x Test	24.09	-4.32	81.50	299.57	-0.18
HC x B2 x M1	23.20	-4.25	81.63	278.22	-0.18
HC x B2 x M2	25.90	-4.26	78.64	344.49	-0.16
HC x B2 x M3	5.64	-0.49	95.80	16.04	-0.09

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

Table 24. Statistical analysis of CIELab parameters of wines after MLF affected by training system (TS). MSW. and their interaction

Treatment	a*	b*	L*	C	H
LC	19.43	-2.11 a	83.34	215.70	-0.10 a
HC	19.22	-3.10 b	84.52	213.17	-0.15 b
TS effect	n.s	*	n.s.	n.s.	***
TEST	20.2 a	-2.90 b	83.57	239.99 a	-0.14 ab
M1	22.8 a	-3.39 b	81.33	277.51 a	-0.15 b
M2	21.9 a	-2.85 b	81.17	253.50 a	-0.13 ab
M3	12.4 b	- 1.29 a	89.64	86.75 b	-0.10 a
Amend. effect	**	**	n.s.	**	*
TS * Amend	n.s.	n.s.	n.s.	n.s.	n.s.

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test – 0 kg/ha; M1 – 5.000 kg/ha; M2 – 10.000 kg/ha; M3 – 20.000 kg/ha. Statistical significance: n.s. = not significant. 5 % level by F test; . . * . ** . *** = significant at $p < 0.1$. $p < 0.05$. $p < 0.01$ and $p < 0.001$ respectively.

Table 25. Total colorimetric differences (ΔE) between wines obtained with two different training systems

Sample	ΔE
LCxB1xT vs HCxB1xT	2.60
LCxB1xM1 vs HCxB1xM1	12.10
LCxB1xM2 vs HCxB1xM2	9.94
LCxB1xM3 vs HCxB1xM3	3.11
LCxB2xT vs HCxB2xT	10.68
LCxB2xM1 vs HCxB2xM1	6.72
LCxB2xM2 vs HCxB2xM2	2.41
LCxB2xM3 vs HCxB2xM3	9.86

Training systems: LC = Low Cordon; HC = High Cordon. Amount of MSW supplied: Test = 0 kg/ha; M1 = 5.000 kg/ha; M2 = 10.000 kg/ha; M3 = 20.000 kg/ha. B = block number.

5 Conclusions

The aim of the present work was to evaluate the effects of mechanical pruning on two different training systems (Low cordon (LC) and high cordon (HC)), and three different concentrations of organic amendment (municipal solid waste compost) on Marselan wines quality. The analyzed parameters were the yield components, main qualitative characteristics of grapes, basic analytical parameters, phenolic composition, and chromatic characteristics of the wines. Regarding the training systems, LC generally led to higher values of Brix, pH and anthocyanins on grapes composition. These results were confirmed with the wine classical analysis which revealed that samples treated with LC had higher alcohol content and pH. Furthermore, according to the phenolic composition, LC samples presented the lower value of non-flavonoids while promoted the tannin power, the copigmentation and polymerization between anthocyanins and flavonols. In term of colour characteristics, LC training negatively affected the blue tonalities of the wine, presenting an increase of b* and H values. The color change between wines obtained from the two different training systems were characterized by a $\Delta E > 2$, confirming that the differences could be observable by human eyes.

The organic treatment with the most significant effect in wine quality was M3 (highest supply of municipal solid waste). This treatment negatively influenced the grape composition in terms of total anthocyanins in comparison with the other concentrations. Regarding the wine composition, M3 led to a decrease of alcohol and total dry extract content. Furthermore, a negative correlation with the colour of the wines obtained with M3 was recorded. This treatment resulted to lower quality wines with lower quantity of anthocyanins, affecting the color intensity, the amount of ionized anthocyanins, the degree of ionization of anthocyanins, the total pigments, the polymerization index, the polymerized pigments and the copigmentation. According to the results of CIELab method, the wines obtained from M3 showed a decrease of general intensity, distinguished by C value, and the colour altered in lower red (a*) and blue (b*; H) tonalities.

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Annex

Annex 1- Reagents

- Distilled water
- Boiled water
- Iodure/Iodate $KIKIO_3$ N/64 obtained with 1 liter of distilled water and Ioddide Iodate concentrate 38064-1EA
- Solution 941 = Sulfuric acid 1:3 v/v (H_2SO_4)
- Solution 908 = NaOH 2N
- Solution 932 = Solfuric acid 1/10 (v/v) (H_2SO_4)
- Tartaric acid. crystalline
- Sodium hydroxide solution. 0.1 M.
- Phenolphthalein solution. 1%. in neutral alcohol. 96% (*m/v*).
- Hydrochloric acid (ρ20 = 1.18 to 1.19 g/mL) diluted 1/4 with distilled water.
- Iodine solution. 0.005 M.
- Potassium iodide. crystalline.
- Starch solution. 5 g/L.
- Saturated solution of sodium tetraborate “Borax”. Na2B4O7.10H2O. about 55 g/L at 20°C
- Bromothymol blue indicator solution. 4 g/l.
- Sodium hydroxide solution. 1M.
- Methanol. >= 99.8%
- Ethyl acetate. >= 99.8%
- Diethyl ether. >= 99%
- Buffer solution pH 7.0 = 9.84g Na_2HPO_4 + 2.73g KH_2PO_4 dissolved in 1 liter of distilled water
- Sulfuric acid. 25:75 v/v with methanol
- Vanillin 99% (MercK. ref[a].8510)
- Vanillin solution. 1% vanillin 99% in methanol

Annex 2- Materials and Instrumentations

- Parafilm
- Volumetric pipette
- Backers
- Volumetric flasks 50 and 100ml
- Vacuum flasks
- Densimeter 45 mN/m – g/dm^3
- Cuvettes with 1mm and 1cm of optical thickness

- Vacuum pump
- Preconditioned neutral Sep-Pack cartridges
- Distillator: Gerhardt VAP300
- pH meter: Thermo Scientific ORION STAR A211
- Mixer: Velp Scientifica
- Centrifugal: HERMLE Z383K.
- Thermal Bath: Precisterm “Selecta”.
- Turbidimeter: “HACH” 2100N IS TURBIDIMETER ISO METHOD 7027.
- Spectrophotometer Cary 100 UV-Vis.
- Sulfimeter: Sulfilyser
- Ultrasounds bath: “Elma” TRANSSONIC 700
- Absorbance detector: Waters 2487 Dual λ Absorbance Detector

Annex 3- Monitored parameters during alcoholic fermentation: Low cordon. Block 1. Test

Date	Densities (g/cm^3)	Temperature °C
16/09	1085	25
17/09	1080	25
18/09	1045	27
19/09	1012	27
20/09	999	26
21/09	998	24
22/09	998	24

Annex 4- Monitored parameters during alcoholic fermentation: Low cordon. Block 1. M1

Date	Densities (g/cm^3)	Temperature °C
16/09	1089	25
17/09	1081	26
18/09	1040	27
19/09	1005	27
20/09	998	26
21/09	998	24
22/09	998	24

Annex 5- Monitored parameters during alcoholic fermentation: Low cordon. Block 1. M2

Date	Densities (g/cm^3)	Temperature °C
16/09	1089	25
17/09	1080	26
18/09	1041	27
19/09	1006	27
20/09	1000	26
21/09	1000	24
22/09	1000	24

Annex 6- Monitored parameters during alcoholic fermentation: Low cordon. Block 1. M3

Date	Densities (g/cm^3)	Temperature °C
16/09	1085	25
17/09	1079	26
18/09	1045	27
19/09	1018	27
20/09	1001	26
21/09	998	24
22/09	998	24

Annex 7- Monitored parameters during alcoholic fermentation: Low cordon. Block 2. Test

Date	Densities (g/cm^3)	Temperature °C
16/09	1099	25
17/09	1095	26
18/09	1062	27
19/09	1022	27
20/09	1004	26
21/09	996	24
22/09	995	24
23/09	995	24

Annex 8- Monitored parameters during alcoholic fermentation: Low cordon. Block 2. M1

Date	Densities (g/cm^3)	Temperature °C
16/09	1098	25
17/09	1093	26
18/09	1063	27
19/09	1032	27
20/09	1010	27
21/09	997	26
22/09	995	24
23/09	995	24

Annex 9- Monitored parameters during alcoholic fermentation: Low cordon. Block 2. M2

Date	Densities (g/cm^3)	Temperature °C
16/09	1102	25
17/09	1098	26
18/09	1056	27
19/09	1017	27
20/09	1001	26
21/09	996	24
22/09	996	24
23/09	996	24

Annex 10- Monitored parameters during alcoholic fermentation: Low cordon. Block 2. M3

Date	Densities (g/cm^3)	Temperature °C
16/09	1090	25
17/09	1086	26
18/09	1044	27
19/09	1006	27
20/09	997	26
21/09	996	24
22/09	996	24
23/09	996	24

Annex 11- Monitored parameters during alcoholic fermentation: High cordon. Block 1. Test

Date	Densities (g/cm^3)	Temperature °C
16/09	1078	25
17/09	1075	26
18/09	1037	27
19/09	1008	27
20/09	999	26
21/09	998	24
22/09	998	24

Annex 12- Monitored parameters during alcoholic fermentation: High cordon. Block 1. M1

Date	Densities (g/cm^3)	Temperature °C
16/09	1089	25
17/09	1086	26
18/09	1040	27
19/09	1007	27
20/09	998	26
21/09	997	24
22/09	997	24

Annex 13- Monitored parameters during alcoholic fermentation: High cordon. Block 1. M2

Date	Densities (g/cm^3)	Temperature °C
16/09	1083	25
17/09	1080	26
18/09	1045	27
19/09	1012	27
20/09	1001	26
21/09	997	24
22/09	997	24

Annex 14- Monitored parameters during alcoholic fermentation: High cordon. Block 1. M3

Date	Densities (g/cm^3)	Temperature °C
16/09	1088	25
17/09	1084	26
18/09	1043	27
19/09	1010	27
20/09	1000	26
21/09	1000	24
22/09	1000	24

Annex 15- Monitored parameters during alcoholic fermentation: High cordon. Block 2. Test

Date	Densities (g/cm^3)	Temperature °C
16/09	1082	25
17/09	1076	26
18/09	1043	27
19/09	1009	27
20/09	999	26
21/09	998	24
22/09	998	24

Annex 16- Monitored parameters during alcoholic fermentation: High cordon. Block 2. M1

Date	Densities (g/cm^3)	Temperature °C
16/09	1087	25
17/09	1085	26
18/09	1048	27
19/09	1019	27
20/09	1001	26
21/09	998	24
22/09	998	24

Annex 17- Monitored parameters during alcoholic fermentation: High cordon. Block 2. M2

Date	Densities (g/cm^3)	Temperature °C
16/09	1088	25
17/09	1086	26
18/09	1046	27
19/09	1009	27
20/09	1000	26
21/09	998	24
22/09	998	24

Annex 18- Monitored parameters during alcoholic fermentation: High cordon. Block 2. M3

Date	Densities (g/cm^3)	Temperature °C
16/09	1073	25
17/09	1070	26
18/09	1027	27
19/09	1013	27
20/09	1001	26
21/09	1000	24
22/09	1000	24