Foliar application of yeast derivatives at *veraison* to grapevines and their effects on the chemical composition

A bibliographic review

Maria Teresa Valadas de Albuquerque

Dissertation to obtain a Master's Degree in

Viticulture and Enology Engineering

Advisor: Doutor Jorge Manuel Rodrigues Ricardo da Silva

Júri:

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

Vogais: Doutor Jorge Manuel Rodrigues Ricardo da Silva, Professor Catedrático do Instituto Superior de Agronomia da Universidade de Lisboa, orientador.
Vogais: Doutor Manuel José de Carvalho Pimenta Malfeito Ferreira, Professor Auxiliar com Agregação do Instituto Superior de Agronomia da Universidade de Lisboa.

2019

Acknowledgments

I would first like to thank my master thesis advisor Professor Jorge Ricardo da Silva, of Instituto Superior de Agronomia at Universidade de Lisboa, who has supported me throughout my thesis with his knowledge and sympathy. He allowed this paper to be my own work and guided me to the right direction to achieve a good result.

Finally, I must express my very profound gratitude to my family, friends and boyfriend, for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this master thesis. This accomplishment would not have been possible without them.

Thank you.

Abstract

The present work was aimed to elaborate a bibliographic review about the importance and effects, in a viticultural and enological point of view, of a new and innovating product, the LalVigne®, that consists in inactive dry yeast (*Saccharomyces cerevisiae*) derivatives. It was discussed the ongoing challenges of climate change in wine industry, as the alterations of flowering, *veraison* and harvest, emphasizing the changes in the ripening process, the metabolism of the vine and also at sensorial level. It was referred in this work, some adaptation measures, being the application of LalVigne® the most important and the one which was developed throughout the work.

Elicitation topic went deeper, being referred what elicitor is and its mode of action. Bridging the gap between elicitors and its mode of action in vines, it was possible to develop the work about LalVigne®. The LalVigne® is an elicitor, consisting in inactive dry yeast derivatives, with foliar application. The characteristics of LalVigne®, it's mode of action, the advantages and disadvantages for viticulture and enology, as well as their side effects, were described.

There are two types of LalVigne®, Mature and Aroma, whose application is made in red and white grapes, respectively. Regarding the side effects, the use of LalVigne® AROMA had: improvements in the skin thickness; developments in the aroma precursors; no impact in berry weight, Brix, pH or Tartaric acid; enhancements in the glutathione concentration; increases in thiolic varieties as 3MH and 3MHA; and reduction of herbaceous or aggressive character. The use of LalVigne® MATURE had: improvements in the skin thickness; no impact in berry weight, Brix, pH or tartaric acid; a reduction of herbaceous or aggressive character; improvements in the concentration of extractable anthocyanins; improvements in the skin tannins; and developments in the degree of polymerization. Several trials were compared in this regard.

Keywords: climate change; elicitor; inactive dry yeasts; foliar application; berry quality.

Resumo

O presente trabalho teve como objetivo a elaboração de uma revisão bibliográfica sobre a importância e os efeitos, do ponto de vista vitícola e enológico, de um produto inovador, o LalVigne®, que consiste em derivados de leveduras inativas (*Saccharomyces cerevisiae*). Foram discutidos os desafios atuais das mudanças climáticas na indústria do vinho tais como: as alterações de floração, *veraison* e colheita, enfatizando as mudanças no processo de amadurecimento, no metabolismo da videira e, também, a nível sensorial. Foram referidas, algumas medidas de adaptação, sendo a aplicação do LalVigne® a mais importante e a que foi desenvolvida ao longo do trabalho.

A elicitação foi um tópico mais aprofundado, tendo sido referido o que é um elicitor e o seu modo de ação. Fazendo a ponte entre elicitores e o seu modo de ação em videiras, foi possível desenvolver o trabalho em volta do produto LalVigne®. LalVigne® sendo um elicitor, consiste em derivados de leveduras secas inativas, e tem uma aplicação foliar. As características do LalVigne®, o seu modo de ação, as vantagens e desvantagens para a viticultura e enologia, e os seus efeitos secundários, foram descritos.

Existem dois tipos de LalVigne®: Mature e Aroma, cuja aplicação é feita em uvas vermelhas e brancas, respetivamente. Em relação aos efeitos secundários, o uso do LalVigne® AROMA apresentou: melhorias na espessura da pele; desenvolvimentos nos precursores de aroma; nenhum impacto no peso do bago, Brix, pH ou ácido tartárico; melhorias na concentração de glutationa; aumentos nas variedades tiólicas como 3MH e 3MHA; e redução do caráter herbáceo ou agressivo. O uso de LalVigne® MATURE teve: melhorias na espessura da pele; nenhum impacto no peso do bago, Brix, pH ou ácido tartárico; uma redução do caráter herbáceo ou agressivo; melhorias na concentração de antocianinas extraíveis; melhorias nos taninos da película; e desenvolvimentos no grau de polimerização. Vários ensaios foram comparados a esse respeito.

Palavras-chave: alterações climáticas; elicitor; leveduras secas inativas; aplicação foliar; qualidade do bago de uva.

Resumo alargado

O presente trabalho tem como objetivo a elaboração de uma revisão bibliográfica sobre a importância e os efeitos, do ponto de vista vitícola e enológico, de um produto inovador, conhecido comercialmente como LalVigne®.

A indústria do vinho enfrenta, atualmente, uma multiplicidade de desafios introduzidos, particularmente, pelas alterações climáticas. Aqui se discutem aquelas que são consideradas como as principais consequências das referidas alterações, nomeadamente, ao nível da floração, da *veraison* e da colheita, enfatizando-se, igualmente, as mudanças mais relevantes introduzidas por esta realidade no processo de amadurecimento do fruto, no metabolismo da videira e, também, ao nível das propriedades organoléticas dos vinhos, o que se reflete em toda a experiência sensorial associada ao seu consumo.

Os desafios referidos exigem a adoção de estratégias que permitam responder e sobrelevar estas novas realidades e obrigam a intervenções devidamente adaptadas aos locais e às especificidades climáticas das regiões. Entre estas inclui-se a aplicação do LalVigne®, que surge como uma recente medida de adaptação e mitigação de algumas das dificuldades surgidas, sendo, por essa razão, a que foi desenvolvida ao longo do trabalho. Este produto, um *elicitor*, totalmente natural, inclui na sua composição frações específicas de leveduras inativas da espécie *Saccharomyces cerevisiae*, sendo aplicado, em pequenas quantidades e por pulverização, ao nível foliar, obrigando a respostas metabólicas das videiras que permitem melhorar diversas características e propriedades da planta, do seu fruto e dos vinhos produzidos.

Abordam-se e discutem-se as características do LalVigne®, o seu modo de ação (nomeadamente ao nível do reconhecimento pelos recetores da videira; da ativação da resposta por parte da planta; do metabolismo secundário por estimulação dos genes envolvidos na síntese de metabolitos; na melhoria da uva e do vinho), as vantagens e desvantagens para a viticultura (ao nível da proteção, da segurança, da homogeneidade, da rentabilidade e ao nível biológico) e para a enologia (referindo-se as características que são aumentadas, as que são reduzidas, a possibilidade de recorrer a menos auxiliares enológicos, a melhoria do vinho e da sua longevidade, o respeito pela diferenciação por parcelas) que resultam da sua utilização assim como os seus efeitos secundários.

São duas as formulações comerciais de LalVigne®: *Mature* (que influencia a maturidade fenólica) e *Aroma* (que influencia a acumulação de percursores de aroma). A primeira fórmula destina-se à aplicação em videiras que produzem uvas tintas e, a segunda, destina-se às que produzem uvas brancas. Apresentam-se, igualmente, os resultados obtidos com a utilização de cada uma destas fórmulas:

LalVigne® AROMA — introduziu melhorias ao nível da espessura da película das uvas, contribuindo para o respetivo aumento; incremento e antecipação da acumulação de precursores aromáticos bem como aumento e melhoria dos compostos varietais presentes; nenhum impacto no peso do bago, no ºBrix, no pH ou no ácido tartárico; melhorias na concentração de glutationa; nas variedades tiólicas, aumento de 3MH e 3MHA em vinhos; maior estabilidade dos compostos aromáticos e redução do caráter herbáceo ou agressivo;

LalVigne® MATURE — introduziu melhorias na espessura da película, não resultando qualquer impacto no peso do bago, no ºBrix, no pH ou no ácido tartárico; redução do caráter herbáceo ou agressivo; melhorias na concentração de antocianinas extraíveis; aumento da acumulação de taninos na película e aumento do grau de polimerização.

Fundamentam-se os resultados acima descritos em diversos ensaios comparativos.

A espessura da película aumentou no tratado de ambos os LalVigne®, o que, apesar de se saber que uma película mais fina caracteriza-se por uma maior libertação de pigmentos vermelhos, permitindo otimizar a maceração, de modo a expressar todo o potencial das uvas na elaboração de vinhos tintos de alta qualidade, para a mesma variedade o tratado teve uma espessura de película superior ao controlo, mas os mesmos resultados para a força de rutura necessária para fragmentar a película. Quer isto dizer que, apesar de mais espessas, as películas continuam a ser adequadas a uma boa maceração, e para uvas brancas e tintas a uva fica mais protegida.

Em relação ao peso do bago, pH, ácidos e ºbrix, nenhuma diferença significativa entre o controlo e o tratamento foi registada, sugerindo que o LalVigne® não tem impacto sobre esses parâmetros e que permanecem inalterados, quando o produto é aplicado.

O LalVigne®AROMA, aumentou as concentrações de glutationa no vinho, pelo fornecimento de precursores para a síntese de glutationa, durante a fermentação.

Também os tióis voláteis sofreram um aumento, sendo compostos importantes para o caracter varietal de um vinho e, consequentemente, os produtores desejam otimizar a formação destes compostos aromáticos.

Relativamente aos taninos, verificou-se um efetivo aumento da sua concentração nas uvas tratadas. A única análise que não teve resultados expectáveis foi a concentração de antocianinas, que apenas em um dos ensaios é que teve um aumento de antocianinas extraíveis.

Também, os vinhos de uvas tratadas foram submetidos a uma análise sensorial e, tal como previsto, houve um aumento da intensidade olfactiva com notas florais mais pronunciadas e uma diminuição considerável das notas vegetais.

Por último, um aumento dos valores dos índices de HCl e gelatina, para os vinhos de uvas tratadas, indicam uma estrutura de tanino mais polimerizada e equilibrada em comparação aos vinhos controlo. Houve uma menor concentração de catequinas monoméricas nos vinhos de uvas tratadas, o que pode ser explicado pela maior concentração de compostos fenólicos polimerizados. Também se descobriu que os polifenóis apresentaram um maior nível de polimerização e maciez nas uvas tratadas, e consequentemente, as notas adstringentes não eram tão evidentes.

Table of contents

List of figures

List of tables

1. Introduction

Grape crops are one of the oldest crops growing in the world. They were started two thousand years before Christ, in the oriental zone of black sea. Vineyard propagation was also largely driven by Greeks and Romans (Reynier, 2004). Such long history of grape growing greatly contributed to the large and geographically distinct viticulture regions worldwide, and thus the fine wines that our society currently enjoys (Unwin, 1991). Grape production has become one of the most important fresh fruit crop in the world, and is the world's most economically important fruit crop (Keller, 2010). It is a crop strongly established in Europe not only because of the tradition and appropriate environmental conditions, but also because of the Catholicism, that had a big importance on the wine consumption culture.

Currently 74% of grape production is routed for the wine production (Magalhães, 2015), whereas the remaining 26% are consumed fresh or as raisin. Winemaking is, therefore, the most important use of grapes, both in terms of production quantity and area (OIV, 2019). According to International Organization of Vine and Wine, (OIV, 2019) the world vine area was 7,4 millions of ha, in 2018, and the world wine production (excluding juice and musts) was estimated at 292.3 million hL (OIV, 2019). The world consumption of wine in 2018 was 246 millions of hectoliters (OIV, 2019).

A major constraint limiting the further growth of wine production is climate change. In the one hand it has been a problem for some areas considered highly appropriate to grape growing. On the other hand it has been a benefiting for regions conventionally inadequate (Moriondo *et al*., 2013).

Additional evidence has been published that contributes to the development of wine production. For instance, the beneficial effects of moderate alcohol consumption have been scientifically investigated because of cardiovascular mortality. Because of that, the "French paradox" was recognized (Fehér *et al.,* 2007) and gave a new interest on the consumption of this product, and for biological studies. Well-structured wines with deep color, fruit scents, soft tannins and pleasing mouthfeel are the demanding of wine consumers (Bruwer *et al.,* 2011), as well as moderate alcohol content, suitable with health concerns and the changing of taste of consumers (Caballero & Segura, 2017). Moreover, the moderate alcohol content must not compromise the color intensity, texture and sensory proprieties, in red wines case, being a new trend in the market, but also seen in white wines when is kept the acidity and primary bouquet (Palliotti *et al.,* 2014). Is possible to produce this type of wines (fresh and fruity) from grapes with a good maturation, an optimal level of phenolic and technological maturity, instead of over ripened grapes (Villangó *et al.,* 2015).

The climate change alongside with the new demands of the consumer are a challenge for the production of wine, therefore new adaptation measures are required in order to satisfy these new trends.

1.1. Climate Change

Grapes are a crop that are dependent of cultural practices, genotype of the cultivar (Keller, 2010), and their interaction with climate and weather (Mozell & Thach, 2014).

Climate has annual variations, that are particular important for wine production and for that matter, any change in climate and weather, might potentially disturb the wine industry (Mozell & Thach, 2014) and influence the olfactory and gustatory notes, that connects a wine to his region (Asproudi *et al.,* 2018). Additionally, these variations between growing seasons also vary the gene expression, more than in pre and post *veraison* (in the same season) (Pilati *et al.*, 2007; Keller 2010).

According to Fraga *et al.* (2012), from 1950 to 2004, the temperatures, during the growing season, have increased 1,7ºC, in Europe, and has been verified a warming pattern more pronounced at north hemisphere (increasing of 2,1ºC in de last 50 years).

Changing weather patterns will lead to a reduction of precipitation in sub-tropical regions, and an increasing in north latitudes and equator, and also a lack of fresh water supplies while some regions will dry more (Mozell & Thach, 2014). Also, more frequent heat events and annual or seasonal precipitation very variable have been confirmed.

1.2. Consequences of climate changes in viticulture

In several viticulture areas, ripening occurs during the warmest part of the season (Asproudi *et al.*, 2016) leading to the advancement of vine phenological stages, caused by climate changes, and hence earlier harvests, due to the temperature increasing of season growing (Kogkou *et al.,* 2017; Jones & Davis 2000; Chuine *et al*., 2004; Duchêne & Schneider, 2005; Wolfe *et al.,* 2005; Webb *et al.,* 2007). These changings in grape vine phenology has been stated in some grape growing areas (Duchêne & Schneider 2005; Petrie & Sadras 2008; Ramos *et al.,* 2008), and the rising temperatures are expected to continue (Dechêne *et al*., 2010). This may lead to future wine production in areas too cold for vineyards and the actual growing grapes regions will have to adapt to these changes (White *et al.,* 2006; Hall & Jones, 2009).

An upward shift in seasonal temperature changed the normal pattern of grape development resulting an earlier flowering, *veraison* and harvest (Keller, 2010). According to Keller (2010), “the timing of *veraison* is of major importance, since earlier *veraison* implies that the critical ripening period to occur during the hottest part of the season".

The balance of berry sensory characters is modified due to warming, water deficit (Palliotti *et al*., 2014), and different distribution of rainfall during ripening phase (Asproudi *et al*., 2018) leading to acceleration of berry shriveling and mesocarp cell death (Bonada *et al*., 2013).

Furthermore, considering that, recently, ripening has been occurring during the warmest part of the season, grape chemistry is adversely affected (Keller, 2010), mainly color and aroma profile (Asproudi *et al*., 2016; Mori *et al*., 2007). Under these conditions is likely to obtain: elevated fruit sugar, lower acid concentrations (especially malic acid), and lower anthocyanins and methoxypyrazine levels.

At high temperatures, more that 35ºC, the metabolism of the vine is inhibited (Mori *et al*., 2007) promoting advanced berry sugar accumulation, but incomplete phenolic ripeness (Kogkou *et al*., 2017) (lower accumulation of polyphenols), leading to an unbalanced timing of sugar/acid and polyphenolic optimal, especially in Mediterranean climates (Mori *et al*., 2007). When the berry exceeds this temperature is verified also a decrease in anthocyanin biosynthesis and/or degradation.

In warmer harvests, the ripening process is faster, therefore is not possible to get a balance between phenolic and technological (sugar) maturity (Hannah *et al*., 2013). There is going to be an increase in the sugar concentration followed by a rapid decrease in the titratable acidity and aromatic potential. As a result, it will be obtained unbalanced wines and too alcoholic (Villangó *et al*., 2015). The high sugar content might stop fermentations and produce a high content of unwanted by-products, like acetic acid and glycerol (Orduña, 2010).

From a sensorial point of view, the high content of alcohol, due to high content of sugar, has several organoleptic consequences: decreases the freshness and the perception of aromatic bouquet, due to the fact that ethanol may increase the perception of sweetness and bitterness while saltiness and sourness are reduced (Asproudi *et al*., 2018) also the ethanol impacts on the volatility of aromatic compounds, which decrease (Le Berre *et al*., 2007) due to the head-space apportioning. Moreover, the lack of optimal phenolic maturity leads to herbaceous and astringent wines (Jones *et al*., 2005).

The vegetative growth, vigor and vine longevity suffer some effects as well, like: uneven budburst caused by lack of cold, reduced growth of roots, leaves, shoots and berries, leaves sunburn, and fruitfulness, vigor and longevity reduction (Anderson, 2008).

At the soil level, the climate change had some effects, as increasing soil erosion and reduction of organic matter content (Brewik, 2013). Also, an altering incidence, timing, severity and type of pests have been verified, as well as a reduced quality and quantity of available water resources/ irrigation, leading to intense and frequent water stress (Keller, 2010).

Assuming that the change scenarios will be different is possible to expect that: the producing regions traditionally inadequate, due to the lack of heat load, are now considered more appropriated, in turn to have a complete ripening of the early grape varieties and also, the less early ripening varieties, can began to grow in these areas, while the other regions, considered highly appropriate to grape growing, now would give an excess of heat load comparing to needs of the presently grown cultivars (Moriondo *et al*., 2013).

In the one hand, the ripening process has been faster in dry and hot harvests, and likewise the balance between phenolic and technological maturity may not be sustained (Hannah et. al, 2013). On the other hand, in rainy and cool harvests, the maturation is slowed, and late ripening varieties cannot reach optimal ripeness (Jackson & Lombard, 2013). Therefore, in the future, areas with cooler temperatures than required for grape growing, will lead to an incomplete ripening, high acid, low sugar, and green flavors. Likewise, areas with warmer temperatures than required will produce over matured grapes, with low acid concentrations (especially malic acid), high sugar, high alcohol, cooked flavors (Santisi, 2011), and lower anthocyanins and methoxypyrazines levels (Keller, 2010).

1.3. Adaptation measures

There are several adaptation measures to climate change, that shouldn't be generalized, but instead, adapted to each terroir, season and production purposes. Some adaptation measures should be considered:

- New vineyards with site selection, rootstocks more resistant to drought, more resistant varieties to water and heat stress, vine spacing, and density adapted to the ecological situation, row orientation and canopy height and trellis system adequate (Van Leevwen *et al.*, 2019).
- Relocation of the vineyards to higher altitudes or to coastal areas (Hannah *et al*., 2013).
- Soil selection with a high available soil water in dry and warm climate with no irrigation possibilities (Van Leevwen *et al.*, 2019).
- Rootstock suitable to water soil status (Van Leevwen *et al.*, 2019).
- Variety selection according to cycle duration, temperature requirements, resistance to water and heat stress, oenological characteristics, and resistance to biotic stress (Magalhães, 2015).
- Changing of training systems avoiding downward shoot positioning systems as it might intensify sunburn in dry and warm regions (Van Leevwen *et al.*, 2019).
- Vine spacing and density according to water availability and radiation interception followed by a right row orientation and canopy height that affects the intercepted

sunlight energy (Van Leevwen *et al.*, 2019).

- Delayed pruning because in influence budburst date and therefore the ripening date (Petrie *et al.*, 2017).
- Doing a soil management in order to prevent soil water evaporation, soil erosion, and increasing of organic matter and infiltration rates (Brewik *et al.,* 2013).
- Canopy management with the objective of an optimal leaf and cluster exposure, by changing the canopy height, movable wires, basal leaf removal, leaf removal above fruit zone (Keller *et al*., 2010).
- Deficit irrigation strategies will lead to periods of water stress at specific phenological stages (Keller *et al*., 2010).
- Precision viticulture practices (Vink *et al.*, 2012)
- Implementation of management techniques based on the use of growth regulators, that might induce a slower ripening, such as: Exogenous auxin (for a delaying ripening); Brassinazol (stimulates maturation); salicylic acid (delays berry softening and color development); exogenous cytokinins (increase berry weights, while reducing pH and anthocyanins accumulation and increasing titratable acidity); Ethylene inhibitors (delayed ripening) (Palliotti *et al*., 2014).
- Application of a foliar spray, based on inactive dry yeast, at *veraison* that provides a uniform *veraison* and a homogenous maturation (Lallemand, 2018).

This last adaptation measure, application of foliar spray, will be deepened in this work, as it is an innovative product.

1.4. Aims of this work

The aim of this work is to elaborate a bibliographic review about the importance and effects, in a viticultural and enological point of view, of a new and innovating product, the LalVigne®, that consists in inactive dry yeast (*Saccharomyces cerevisiae*) derivatives. Is going to be discussed the ongoing challenges of climate change in wine industry and relate that with the potential of the LalVigne®, as a mitigation measure. Several trials will be compared in this regard.

2. Elicitors

Plants are subject to various threats such as pathogen attacks (fungi, viruses, insects, nematodes) and harsh physical conditions (drought, salinity, temperature, exposure to UV radiation) (Thakur *et al.*, 2019). Plants have receptors and sensors to recognize a threat, elicitors or signal molecule, activating defense responses to stabilized against these stresses (Thakur *et al.*, 2019).

These responses involve accumulation of secondary metabolites. In the vine plant, secondary metabolites are produced and accumulated in grape berries (Coombe & McCarthy, 2000) and includes: volatile compounds, flavonoids, glycosides, tannins and flavors (Thakur *et al.*, 2019). Plant secondary metabolites act as defense chemicals (Ramakrishna & Ravishankar, 2011) being essential for the protection of plants from insect, pests, herbivores, phytopathogens and adaptation of the plants to the environment (Thakur *et al.*, 2019), even though they have no substantial role in the preservation of vital life processes of plants.

Furthermore, a very important protective barrier against physical damage and pathogens attack, and also synthesizes phenolic and volatile compounds is the grape skin (Fournand *et al.*, 2006; González-Barreiro, 2015). Phenolic compounds have an effect on grape and wine quality, regarding the color, mouthfeel, aging potential and stability.

2.1. What is an elicitor

According to Radman *et al.* (2003) an elicitor may be defined as a substance which induces or increases the biosynthesis of specific compound in order to adapt the plants to stressful conditions, when applied in small amount to a living system (Thakur *et al.*, 2019). They are distinguished from toxins, because toxins only act at higher concentrations and/or affect the plant destructively, without activating the plant metabolism (Boller, 1995).

Stimulation of stress responses in plants can be produced by elicitors, that are chemical compounds from abiotic and biotic sources (Goetz *et al.*, 1999; Song *et al.*, 2015), whose responses leads to the production of new secondary metabolites and to the enhanced production and accumulation of secondary metabolites (Thakur *et al.*, 2019), a response similar to a plant under microbial pathogens attack (Ferrrari, 2010).

Biotic elicitors are substances with biological origin and/ or within living organisms, either from pathogens or by the plant itself (Patel & Krishnamurthy, 2013). The receptors of the plant are linked to the elicitor's functions, being that they activate or inactivate a number of enzymes or ion channels. Elicitors can be: fungi, bacteria or herbivores, plant cell wall fragments, and chemicals released on attack site, when a plant is under pathogen or herbivore attack (Namdeo, 2007)

Abiotic elicitors are substances without biological origin, being mostly inorganic salts (Cu, Ca, Cd ions), and physical factors (pH, salt stress, water stress, cold, heat) (Veersham, 2004), and act by triggering the synthesis of phytochemicals in plants by using chemical or physical stimulus (Owolabi *et al*., 2018).

2.2. Mode of action

Elicitors must be recognized by plant receptors or proteins (with resistant genes) localized in the plasma membrane or in the cytoplasm, before initiating signaling pathways, that will lead to defense reactions like synthesis of pathogenesis-related proteins or secondary metabolites (Zhao *et al*., 2005).

Plant receptors are activated after the perception of elicitor signals, and then is activated they executers, as well as: ion channels; biding proteins (G-proteins); and protein kinases. These activated executers transmit the signals to second messengers, that likewise will amplify the elicitor signal to other downstream reactions (Blume *et al*., 2000). An elicitor signaling pathway may vary with the different perception of elicitor signals leading to different target defense responses (Zhao *et al*., 2005).

In the end, the elicitation mechanisms lead to a wide spectrum of metabolic modifications, such as: cell-wall reinforcement; accumulation of antimicrobial compounds; synthesis of pathogenesis-related protein for plant protection; lignification; and a hypersensitive response similar to a programmed cell death at the infection site, which blocks pathogen development (Huckelhoven, 2007).

2.3. Inactive dry Yeasts (LalVigne®)

Among biological elicitors, yeast extracts contain several compounds (as triggers of various modes of plant defense) (Ferrari, 2010) that may act as elicitors (Giacosa *et al*., 2019). Yeast cell walls are made up of mannoproteins, β-1,3- and β- 1,6-glucans and chitin, while yeast plasmatic membrane contains lipids, sterols, and proteins (Portu *et al*., 2016). Yeast extracts have been used in plant tissue culture because of their capacity to stimulate the defense mechanisms of plants.

Recently, inactive dry yeast preparations have been used as commercial products, during the winemaking process in order to improve or preserve wine aromatic composition and/or develop required mouthfeel properties (Šuklje *et al*., 2016). The inactive dry yeasts are typically *Saccharomyces cerevisiae* derivatives, that can be divided in four commercial groups, according to their different manufacturing processes: inactive yeasts, yeast autolysates, yeast hulls and yeast extracts (Pozo-Bayón *et al*., 2009; Rodríguez-Bencomo *et al*., 2014) and they have the ability to bind to pathogens and/or stimulate immune pathways in the host (Bertaud, 2016). The inactive dry yeasts additions are generally made to juice before, during or after fermentation (Comuzzo *et al*., 2012; Del Barrio-Galán *et al*., 2011).

Even if this is well-known by the viticultural and oenological sector, still few studies have been published nowadays on the inactive dry yeasts elicitor effect of application on grapevine under field conditions (Giacosa *et al*., 2019). The Lallemand's investment, was intended to develop the relation between composition and function of yeasts derivatives. Having had also, the intention of selecting a new generation of yeasts fractions that have characteristics enhanced regarding interaction with bacteria (pathogen biding) and modulation of the host's immune system (Bertaud, 2016).

Therefore, yeasts derivatives are elicitors, once they change the phenolic content of treated berries through a vine-pathogen interaction, as yeasts are recognized as pathogens and activate the plant defense mechanisms (Santamaría *et al*., 2011), enhancing secondary metabolism in the ripening fruit (Zhao *et al*., 2005)

2.3.1. What is LalVigne®

Recently, a new foliar spray was developed by Lallemand Inc., named commercially, LalVigne®. It consists in inactivated wine yeast (*Saccharomyces cerevisiae*) derivatives, and is a formulation 100% natural (Kogkou *et al*., 2017; Pastore *et al*., 2019; Villangó *et al*., 2015). It was specially designed to be used with the patent foliar application technology WO/2014/024039, Lallemand Inc., Canada (Villangó *et al*., 2015).

According to Lallemand, this product improves ripening from *veraison,* providing a uniform *veraison* and, subsequently, a homogeneous maturation (Lallemand, 2018). Some experiences have been made in different countries with different cultivars, and the results were not only seen for viticulture, but also for oenology, at the sensory level.

It is a product that is produced in two formulas, and used according to what we want to improve, even though both can be applied in any variety, LalVigne® MATURE is recommended for red varieties, and LalVigne® AROMA is recommended for white varieties (Lallemand, 2018). The LalVigne® MATURE aim is to increase and advanced phenolic maturity, and the LalVigne® AROMA purpose is to increase and advanced the accumulation of aroma precursors (Lallemand, 2018).

2.3.2. Characteristics of LalVigne®

The LalVigne, as said earlier, is composed by 100% specific fractions of selected inactivated yeast (*Saccharomyces cerevisiae*) and not genetic modified (Lallemand, 2018). It is non-pathogenic, non-hazardous, non-genetic modified organism (Pastore *et al*., 2019), and suitable in organic farming according to CE nº834/2007 and 889/2008 regulation.

The use of LalVigne® AROMA has: improvements in the skin thickness (Río Segade *et al*., 2016); developments in the aroma precursors (Tomasi *et al*., 2017); no impact in berry weight, Brix, pH or Tartaric acid (Téllez *et al*., 2015); enhancements in the glutathione concentration (Šuklje *et al*., 2016); increases in thiolic varieties as 3MH and 3MHA (Šuklje *et al*., 2016); and reduction of herbaceous or aggressive character (Tomasi *et al*., 2017).

The use of LalVigne® MATURE has: improvements in the skin thickness (Río Segade *et al*., 2016); no impact in berry weight, Brix, pH or tartaric acid (González *et al*., 2016); a reduction of herbaceous or aggressive character (Tomasi *et al*., 2017); improvements in the concentration of extractable anthocyanins (Villangó *et al*., 2015); improvements in the skin tannins (Lissarrague *et al*., 2014); and developments in the degree of polymerization (Villangó *et al*., 2015).

LalVigne® AROMA is sold in boxes with a total weight of 6kg, having two bags of 3kg inside, because has two applications, whereas LalVigne® MATURE is sold in boxes with a total weight of 2kg, having two bags of 1kg inside, for the same reason of AROMA. Is recommended to store in sealed original packaging and avoid extreme storage conditions. Also, is not flammable (Lallemand, 2018).

2.3.3. Foliar application and dose

The recommended dose by application on vines is 3kg or 1kg per ha (LalVigne® AROMA and MATURE, correspondingly), being that is one treatment composed by two foliar applications. The first application is made on the beginning of *veraison*, and the second is made between seven to fourteen days after the first application (best time will be ten to twelve days after) (Lallemand, 2018).

In order to get a perfect solution, is recommended suspend the product in approximately ten times its weight in water. The solution is then added to the tank of the spray machine with the minimum amount of water, that allows to perform a homogeneous spray, avoiding excess of water that can cause loss of product by dripping. Water acts as the treatment vehicle, what is important is the dose of the product used. The agitator should be running during the application (Lallemand, 2018).

Is conceivable to mix both LalVigne®, but when possible, avoid that. It is not recommended to mix it with oils, alkaline products and lime sulfur solutions (used in pest control), and also it was not found incompatibilities with other products. In case of mix, it is advisable to check the recommendations of the other product used and perform phytotoxicity test. If a treatment with another product is going to be done, but without mixing products, LalVigne should be the first one to be applied and after 48 hours we can apply the other product (Lallemand, 2018).

The efficacy of the product is affected if rains in the 48 hours after the application, so if it happens after the first application we should do the second application 7 days right after the first, and if the raining event happens after the second application, is just necessary to repeat it (Lallemand, 2018).

The LalVigne® products are food quality, therefore they do not have Maximum Residual Limits, and grapes could be harvested just after the application, even though is not usual. Also, when mixed with other products, be aware with the Maximum Residual Limits (Lallemand, 2018).

LalVigne® AROMA can be mixed with UREA. In fact, it has a complementary effect. Increases skin thickness and compounds present in it. It will improve wine sensory characteristics and provide greater longevity and complexity (Lallemand, 2018).

LalVigne® MATURE can be applied in white varieties seeking the improvement of mouthfeel and LalVigne® AROMA can be applied in red varieties to increase the aroma. This is usual since in these cases it is a matter of wine style. In general, the recommendations for grapes intended to make rosé wine is the use of LalVigne® AROMA if the vineyard management and wine objective is to make rosé but if rosé is made to concentrate the red wines, LalVigne® MATURE will give better results (Lallemand, 2018).

2.3.4. Mode of action of LalVigne®

As previously explained, yeasts derivatives are elicitors, once they change the phenolic content of treated berries through a vine-pathogen interaction, as yeasts are recognized as pathogens and activate the plant defense mechanisms (Santamaría *et al.*, 2011), enhancing secondary metabolism in the ripening fruit (Zhao *et al.*, 2005).

Studies shown that the exogenous application of elicitors (abiotic or biotic) – in the case of LalVigne®, foliar application - triggers a stress response by plants. A response similar to a plant under attack by microbial pathogens (Ferrari, 2010), due to the perception of a simulation of pathogen attack, there is an accumulation of defense compounds in the tissues (Giacosa *et al.*, 2019) and a development in secondary metabolite production (Pastore *et al.*, 2019).

Among biological elicitors, inactivated yeasts extracts are known to induce secondary biosynthetic pathways due to the plant defense responses stimulated by their content of several components, including chitin, N-acetylglucosamine oligomers, β-glucan, glycopeptides, and ergosterol (Ferrari, 2010; Granado *et al.*, 1995).

The application of inactivated dry yeasts in grape berries, could be of great importance for the skin. The skin is a protective barrier against physical injuries and pathogens attack, and it is involved in the synthesis of important metabolites, such as phenolic and volatile compounds (Fournand *et al.*, 2006; González-Barreiro, 2015; Gabler *et al.*, 2003). Therefore, the application of yeast extract increases grape skin resistance. Furthermore, the extractability of anthocyanins can be measured by the estimation of the skin cell wall degradability, and because of that, skin mechanical properties are so important (Rolle *et al.*, 2008). Also, the increase of berry skin thickness, due to a defense against the presence of yeasts derivatives (Villangó *et al.*, 2015) could influence the anthocyanin release during the maceration process (Río Segade *et al.*, 2016).

Elicitors induce stress, leading to the activation of several genes related to defenses or inactivation of genes not related to defenses, transient protein phosphorylation/ dephosphorylation, and expression of enzymes whose information can be used to establish the biosynthetic pathways of many secondary metabolites (Pastore *et al.*, 2019). The secondary metabolite pathways are very specific, according to the type of elicitor that the cells are exposed. There is variability in the mechanism of action involving a wide range of metabolic responses to stress in plants (Pastore *et al.*, 2019). The different composition of LalVigne® products is found on the type of elicitor that the skin is exposed to, thus there is one product designated for red or white varieties, being that both can be used in any variety once metabolic responses to stress are always different.

2.4. How is this inactivated dry yeasts going to address these challenges (mentioned in previous sections)

2.4.1. Advantages for Viticulture

The winemaking process starts in vineyards, so the interventions taken in the plant are of major importance. The application of the foliar spray LalVigne® brings some advantages regarding protection of the berry, safety in the development of the plant, homogeneity of grape maturation, ecological friendly and profitable (Lallemand, 2018).

There is an improvement of the protection of the plant due to the increase of berry skin thickness, when both LalVigne® are applied (Lallemand, 2018). The concentration and extractability of anthocyanins and tannins are in the grapes skin, which affects the quality of the wine (Ribéreau-Gayon *et al.,* 1999). Moreover, grape skin constitutes the defense against damage by physical injury and attack by pathogens (Negri *et al.*, 2008), therefore there will be a lower berry break in the case of mechanical harvesting (Lallemand, 2018) and a lower incidence of pests and diseases due to climate change.

Additionally, an advancement on phenolic maturity and synthesis of aroma precursors is granted (Lallemand, 2018), which gives a balance between phenolic and technological maturity, and less greeny and adstringent wines. This advancement on the phenolic maturity may enable an advancing harvest, reducing the risks of late harvests (rainfall, frost, animals eating the sweetest grapes) (Lallemand, 2018).

According to Lallemand (2018), LalVigne® gives an homogeneous maturation of the grapes which facilitates the decision of the optimal moment of harvest, and also have a reduction on heterogeneity after weather incidents (e.g. frost). Is a product authorized in organic viticulture, which allows a large range of application, on the part of winegrowers.

Lallemand (2018), also claim more profitability, because LalVigne® may be a complementary or alternative practice to cluster thinning (in cold regions), it allows to advance harvests and also reduces the loss of yield due to dehydration.

2.4.2. Advantages for Oenology

Although the application of LalVigne® is done in the vineyard, it has positive repercussions in the elaboration of wine. The product increases the concentration and improves several compounds, reduces unwanted flavors and aggressive characters, saves adding of enological products, facilitates some processes, improves the wine mouthfeel, and improves wine longevity (Lallemand, 2018).

The application of LalVigne® increases the grape balance, because skin compounds like anthocyanins, skin tannins, aromatic precursors and glutathione (GSH) rise and improve. Anthocyanins are very sensitive to temperatures variations, that influences the color of the wine, therefore for cold temperatures LalVigne® will help to achieve more colored wines. An improvement in skin tannins will decrease the herbaceous and aggressive character and also increase color stability and antioxidant capacity (Kritzinger & du Toit, 2012). Instead of adding GSH to the wine in order to prevent browning, increase production of some volatile thiols during fermentation and provide a protective role against the loss of terpenes, esters and thiols during ageing, LalVigne® may enhance this compound in grapes. The aromatic precursors are mostly improved through LalVigne®AROMA, and the aromatic compounds that characterized a variety are enhanced (Lallemand, 2018).

Alongside the improvements described above, there is a reduction on herbaceous and vegetable character of the grapes, and of the aggressiveness in mouthfeel, that can be caused by warmer climates (Lallemand, 2018). Furthermore, there is no need for oenological inputs as, addition of skin tannins and GSH, which saves some money.

This product also simplifies some processes. According to Lallemand (2018), the maceration is optimized because of the enhancements on skin thickness and anthocyanins concentration and extractability, compounds are released faster, and the fermentation risks associated to excessively mature grapes are reduced.

Undoubtedly, there is a wine improvement, as it gets more balanced and complex, and also allows the production of lower alcohol wines (Lallemand, 2018) as it is the new trend (Caballero & Segura, 2017). Furthermore, LalVigne® enhance the differentiation provided for each plot, for each origin, giving character to the wine, and also more longevity of stability of wine aromas (Lallemand, 2018).

3. Effects

The foliar application of yeast derivatives has an elicitor effect, by inducing the secondary metabolism. This response has side effects physically and chemically. Therefore, it is important to show how the berry formation and ripening occurs and when is LalVigne® a favorable factor for its development.

Berry development comprises three stages (Keller, 2010; Magalhães, 2015). The first stage starts at flowering and ends in maturation (Figure 1). During the first stage, berries grow through cell division. Afterwards, the second stage starts at lag phase with a pause in berry growth and goes until the beginning of *veraison*. The third, and last stage, starts at *veraison*, when berries change color, soften, accumulate sugars, and metabolize acids (Magalhães, 2015). Because the acting period of LalVigne® goes from *veraison* to the end of maturation, this third stage is critical in what regards the effects of this product for sensory analysis.

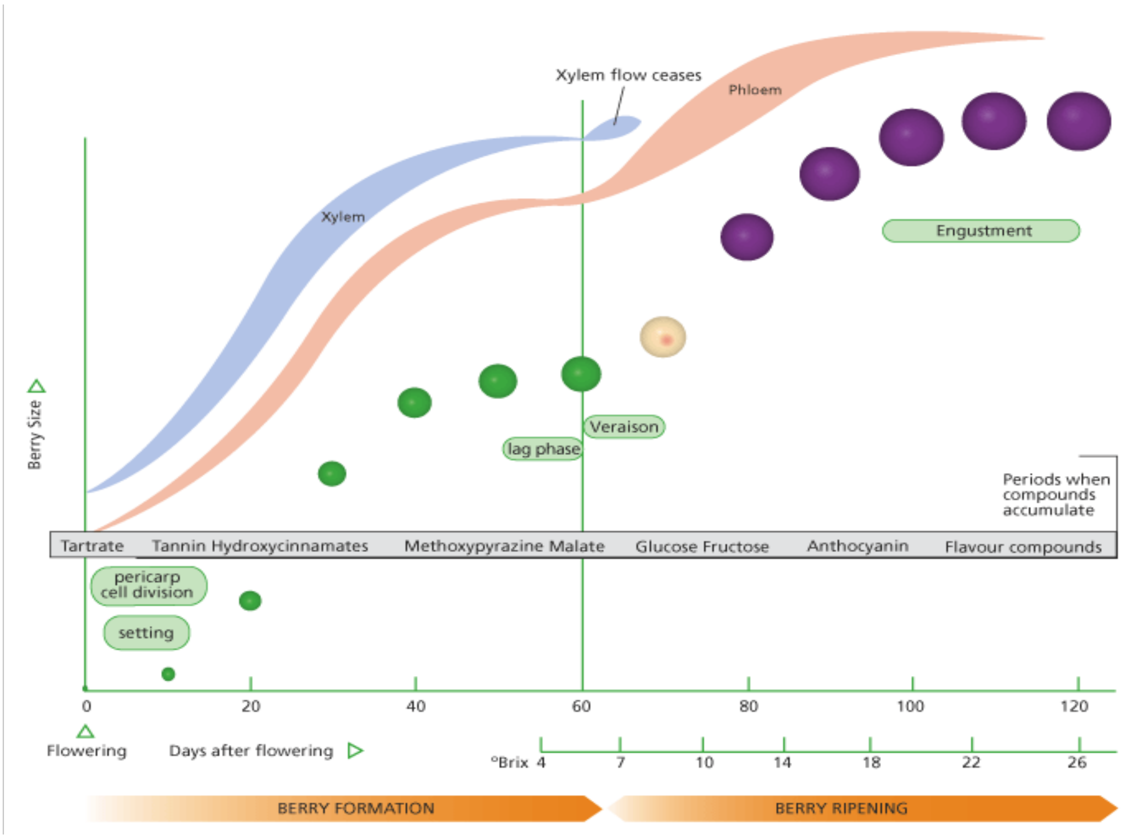

Figure 1. Grape berry formation and ripening.

Source: Kennedy J., 2002.

The boundary between the lag phase and the beginning of *veraison* is often unclear. Consequently, the end of the lag phase is important because of the accumulation of acids and tannins, which achieves their maximum level at the start of *veraison*. During the third phase, illustrated in Figure 2, berries doubles in size and go through several additional changes. For instance, the production of aromas, polyphenols and other important components occurs in the third phase. In this phase, the berry is rich in acids (malic acid and tartaric acid), while the sugar content is low. Grapes of white or red varieties, gradually get yellowish because of flavones, or pinky because of anthocyanins, respectively (Magalhães, 2015). The *veraison* ends when, at least, fifty percent of the grapes are colored, and then the maturation begins.

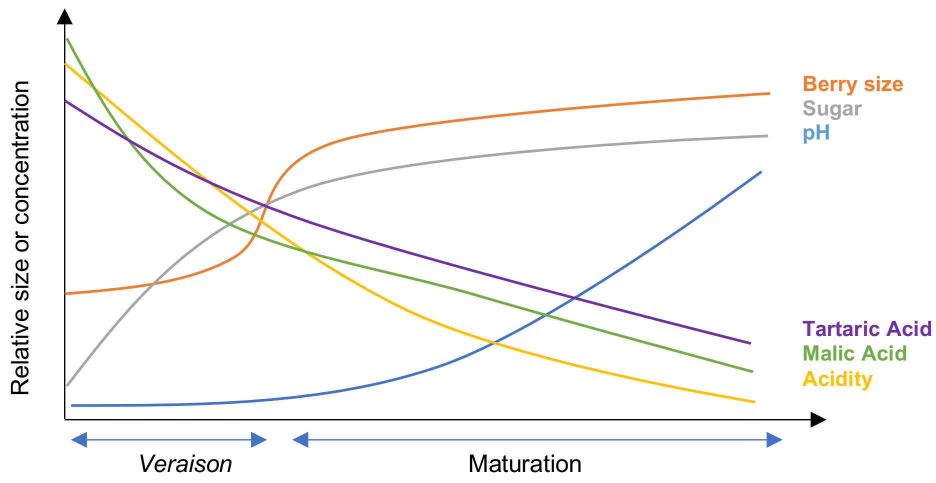

Figure 2. Evolution of different components of the berry during *veraison* and maturation.

Source: from the author.

3.1. Effects on bunch and berry

3.1.1. Skin thickness

Grape skin constitutes a fundamental protective barrier against damage by physical injury and attack by pathogens. It also has an important role concerning the synthesis of many compounds of interest (Negri *et al.*, 2008). For instance, skin tannins and aroma compounds and anthocyanins are some of the compounds synthesized in the skin, with anthocyanins generally restricted to skin tissue (González-Barreiro *et al.*, 2015). Among these compounds, particular interest attaches to tartaric acid, which accumulates in the skin early in development (González-Barreiro *et al.*, 2015). There is also an accumulation of resveratrol in the early phase, that, along with skin thickness, plays an important role in plant protection (González-Barreiro *et al.*, 2015).

According to González-Barreiro *et al.* (2015), the berries treated with LalVigne® MATURE get higher anthocyanin content, and lower skin hardness but thicker skins. However, this goes against other authors, describing that thinner (Río Segade *et al.*, 2011) and harder skins (Rolle *et al.*, 2008) contained more anthocyanins. Moreover, Río Segade *et al.* (2016) found that there is no correlation between skin break force and skin thickness, in other words for the same grape variety, control and treated had different values of skin thickness, but the same values for skin break force. More recently, Lallemand (2018) suggests that LalVigne® would increase skin thickness, which was confirmed in the three trials whose results are shown in Figure 3.

Furthermore, besides the importance regarding synthesis of components, grape skin plays an important role in the prediction index of anthocyanin extractability, by measuring the skin thickness. Thinner skins are characterized by a greater release of red pigments which allows optimizing the maceration management in order to express all the potential of grapes in the elaboration of high-quality red wines (Río Segade *et al.*, 2011).

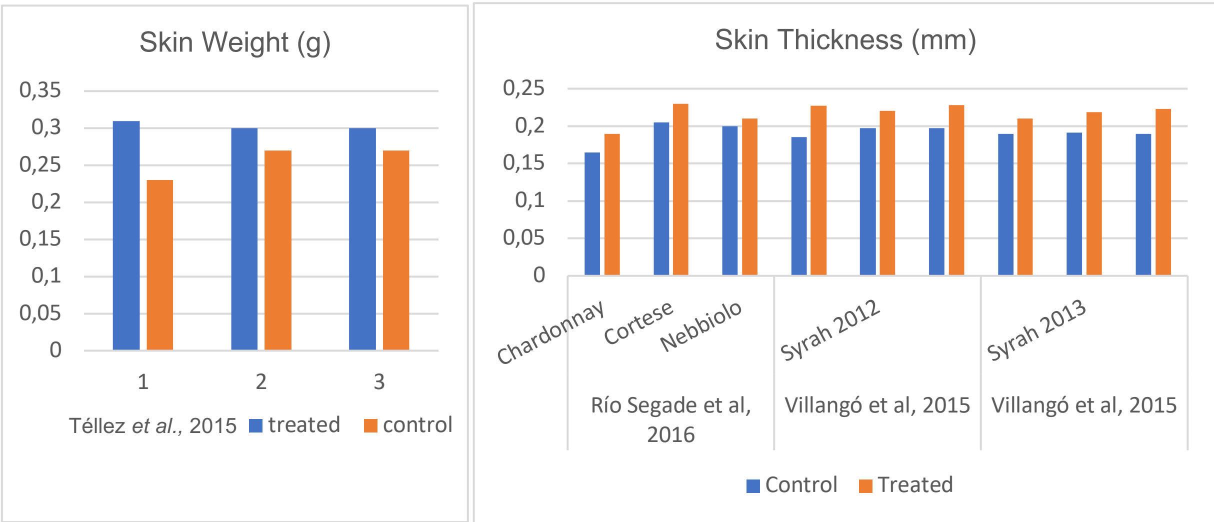

Figure 3. Comparison between Treated (LalVigne® AROMA) and Control regarding Skin Weight (left) of Téllez *et al.,* (2015) Sauvignon Blanc trials. Comparison between Treated (LalVigne® MATURE and AROMA) and Control regarding Skin Thickness (right) of Río Segade *et al.,* (2016) and Villangó *et al.,* (2015) trials.

Abbreviations: Dates of harvest: 1, 20/08/2015; 2, 03/09/2015; 3, 10/09/2015.

The skin thickness had an increase in both trials, Río Segade *et al.,* (2016) and Villangó *et al.,* (2015), for red and white varieties. Río Segade *et al.,* (2016) applied LalVigne® AROMA for Chardonnay and Cortese and LalVigne® MATURE for Nebbiolo, while and Villangó *et al.,* (2015) applied only LalVigne® MATURE in 2012 and 2013, for Syrah.

3.2. Effects on Chemical Composition

Summarizing the current literature and as suggested by Lallemand (2018), based on the grape variety (red or white), there are side effects on chemical composition regarding the application of LalVigne® MATURE and AROMA. Sugar content, pH and acid content had no modifications (Río Segade *et al*., 2016; Tomasí *et al*., 2017; Téllez *et al*., 2015; Villangó *et al*., 2015; Lissarrague *et al*., 2014), while Glutathione (GSH), thiols, anthocyanins concentrations and skin tannins has improvements (Šuklje *et al*., 2016; González *et al.,* 2016; Río Segade *et al*., 2016; Tomasí *et al*., 2017; Téllez *et al*., 2015; Villangó *et al*., 2015; Lissarrague *et al*., 2014).

3.2.1. Impact on berry composition

The primary fruit maturity indicators are pH, acid contents and sugar, because of their abundance and easy measurement (Dami, 2014).

Acids, upon dissociation in a juice solution, or wine, release H+ ions, which are measured and expressed as pH (Dami, 2014), and are a form of measure active acidity. An extensive range of factors in the wine are affected by the pH level, such as: microbial stability (spoilage), physical stability (protein, tartrate, metal), oxidation level, SO_2 activity, wine color and flavor, and malolactic fermentation (Dami, 2014).

Is important to know the evolution of malic and tartaric acid during *veraison* and maturation periods, since their concentrations will influence the wine production. As shown in Figure 2, initially, the malic acid content in berry can be superior to the tartaric acid, but then malic acid suffers a bigger degradation during maturation, therefore is values are lower than tartaric acid through the harvest (Huglin & Schneider, 1998). In Table 1, there are the expecting values of acidity in berry, from *veraison* to maturation.

Table 1. Average values of berry acidity variation, during *veraison* and maturation.

	Veraison	Maturation
Malic Acid	13,4 – 26,8 g/L	0,7 – 2,7 g/L
Tartaric Acid	7,5 – 15,0 g/L	6,0 – 9,0 g/L
pH	2,3 – 2,9	3,2 – 4,0

Source: Adapted from Magalhães (2015).

During maturation, the most important phenomena is sugar accumulation on berry, not only because it translates the potential alcohol content but also because it is an important starting point for the synthesis of: polyphenols, anthocyanins and aroma. Sugar levels are usually expressed in degree Brix, which represents grams of sugars per 100 grams of juice. Desirable Brix levels should be between 18 and 24, dependent on grape variety and wine style (Dami, 2014). Sugars and anthocyanins accumulate, and malate declines rapidly during the early ripening phase, and the sugar concentration may continue to increase due to berry shrinkage.

Table 2. Results of the application of LalVigne®, regarding basic parameters of grape ripening.

Source		Río Segade *et al.*, 2016		Tomasí *et al.*, 2017								Téllez *et al.*, 2015		Villangó *et al.*, 2015						Lissarrague *et al.*, 2014	
		a	b	c. 13	c. 14	c. 15	c. 16	d. 13	d.14	d.15	d.16	e	f	h			I			j	k
Brix	C	21,7	23,8	22,1	19,83	21,3	22,8	16,2	13,5	16,7	15,9	25,3	27,58	22,9	23,7	24,3	18,5	19	21,2	23,13	24,55
	T	21,7	24,6	22,6	19,73	21,13	22,7	16,4	13,7	17,2	16,3	24,2	26,9	23,6	24	24,3	18,2	20,4	21	22,78	24,82
Berry weight (g)	C	1,7	2,2	-	-	-	-	-	-	-	-	1,18	0,96	1,28	1,25	1,35	1,73	1,71	1,47	0,834	1,024
	T	1,6	2,17	-	-	-	-	-	-	-	-	1,28	0,95	1,34	1,22	1,37	1,79	1,76	1,47	0,82	0,988
pH	C	3,35	3,19	3,34	3,14	3,43	3,35	3,2	3,15	3,16	3,22	3,7	3,54	3,14	3,32	3,25	2,9	2,93	2,92	3,59	3,57
	T	3,32	3,18	3,37	3,13	3,38	3,36	3,2	3,13	3,18	3,18	3,66	3,5	3,23	3,34	3,34	2,89	3,02	2,91	3,57	3,54
Titratable acidity (g/L)	C	5,18	5,13	5,5	8,52	6,57	5,94	7,7	7,1	6,4	6,6	-	-	7,6	5,2	5,5	10,8	10,2	8,6	-	-
	T	5,48	4,61	5,3	8,32	6,45	5,98	7,5	6,9	6,8	6,7	-	-	6,3	5,3	5,9	9,4	8,9	9,2	-	-
Citric Acid (g/L)	C	nd	0,11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	T	nd	0,11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tartaric Acid (g/L)	C	6,91	6,7	-	6,48	5,7	7,34	6,6	4,9	6,5	7,3	-	-	-	-	-	-	-	-	-	-
	T	7,11	6,99	-	6,17	5,72	7,26	6,5	4,3	6,5	6,9	-	-	-	-	-	-	-	-	-	-
Malic Acid (g/L)	C	1,17	0,9	-	3,86	2,05	1,87	3,6	4,2	2,3	2,9	-	-	-	-	-	-	-	-	-	-
	T	1,25	0,91	-	3,94	2,1	1,94	3,3	4,6	2,6	3,2	-	-	-	-	-	-	-	-	-	-

Abbreviations: a, Chardonnay 2016; b, Nebbiolo 2016; c, Merlot 2013, 2014, 2015 and 2016; d, Glera 2013, 2014, 2015 and 2016; e, Sauvignon Blanc 2015; f, Cabernet Sauvignon 2015; h, Syrah 2012 (harvest 3 times); i, Syrah 2013 (harvest 3 times); j, Sauvignon Blanc 2013; k, Syrah 2013; C, Control; T, Treated.

Following sugars, organic acids are the most abundant solids present in grape juice. They give the sour taste of must and wine and also have a large influence on wine stability, color and pH. The tartaric, malic and citric acids are the main acids found in grapes. At *veraison*, the soluble solids accumulate in the berry and therefore the acid content decreases. Titratable acidity is the actual amount of acid reserve in the wine, and at harvest the levels are found between 0,6 and 0,8 grams of titratable acids/ 100 mL (%TA) (Dami, 2014). Several trials were taken concerning the application of LalVigne®, and the results regarding impact on berry are illustrated in Table 2 and summarized in Figure 4 and 5.

According to Lallemand (2018), the application of LalVigne® shows no repercussions on berry basic parameters, and the results of some trials comparing the treated vine with LalVigne® with the control treatment are positive. There was no impact on berry, no significant differences between experimental treatments regarding Malic acid, Tartaric acid and Citric acid. Figure 4 and 5, shows the comparison between control and treated groups of the several trials concerning ºBrix, Berry Weight and pH, respectively. No significant difference between control and treated were recorded, suggesting that LalVigne® has no impact on these parameters that stay unaltered when the product is applied.

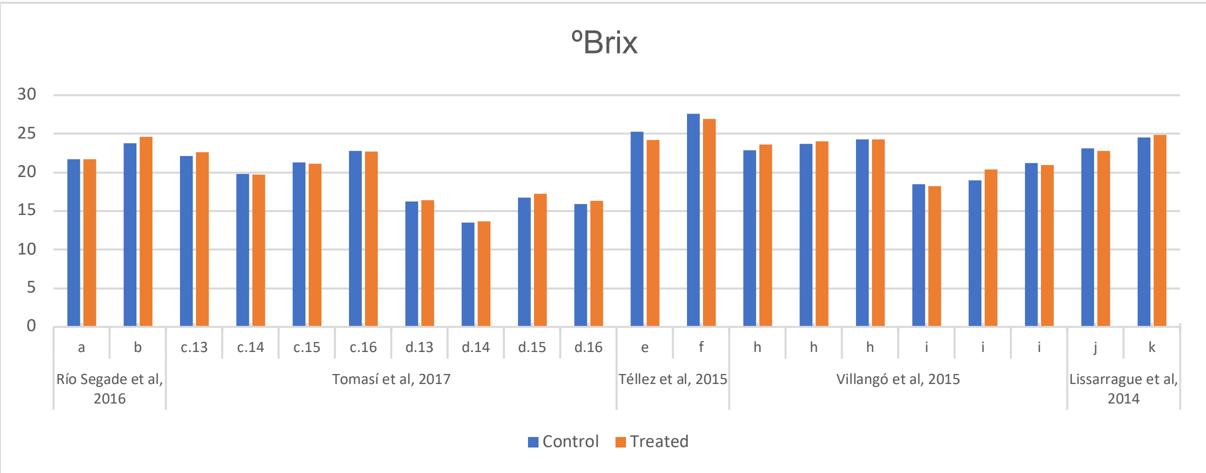

Figure 4. Comparison concerning ºBrix of control and treated vines of the several trials.

Abbreviations: a, Chardonnay 2016; b, Nebbiolo 2016; c, Merlot 2013, 2014, 2015 and 2016; d, Glera 2013, 2014, 2015 and 2016; e, Sauvignon Blanc 2015; f, Cabernet Sauvignon 2015; h, Syrah 2012 (harvest 3 times); i, Syrah 2013 (harvest 3 times); j, Sauvignon Blanc 2013; k, Syrah 2013.

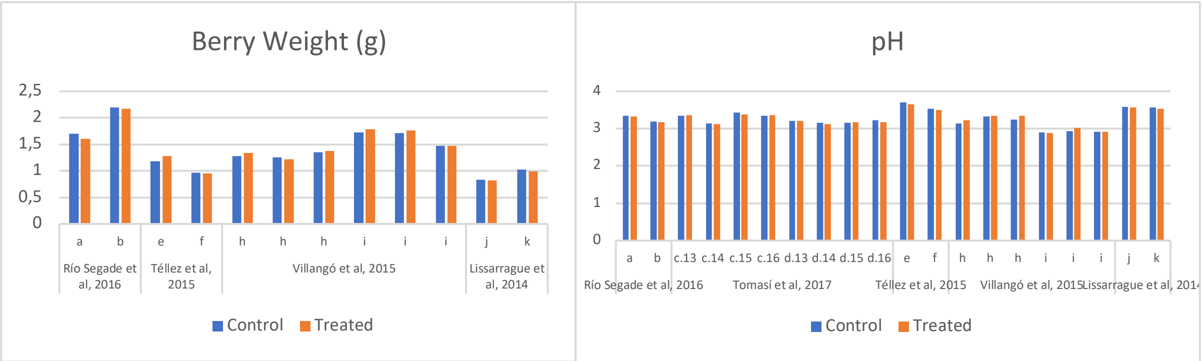

Figure 5. Comparison concerning Berry Weight and pH of control and treated vines of the several trials. **Abbreviations:** a, Chardonnay 2016; b, Nebbiolo 2016; c, Merlot 2013, 2014, 2015 and 2016; d, Glera 2013, 2014, 2015 and 2016; e, Sauvignon Blanc 2015; f, Cabernet Sauvignon 2015; h, Syrah 2012 (harvest 3 times); i, Syrah 2013 (harvest 3 times); j, Sauvignon Blanc 2013; k, Syrah 2013.

Alongside the rising temperatures, this non-impact on berry may not be beneficial regarding some parameters. For higher environmental temperatures there is a bigger oxidation of acids and consequently an increase of pH (Orduña, 2010), which might originate wines with a low fixed acidity as well as microbiologically instable, thereby leading to an accelerated bacterial contamination of wine (Orduña, 2010; Asproudi *et al.*, 2018). In this situation, since LalVigne® has no impact in pH, the addition of tartaric acid before fermentation would be required in order to avoid such microbiological instability and improve mouth feel (Keller, 2010), especially in white wines with lower malic acid, taking in account malolactic fermentation does not take place. Similarly, malic acid levels depend on maturity and temperature, having a decrease when high temperatures arise (Huglin & Schneider, 1998; Kliewer, 1971), so LalVigne® will not influence this trend. Nevertheless, tartaric acid is reasonably stable regarding to temperature effects (Buttrose *et al.*, 1971).

In the one hand large thermal ranges, combined with high insolation values, are propitious not only to a bigger sugar accumulation, but also, indirectly, to an intensification of the aromas and anthocyanins concentrations. On the other hand, medium to low temperatures (lower than 20ºC), regardless of the thermal temperature, reduce photosynthetic activity to lower levels, consequently, with a reducing of sugar synthesis (Dami, 2014). At harvest, if the climate is warmer than ideal, earlier sugar ripeness may be achieved while acids are lost through respiration, therefore resulting in unbalanced wines (Jones *et al.*, 2005). It is difficult to do the fermentation with high sugar content, beyond the natural maximum of less than or equal to 25°Brix (Jones *et al.*, 2005), and also wines get to alcoholic, which is not desired. Regardless of temperature during maturation, earlier sugar ripeness will not occur when LalVigne® is applied because it guaranties a homogeneous maturation (Lallemand, 2018). Despite there is no impact on sugar content, there is a stimulation effect in the plant, that allows sugar content to rise at the same time as the other components, in order to get balanced wines and normal fermentations. Furthermore, no impact on berry weight is positive as there is no berry growth without the maturation of the grape occurring at the same time. In this way, a well-matured grape is obtained, instead of a bunch with the right size but not complete colored and with the right acidity and aroma.

3.2.2. Reduced Glutathione (GSH)

Reduced glutathione (GSH) can be added to juice and wine or come naturally with the yeasts (*Saccharomyces Cerevisiae*). It is an important antioxidant that have been reported to prevent browning, increase production of some volatile thiols during fermentations and provide a proposed protective role against the loss of certain terpenes, esters and thiols in wine during ageing (Makhotkina *et al.*, 2014), having a decrease during that period. Also, GSSG (oxidized glutathione) forms on the oxidation of GSH and does not have anti-oxidant properties, therefore the measure of Glutathione is important to be quantified in both forms, GSH and GSSG. Lallemand (2018) claimed that LalVigne®AROMA increase wine GSH concentrations by providing precursors for GSH synthesis during fermentation (Kritzinger & du Toit, 2012).

Šuklje *et al*. (2016) measured the GSH concentrations before fermentations, after fermentations and after two months of storage (Table 3). It was measured significantly lower GSH concentrations, in the juice, before fermentation, comparing the control with treated (LalVigne®AROMA). The trend was maintained until the end of fermentation (Figure 6), being that after two months of storage the difference was irrelevant. The GSSG concentrations in juice before the fermentation were between 0,33 and 0,45 mg/L and the differences were irrelevant between treatments (Table 3) (Šuklje *et al.*, 2016), which is good because GSSG increasing is not desired.

Table 3. Comparison between control and Aroma (LalVigne®AROMA) regarding GSH and GSSG concentrations in mg/L in Sauvignon Blanc vines.

Sauvignon Blanc	concentration GSH (mg/L)		concentration GSSG (mg/L)	
	Aroma	Control	Aroma	Control
Before Fermentations	52,2	40,6	0,35	0,45
After Fermentations	47,6	38,1	0,53	0,77
2 months of storage	7,2	5,9	0,19	0,16

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; Aroma, LalVigne®AROMA
Source: *Šuklje* et al.*, 2016*

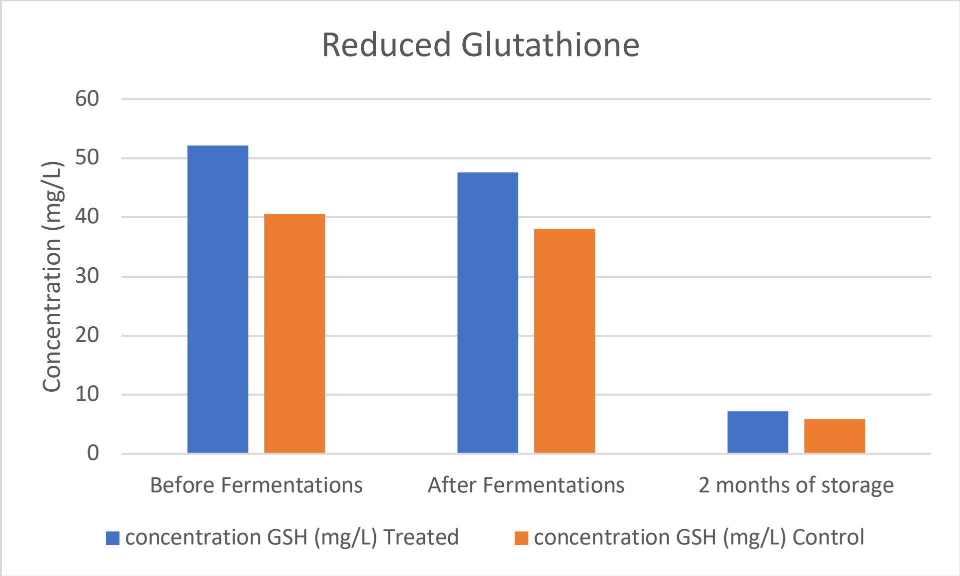

Figure 6. Graphic of comparison between concentration of reduced glutathione in treated vines with LalVigne®AROMA, and Control in Sauvignon Blanc vines.

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione.

Source: Šuklje *et al*., 2016

3.2.3. Thiols

Volatile thiols are important compounds for wine once they contribute to the varietal character, manly Sauvignon Blanc. These sulfur compounds are a strong attribute in a wine, therefore winemakers want to optimize the formation of these aroma compounds.

Šuklje *et al*., (2016) measured thiols content in wines after two months of ageing (Table 4) and there were observed significant differences between the control values and treated. Complex interactions between different amino acids and carboxylic acids have been shown to influence the release of 3SH (3-sulfanylhexan-1-ol) during fermentation (Pinu *et al.,* 2014). It was found in wines from the LalVigne®AROMA treatment, higher 3SHA (3-sulfanylhexyl acetate) concentrations, but the exact mechanism cannot be explained with Šuklje *et al*. (2016) data.

Table 4. Thiols concentration (mg/L) regarding control and Aroma (LalVigne®AROMA) after two months of ageing and the perception threshold (µg/L), in Sauvignon Blanc vines.

Thiols	Perception threshold (µg/L)	2 months storage (mg/L)	
		Aroma	Control
3SH	0,06	969,7	747,1
3SHA	0,004	133,3	97,9

Abbreviations: 3SH, 3-sulfanylhexan-1-ol; 3SHA, 3-sulfanylhexyl acetate; Aroma, LalVigne®AROMA

3.2.4. Concentration of anthocyanins

The anthocyanins represent the reddish pigments of the red grape varieties, particularly in the skin and also in the pulp, for some teinturier grape varieties. They are chemically composed of delfinidine, petunidine, cyanidine, peonidine and malvidine molecules (Table 5). The synthesis of anthocyanins is related to environmental and cultural factors, and they are very sensitive to temperature variations. On the one hand, in cold and weakly insolated areas, the concentration of anthocyanins is lower, even those generally unfavorable to the production of high-quality red wines (Magalhães, 2015). On the other hand, in areas with high temperatures, when below 35ºC, and large diurnal thermal amplitudes, are favorable for the synthesis of anthocyanins, producing more colored wines. Also, the trilling system influences the concentration of anthocyanins, according to the vigor and hedge density, as well as the water availability in the soil (Magalhães, 2015). Furthermore, studies shown that temperatures above 35ºC can cause a reduction in the accumulation of these compounds, when occur throughout long periods during ripening. The main reason for this reduction on the accumulation is owing to a decrease of their synthesis, which often translates a higher degradation (Pastore *et al*., 2017; Mori *et al*., 2007).

The first treatment with LalVigne®MATURE was performed in the beginning of anthocyanin accumulation, in berry skin. Concerning individual anthocyanins (Dp, delphinidin-3-*O*- glucoside; Cn, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*- glucoside; Pn, peonidin-3-*O*-glucoside; Mv, MvAc, malvidin 3-*O*-acetate-glucoside; MvCm, malvidin 3-*O*-coumarate-glucoside), there were few differences among treatments in the levels of monomeric anthocyanins (Figure 7) in accordance with the results of grapes, foliar yeast spraying had no influence on anthocyanin concentration across different harvest dates and vintages (Table 5), with the exception of Lissarrague *et al*. (2014), that found higher levels of extractable anthocyanins, namely the fraction that will be extracted during winemaking.

Table 5. Comparison between control and treated regarding Total anthocyanins and Extractable anthocyanins.

Source		Villangó et al., 2015						Tomasí et al., 2017				Río Segade et al., 2016				Lissarrague et al., 2014		
		2012			2013			2013	2014	2015	2016	maceration hours				a	b	c
												25	50	100	150			
Total anthocyanins (mg/L)	C	1754	1781	1834	1084	1038	1356	-	-	-	-	-	-	-	-	1800	2500	1900
	T	1781	1888	1736	1273	1386	1433	-	-	-	-	-	-	-	-	2000	3000	2000
Total anthocyanins (mg/kg)	C	-	-	-	-	-	-	789	317	530	674	350	320	300	290	-	-	-
	T	-	-	-	-	-	-	896	290	639	801	410	400	370	320	-	-	-
Extratable anthocyanins (mg/L)	C	828	801	725	559	593	602	-	-	-	-	-	-	-	-	1100	900	1000
	T	958	839	792	702	734	761	-	-	-	-	-	-	-	-	1300	1350	1200
Extratable anthocyanins (mg/kg)	C	-	-	-	-	-	-	312	225	236	313	-	-	-	-	-	-	-
	T	-	-	-	-	-	-	337	215	284	394	-	-	-	-	-	-	-

Abbreviations: a, 1st harvest 2014; b, 2nd harvest 2014; c, 3rd harvest 2014; C, control; T, treated.

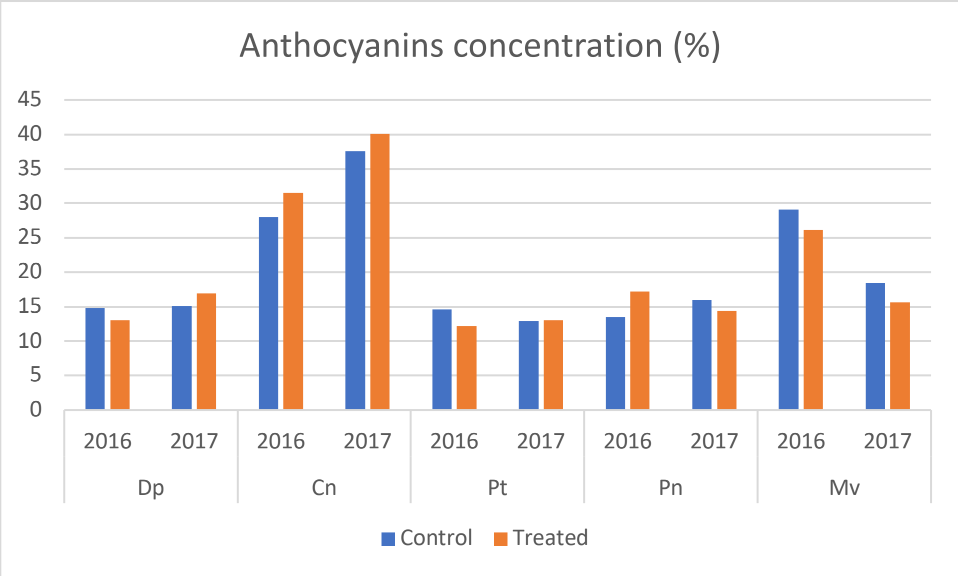

Figure 7. Anthocyanin concentration in percentage.

Abbreviations: Dp, delphinidin-3-0-glucoside; Cn, cyanidin-3-0-glucoside; Pt, petunidin-3-0-glucoside; Pn, peonidin-3-0-glucoside; Mv, malvidin-3-0-glucoside.

Source: Pastore *et al.*, 2019.

Table 6. Anthocyanin profile in percentage regarding Control and Treated vines.

Anthocyanin profile (%)		Río Segade et al., 2016				Pastore et al., 2019		Kogkou et al., 2017
		maceration hours				2016	2017	2017
		25	50	100	150			
Dp	C	-	-	-	-	14,8	15,1	4,9
	T	-	-	-	-	13	16,9	5,8
Cn	C	-	-	-	-	28	37,6	3,4
	T	-	-	-	-	31,5	40,1	3,6
Pt	C	-	-	-	-	14,6	12,9	6,1
	T	-	-	-	-	12,2	13	6,7
Pn	C	35	33	33	33	13,5	16	5,7
	T	37	37	38	39	17,2	14,4	6,9
Mv	C	31	32	35	39	29,1	18,4	68,4
	T	26	27	28	29	26,1	15,6	68,3

Abbreviations: Dp, delphinidin-3-0-glucoside; Cn, cyanidin-3-0-glucoside; Pt, petunidin-3-0-glucoside; Pn, peonidin-3-0-glucoside; Mv, malvidin-3-0-glucoside; C, control; T, Treated.

3.2.5. Skin Tannins

Condensed tannins (proanthocyanidins or flavanols) are high molecular weight polymers of flavan-3-ol and copolymers of flavane-3-ol-anthocyanidins, found mainly in the stems of the bunch, grains, and the skin, giving astringent characteristics to the wine. Of the tannins stand out the procyanidins, which include catechins and epicatechins (important compounds in the definition of quality of maturation). Tannins also participate in color stability, have an antioxidant capacity and give astringency and bitterness.

Depending on the molecular weight of the tannins, the rapid properties vary from herbaceous, hard or aggressive and astringent to round and velvety. Tannins can also be combined with proteins, and protein metabolism favors the formation of polymerized nitrogenous compounds and aggressive herbaceous tannins by increasing coarse-flavored tannin-protein compounds. This aggressive characteristic will be deepened later.

According to Río Segade *et al.* (2016), it was found a significant increase in flavanols extracted during the simulated maceration in the treated samples (with LalVigne®MATURE) (Table 7). The evolution of grape tannins during ripening revealed a higher concentration in these phenolics in treated grapes than in control ones. Same results were observed by Lissarrague *et al.* (2014) and Téllez *et al.* (2015) by the application of LalVigne®MATURE in red grapes (Figure 8).

Table 7. Tannin polymerization results regarding the application of LalVigne®MATURE

Tannins		Lissarrague et al., 2014			Río Segade et al., 2016				Téllez et al, 2015		
					maceration hours						
		a	b	c	25	50	100	150	d	e	f
mg epicathechin/ g	C	2	2,5	2,7	-	-	-	-	6,5	6,1	4
	T	5	3,7	3	-	-	-	-	7	7,4	6
mg proanthocyanidins/ kg	C	-	-	-	2000	2000	1900	1850	-	-	-
	T	-	-	-	2200	2300	2100	2000	-	-	-
mg vanilinassay/ kg	C	-	-	-	800	800	800	800	-	-	-
	T	-	-	-	1100	1050	1050	1050	-	-	-

Abbreviations: a, 1st harvest 2014; b, 2nd harvest 2014; c, 3rd harvest 2014; d, 1st harvest 2015; e, 2nd harvest 2015; f, 3rd harvest 2015; C, control; T, Treated.

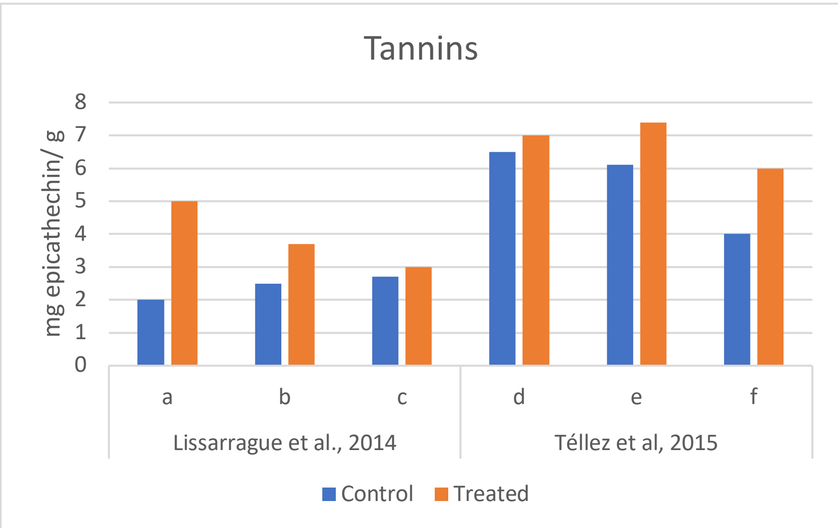

Figure 8. Evolution of grape tannins during ripening, regarding the application of LalVigne®MATURE, comparing Control and Treated in two different trials.

Abbreviations: a, 1st harvest 2014; b, 2nd harvest 2014; c, 3rd harvest 2014; d, 1st harvest 2015; e, 2nd harvest 2015; f, 3rd harvest 2015.

Source: Téllez *et al.*, 2015; Lissarrague *et al.*, 2014.

3.2.6. Degree of polymerization

Tannins that depend on phenolic maturation and origin (skin or seed) modify the perception of bitterness and astringency. The skin tannins have a high degree of polymerization and have a small proportion of epicatechin. On the other hand, seed tannins have a lower degree of polymerization and a high percentage of epicatechins. So, an immature grape will tend towards very reactive, low polymerized tannins.

According to Villangó *et al.* (2015), the increased values of HCl and gelatine indices for the wines from treated grapes, indicate a more polymerized and balanced tannin structure compared to the control wines. This capacity to achieve a higher phenolic maturity is a potential benefit of the foliar spray treatment. There was a lower concentration of monomeric catechins in wines from treated grapes that may be explained by the higher polymerized phenolic compound concentration.

Tomasí *et al.* (2017) also found that polyphenols were found to have a higher level of polymerization and softness in treated grapes, therefore the astringent notes were not as evident.

3.2.7. Aroma Precursors

The aromatic potential of grapes consists on free and volatile compounds (odorants) directly accessible to the olfactory mucosa and, on the other hand, of bound and nonvolatile compounds (odorants) called aroma precursors (Gunata, 1994).

In the beginning of *veraison* forwards, the production of aromas in the skins starts, with a constant increase in relation to the maturation trend (Tomasí *et al.,* 2015). The main classes of aromatic compounds present in white grapes are terpeneols (floral, fruity and citrus notes), norisoprenoids (spice, tropical fruit, floral and violet), and benzenoid compounds (balsamic and spicy notes) (Tomasí *et al.,* 2015).

In Figure 9, data show that all harvests recorded better synthesis of all main aromatic precursors in treated Glera (Tomasí *et al.*, 2015) compared with the control sample. As Lallemand (2018) have stated, there is indeed an increase on aroma precursors, as well as an increase of their sensory quality. Sensory analysis results shown in Figure 10, confirmed that wines produced with treated grapes showed improved olfactory intensity with more noticeable floral notes, and a better palate balance and less bitter notes.

The fruity and floral attributes in wine are due to norisoprenoids (Winterhalter & Schereier, 1994) like b-damascenone or b-ionone, that are slightly insensitive to temperature variations (Keller, 2010), although methoxypyrazines can masked them under cool conditions, since methoxypyrazines have a higher accumulation under these cooler conditions (Roujou de

Boubée *et al.*, 2000), in Tomasí *et al.* (2015) trials, norisopernoids had always more expression in treated grapes, comparing to control.

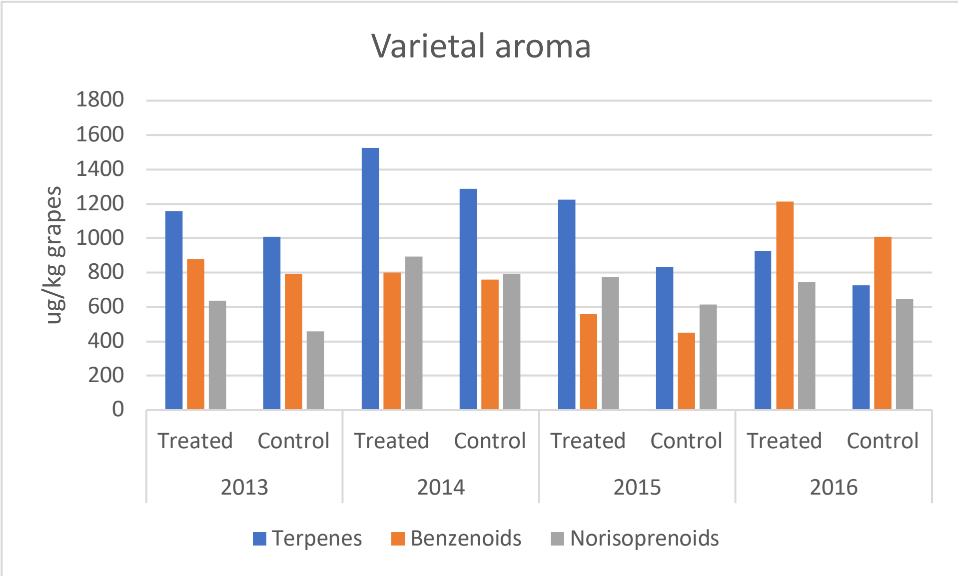

Figure 9. Glycosylated aromatic precursors were (µg/Kg grapes) measured in Glera grapes treated with LalVigne® AROMA and in the control samples for Tomasí *et al.* (2017) trials.

Source: Tomasí *et al.*, 2015

3.3. Effects on sensory analysis

Regarding sensory analysis, few studies were made. It is a very important analysis because, despite of this product being applied in the vineyards, their repercussions in sensory analysis define whether a wine is better for the consumer when treated, or not.

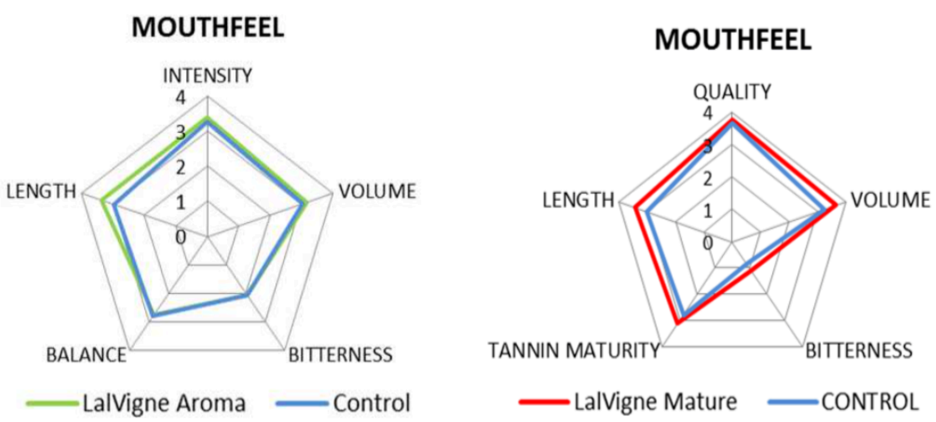

Figure 10. Taste analysis in Téllez *et al.* (2015) trials. Sauvignon Blanc (Left) and Cabernet Sauvignon (Right).

Source: Téllez *et al.,* 2015. Figure 3.

Téllez *et al.* (2015), made taste analysis with Sauvignon Blanc and Cabernet Sauvignon, comparing the mouthfeel of treated wines and the control, as shown in Figure 10.

The results of Sauvignon Blanc doesn´t show a significant difference between the treated (LalVigne®AROMA) and the Control. The taste analysis of Sauvignon Blanc indicated a bigger length on mouthfeel, which is a feature increasingly appreciated by the consumer with regard to white wines. Besides that, a small increase on intensity and volume was verified.

The taste analysis of Cabernet Sauvignon suggested bigger differences between Treated and Control, comparing to Sauvignon Blanc analysis. The length, volume and bitterness had a significant increase. Tannin maturity increased as well, which was expected since the application of LalVigne®MATURE showed an evolution on the grape tannin's concentration, during ripening. The quality of the mouthfeel remained the same.

Another side effect that affect mouthfeel is the herbaceous and/or aggressiveness. Therefore, it will be deepened in the next paragraphs.

3.3.1. Herbaceous/aggressive and mouthfeel

Methoxypyrazines are nitrogenated heterocyclic products of amino acid metabolism which originate in the grape and are associated with vegetal characteristics of wine (González *et al.,* 2016). Also, protein metabolism favors the formation of polymerized nitrogenous compounds and aggressive herbaceous tannins. Both contributes to this herbaceous and aggressive flavor.

Also, pyrazine accumulation tends to be depressed because of warmer temperatures, leading to their degradation, which gives wines with "veggie, herbaceous notes" (Keller, 2010).

The fruity and floral attributes in wine are due to norisoprenoids (Winterhalter & Schereier, 1994) like b-damascenone or b-ionone, that are slightly insensitive to temperature variations (Keller, 2010), although methoxypyrazines can masked them under cool conditions, since methoxypyrazines have a higher accumulation under these cooler conditions (Roujou de Boubée *et al.*, 2000)

The wine from treated wines were subjected to sensory analysis, and according to Lallemand (2018) there is an increased olfactory intensity with more pronounced floral notes and a considerable decrease of vegetal notes. According to Tomasí *et al*. (2015) there was bigger intensity on the nose, with more fruity notes and decreased vegetal notes (Figure 12) since it was reported higher degradation of methoxypyrazines following the treatment with LalVigne®MATURE. The same results were observed in the grapes treated with LalVigne®AROMA, more pronounced floral notes and decrease of vegetal, resulting wines with better palate balance and less bitter notes (Figure 11).

The same results were found in Téllez *et al*. (2015), trials (Figure 10), where is verified a bigger tannin maturity in grapes treated with LalVigne®MATURE, and therefore tannins are not very reactive because they are polymerized, decreasing the herbaceous aggressive mouthfeel.

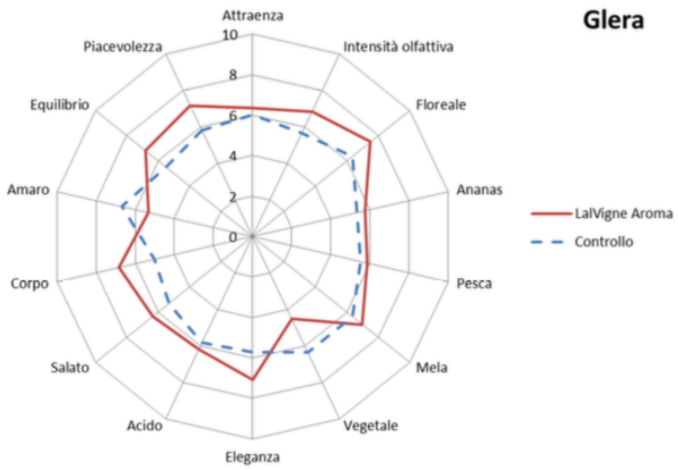

Figure 12. Comparison of the average sensorial profile of Glera wines produced with grapes treated with LalVigne®AROMA and a control sample. The data are the average of the years studied (2013, 2014, 2015, and 2016).

Source: Tomasí *et al*., 2015. Figure 3.

Translation: controllo - control; attraenza - attractiveness; intensità olfattiva - olfactory intensity; floreale - floral; ananas - pineapple; pesca - peach; mela - apple; vegetale - vegetal; elegance - eleganza; acido - acid; salato - salty; corpo - body; amaro - bitter; equilibrio - balance; piacevolezza - pleasurability.

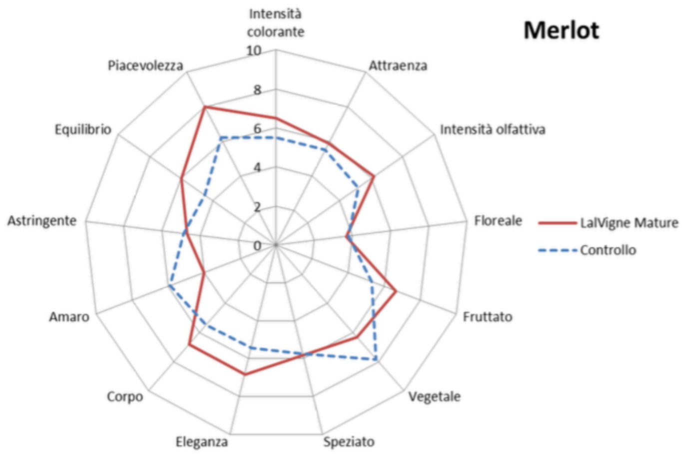

Figure 11. Comparison of the average sensorial profile of Merlot wines produced with grapes treated with LalVigne®MATURE and a control sample. The data are the average of the years studied (2013, 2014, 2015, and 2016).

Source: Tomasí *et al*., 2015. Figure 2.

Translation: controllo - control; attraenza - attractiveness; intensità olfattiva - olfactory intensity; floreale - floral; ananas - pineapple; pesca - peach; mela - apple; vegetale - vegetal; elegance - eleganza; acido - acid; salato - salty; corpo - body; amaro - bitter; equilibrio - balance; piacevolezza - pleasurability.
ensity; floreale - floral; ananas - pineapple; pesca - peach; mela - apple; vegetale - vegetal; elegance - eleganza; acido - acid; salato - salty; corpo - body; amaro - bitter; equilibrio - balance; piacevolezza - pleasurability.

4. Conclusion

The bibliographic review carried out in this work, was aimed to understand the importance of inactive dry yeast derivatives application, in vineyards. Therefore, this work was focused in the application of this product, as a climate change adaptation measure, and focused in is elicitation characteristic, regarding side effects.

The comparison between the different trials showed that LalVigne® increased skin thickness, GSH, thiols, concentration of anthocyanins and skin tannins concentration. Also, varietal aroma, and sensory analysis had positive results, with verified improvements in both. There was no impact verified on berries composition, even with all the changes seen on other characteristics.

The climate change alongside with the new demands of the consumer are a challenge for the production of wine, therefore LalVigne® is a good option once it gives an homogeneous maturation of the grapes facilitating the decision of the optimal moment of harvest, and also having a reduction on heterogeneity after weather incidents. The product increases the concentration, and improves, several compounds, reduces unwanted flavors and aggressive characters, saves adding of enological products, facilitates some processes, improves the wine mouthfeel, and improves wine longevity.

In the future the application of LalVigne® in cooler and less optimal harvests could be an advantage, once it enhances the ripening process. Consequently, it will be possible to obtain wines with superior oenological potencial.

5. References

Anderson, K., 2008. Terroir Rising in the New World? Varietal and Quality Distinctiveness of Australia's Wine Regions. *European Association of Wine Economists* - Annual Conference, 29 – 31.

Asproudi, A., Ferrandino, A., Bonello, F., Vaudano, E., Pollon, M. & Petrozziello, M., 2018. Key norisoprenoid compounds in wines from early-harvested grapes in view of climate change. *Food Chemistry* **268**, 143 – 152.

Asproudi, A., Petrozziello, M., Cavalletto, S., & Guidoni, S., 2016. Grape aroma precursors in cv. Nebbiolo as affected by vine microclimate. *Food Chemistry* **211**, 947 – 956.

Bertaud, B., 2016. Developing innovative yeast derivatives. *All About Feed* **24**, 9 – 11.

Blume, B., Nqrnberger, T., Nass, N., Scheel, D., 2000. Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. *Plant Cell* **12**, 1425 – 40.

Bollet, T., 1995. Chemoperception of microbial signals in plant cells. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 189 – 214.

Bonada, M., Sadras, V.O., Fuentes, S., 2013. Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Australian Journal of Grape and Wine Research* **19**, 87 – 94.

Brewik, E.C., 2013. The potential impact of climate change on soil properties and process and corresponding influence on food security. *Agriculture* **3**, 398 – 417.

Bruwer, J., Saliba, A. & Miller, B., 2011. Consumer behaviour and sensory preference differences: implications for wine product marketing. *Journal Consumer Marketing* **28**, 5 – 18.

Buttrose, M.S., Hale, C. R., & Kliewer, W.M., 1971. Effect of temperature on composition of Cabernet Sauvignon berries. *American Journal of Enology and Viticulture* **22**, 71 – 75.

Caballero, A. & Segura, A., 2017. The quest for lower alcoholic wines. *Microbial Biotechnology* **10**, 238 – 241.

Comuzzo, P., Tat, L., Tonizzo, A., & Battistutta, F., 2006. Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. *Food Chemistry* **99**, 217 – 230.

Coombe, B. G., & McCarthy, M. G., 2000. Dynamics of grape berry growth and physiology of ripening. *Australian Journal of Grape and Wine Research* **6**, 131 – 135.

Chuine, I., Yiou, P., Viovy, N., Seguin, B., Daux, V. and Le Roy Ladurie, E., 2004. Grape ripening as a past climate indicator. *Nature* **432**, 289 – 290.

Dami I., 2014. Determining Grapes Maturity and Fruit Sampling. Available in: https://ohioline.osu.edu/factsheet/HYG-1436. Accessed in: 25/08/2019.

Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Williams, P., & Doco, T., 2011. Effect of aging on lees and of three different dry yeast derivative products on Verdejo white wine composition and sensorial characteristics. *Journal of Agricultural and Food Chemistry* **59**, 12433 – 12442.

Duchêne, E., Huard, F., Dumas, V., Schneider, C. & Merdinoglu, D., 2010. The challenge of adapting grapevine varieties to climate change. *Climate Research* **41**, 193 – 204.

Duchêne, E., Schneider, C., 2005. Grapevine and climatic changes: a glance at the situation in Alsace. *Agronomy for Sustainable Development* **25**, 93 – 99.

Fehér, J., Lengyel, G., & Lugasi, A., 2007. The cultural history of wine – theoretical background to wine therapy. *Central European Journal of Medicine* **2**, 379 – 391.

Ferrari, S., 2010. Biological elicitors of plant secondary metabolites: Mode of action and use in the production of nutraceutics. *Biofarms for nutraceuticals: Functional food and safety control by biosensors*, 152 – 166.

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J. M., Moutounet, M., & Cheynier, V., 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry* **54**, 7331 – 7338.

Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Santos, J.A., 2012. An overview of climate change impacts on European viticulture. *Food Energy Secure* **1**, 94 – 110.

Gabler, F. M., Smilanick, J. L., Mansour, M., Ramming, D. W., & Mackey, B. E., 2003. Correlations of morphological, anatomical, and chemical features of grapes berries with resistance to Botrytis cinerea. *Phytopathology* **93**, 1263 – 1273.

Giacosa, S., Ossola, C., Botto, R., Segade, S.R., Paissoni, M.A., Pollon, M., Gerbi, V. & Rolle, L., 2019. Impact of specific inactive dry yeast application on grape skin mechanical properties, phenolic compounds extractability, and wine composition. *Food Research International* **116**, 1084 – 1093.

Goetz, G., Fkyerat, A., Métais, N., Kunz, M., Tabacchi, R., Pezet, R., & Pont, V., 1999. Resistance factors to grey mould in grape berries: Identification of some phenolics inhibitors of *Botrytis cinerea* stilbene oxidase. *Phytochemistry* **52**, 759 – 767.

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., & Simal-Gándara, J., 2015. Wine aroma compounds in grapes: A critical review. *Food Science and Nutrition* **55**, 202 – 218.

Granado, J., Felix, G., & Boller, T., 1995. Perception of fungal sterols in plants. subnanomolar concentrations of ergosterol elicit extracellular alkalinization in tomato cells. *Plant Physiology* **107**, 485 – 490.

Gunata Z., 1994. Étude et exploitation par voie enzy-matique des précurseurs d'arômes du raisin de nature glycosidique. *Rev. Œnol.* **74**, 22 – 27.

Hall, A., Jones, G.V., 2009. Effect of potential atmospheric warming on temperature-based indices describing Australian winegrape growing conditions. *Australian Journal of Grape and Wine Research* **15**, 97 – 119.

Hall, C.M., 1996. Wine Tourism in New Zealand. Tourism and Services Management.

Hannah, L., Roehrdanz, P., Ikegami, M., Shepard, A., Shaw, M., Tabo, G., Zhi, L., Marquet, P., Hijmans, R., 2013. Climate change, wine, and conservation. *Proceedings of the National Academy of Sciences* **110**, 6907 – 6912.

Huglin, P., & Schneider, C., 1998. Biologie et écologie de la vigne. Cachan, France: Éditions Tec & Doc Lavoisier.

Jackson, D.I. & Lombard, P.B., 1993. Environmental and management practices affecting grape composition and wine quality – A review. *American Journal of Enology Viticulture* **44**, 409 – 430.

Jackson, R.S., 1994. Wine Science: Principles and Applications. 1st edition, Academic Press. California.

Jones, G.V., White, M.A., Cooper, O.R. & Storchmann, K., 2005. Climate Change and Global Wine Quality. *Climate Change* **73**, 319 – 343.

Jones, G.V., Webb, L.B., 2010. Climate change, viticulture, and wine: challenges and opportunities. *Journal of Wine Research* **21**, 103 – 106.

Jones, G.V. & Davis, R.E., 2000. Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. *American Journal of Enology and Viticulture* **51**, 249 – 261.

Keller, M.M., 2010. Managing grapevines to optimize fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research* **16,** 56 – 69.

Kennedy J., 2002. Understanding Grape Berry Development. *Practical winery and vineyard Journal,*1 – 5.

Kliewer, W.M., 1971. Effect of day temperature and light intensity on concentration of malic and tartaric acids in Vitis vinifera L. grapes. *Journal of the American Society for Horticultural Science* **96**, 372 – 377.

Koblet, W., Zanier, C., Tanner, H., Vautier, Ph., Simon, J.L., & Gnägi, F., 1977. Reifeverlauf von Sonnenund Schattentrauben. *Schweizerische Zeitschrift für Obstund Weinbau* **113**, 558 – 567.

Kogkou, C., Chorti, E., Kyraleou, M., Kallithraka, S., Koundouras S., Logan, G., Kanakis, I. & Kotseridis, Y., 2017. Effects of foliar application of inactivated yeast on the phenolic composition of *Vitis vinifera* L. cv. Agiorgitiko grapes under different irrigation levels. *International Journal of Wine Research* **9**, 23 – 33.

Kritzinger, E. C., Bauer, F. F., & du Toit, W. J., 2012. Role of glutathione in winemaking: A review. *Journal of Agricultural and Food Chemistry* **61**, 269 – 277.

Lallemand, 2018. LalVigne: Grow your wine. *Catalogue Lallemand*

Le Berre, E., Atanasova, B., Langlois, D., Etiévant, P., & Thomas-Danguin, T., 2007. Impact of ethanol on the perception of wine odorant mixtures. *Food Quality and Preference* **18**, 901 – 908.

Lissarrague, J.R., Téllez, J., García, E., Peiro, E., 2014. Impact on agronomic parameters in Vines and wine quality of foliar treatments with specific fractions of yeast derivatives. *American Society Enology Viticulture*. 65th ASEV National Conference & 39th ASEV Eastern Section Annual Meeting, Hyatt Regency Austin, Austin Texas.

Magalhães, N., 2015. *Tratado de Viticultura – A Videira, A vinha e o Terroir*. Esfera Poética.

Makhotkina, O., Araujo, L. D., Olejar, K., Herbst-Johnstone, M., Fedrizzi, B., & Kilmartin, P. A., 2014. Aroma impact of ascorbic acid and glutathione additions to Sauvignon blanc at harvest to supplement sulfur dioxide. *American Journal of Enology and Viticulture* **65**, 388 – 393.

Mori, K., Goto-Yamamoto, N., Kitayama, M., & Hashizume, K., 2007. Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany* **58**, 1935 – 1945.

Moriondo, M., Jones, G.V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., Bindi, M., 2013. Projected shifts of wine regions in response to climate change. *Climate Change* **119,** 825 – 839.

Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., Cavallini, E., D'Incà, E., Tornielli, G.B., Filippetti, I., 2016. *Journal Plant Research* **129**, 513 – 526.

Mozell, M.R. & Thach, L., 2014. The impact of climate change on the global wine industry: challenges & solutions. *Wine Economics and Policy* **3**, 81 – 89.

Namdeo, A.G., 2007. Plant cell elicitation for production of secondary metabolites: a review. *Pharmacognosy Reviews* **1**, 69 – 79.

Negri, A.S., Prinsi, B., Rossoni, M., Failla, O., Scienza, A., Cocucci, M. & Espen, L., 2008. Proteome changes in the skin of the grape cultivar Barbera among different stages of ripening. *BMC Genomics* **9**, 378.

OIV, 2019. State of the Viticulture world market. *International Organization of Vine and Wine*.

Orduña, R.M., 2010. Climate change associated effects on grape and wine quality and production. *Food Research International* **43**, 1844 – 1855.

Owolabi, L.O., Yupanqui, C.T., Siripongvutikorn, S., 2018. Enhancing secondary meta- bolites (emphasis on phenolics and antioxidants) in plants through elicitation and metabolomics. *Pakistan Journal of Nutrition* **17**, 411 – 420.

Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., Poni, S., 2014. Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. *Scientia Horticulturae* **178**, 43 – 54.

Pastore, C., Allegro G., Valentini G., Colucci E., Battista, F. & Filippetti, I., 2019. Biochemical and molecular effects of yeast extract applications on anthocyanin accumulation in cv. Sangiovese. *BIO Web of Conferences* **13.**

Pastore, C., Dal Santo, S., Zenoni, S., Movahed, N., Allegro, G., Valentini, G., Filippetti I., Tornielli, G.B., 2017. *Frontiers in Plant Science* **8**, 929.

Patel, H., Krishnamurthy, R., 2013. Elicitors in plant tissue culture. *Journal of Pharmacognosy and Phytochemistry* **2**, 2278 – 4136.

Petrie, P.R. & Sadras, V.O., 2008. Advancement of grapevine maturity in Australia between 1993 and 2006: putative causes, magnitude of trends and viticultural consequences. *Australian Journal of Grape and Wine Research* **14**, 33 – 45.

Petrie, P.R., Brooke, S.J., Moran, M.A., Sadres, V.O., 2017. Pruning after budburst to delay and spread grape maturity: delaying pruning and grapevine phenology. *Australian Journal of Grape and Wine Research* **23**, 305 – 460**.**

Pilati, S., Perazzolli, M., Malossini, A., Cestaro, A., Demattè, L., Fontana, P., Dal Ri, A., Viola, R., Velasco, R. and Moser, C., 2007. Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at *veraison*. *BMC Genomics* **8**, 428.

Pinu, F., Edwards, P. B., Jouanneau, S., Kilmartin, P., Gardner, R., & Villas-Boas, S., 2014. Sauvignon blanc metabolomics: Grape juice metabolites affecting the development of varietal thiols and other aroma compounds in wines. *Metabolomics* **10**, 556 – 573.

Portu, J., López, R., Baroja, E., Santamaría, P., Garde-Cerdán, T., 2016. Improvement of grape and wine phenolic content by foliar application to grape- vine of three different elicitors: methyl jasmonate, chitosan, and yeast extract. *Food Chemistry* **201**, 213 – 221.

Pozo-Bayón, M. Á., Andujar-Ortiz, I., Alcaide-Hidalgo, J. M., Martín-Álvarez, P. J., & Moreno-Arribas, M.V., 2009. Characterization of commercial inactive dry yeast preparations for enological use based on their ability to release soluble compounds and their behavior toward aroma compounds in model wines. *Journal of Agricultural and Food Chemistry* **57**, 10784 – 10792.

Radman, R., Saez, T., Bucke, C., Keshavarz, T., 2003. Elicitation of plants and microbial cell systems. *Biotechnology and Applied Biochemistry* **37**, 91 – 102.

Ramakrishna, A., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior* **6**, 1720 – 1731.

Ramos, M.C., Jones, G.V., Martinez-Casasnovas, J.A., 2008. Structure and trends in climate parameters affecting winegrape production in northeast Spain. *Climate Research* **38**, 1 – 15.

Reynier, A., 2004. Manual de Viticultura. 3rd edition, Publicações Europa-América, Portugal.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D., 1999. The Chemistry of Wine Stabilization and Treatments: Phenolic Compounds. *Handbook of Enology* **2**, 129 – 186.

Río Segade, S., Giacosa, S., Paissoni, M.A., Ossola, C., Gerbi, V., Suárez Martínez, C., Battista, F., Téllez Quemada, J., Vagnoli, P., Rolle, L., 2016. Influence of specific inactive dry yeast treatments during grape ripening on postharvest berry skin texture parameters and phenolic compounds extractability**.** *Macrowine.* Poster.

Río Segade, S., Giacosa, S., Gerbi, V. & Rolle, L., 2011. Berry skin thickness as main texture parameter to predict anthocyanin extractability in winegrapes. *LWT-Food Science Technology* **44**, 392 – 398.

Rodríguez-Bencomo, J.J., Andújar-Ortiz, I., Moreno-Arribas, M.V., Simó, C., González, J., Chana, A., 2014. Impact of glutathione-enriched inactive dry yeast preparations on the stability of terpenes during model wine aging. *Journal of Agricultural and Food Chemistry* **62**, 1373 – 1383.

Rolle, L., Torchio, F., Zeppa, G., & Gerbi, V., 2008. Anthocyanin extractability assessment of grape skins by texture analysis. *Journal International des Sciences de la Vigne et du Vin* **42**, 157 – 162.

Roujou de Boubée, D., Van Leeuwen, C. and Dubourdieu, D., 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *Journal of Agricultural and Food Chemistry* **48**, 4830 – 4834.

Ruffner, H.P., Hawker, J.S., & Hale, C. R., 1976. Temperature and enzymic control of malate metabolism in berries of Vitis vinifera. *Phytochemistry* **15**, 1877 – 1880.

Santamaría, A.R., Mulinacci, N., Valletta, A., Innocenti, M., Pasqua, G., 2011. Effects of elicitors on the production of resveratrol and viniferins in cell cultures of *Vitis vinifera* L. cv Italia. *Journal of Agricultural and Food Chemistry* **59**, 9094 – 9101.

Santisi, J., 2011. Warming up the wine industry. *Emagazine: Environment Magazine* **22**, 15 – 17.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A., & Qian, M. C., 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. *Food Chemistry* **173**, 424 – 431.

Spayd, S.E., Tarara, J.M., Mee, D.L., Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. *American Journal of Enology and Viticulture* **53**, 171 – 182.

Šuklje, K., Antalick, G., Buica, A., Coetzee, Z.A., Brand, J., Schmidtke, L.M. & Vivier, M.A., 2016. Inactive dry yeast application on grapes modify Sauvignon Blanc wine aroma. *Food Chemistry* **197**, 1073 – 1084.

Tate, A.B., 2001. Global warming's impact on wine. *Journal Wine Research* **12**, 95 – 109.

Téllez, J., González, V., García, E., Peiro, E., Lissarrague, J.R., 2015. Foliar application of yeast derivatives on grape quality and resulting wines. *American Society Enology Viticulture*. Poster.

Thakur, M., Bhattacharya, S., Khosla, P.K., Puri, S., 2019. Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants* **12**, 1 – 12.

Tomasi, D., Panighel, A., Flamini, R., Lovat, L., Battista, F., 2017. Foliar application of specific inactivated yeast with action on phenolic and aromatic metabolism of grapes. *OIV congress*.

Unwin, T., 1991. Wine and the Vine: An Historical Geography of Viticulture and the Wine Trade. Routledge, London and New York.

Van Leevwen, C., Pieri, P., Ollat, N., Gaudy, M., 2019. Reduced density is an environmentally friendly and cost-effective solution to increase resistance to drought in vineyards in a context of climate change. *International Viticulture and Enology Society OENO* **2**, 129 – 146.

Veersham, C., 2004. Elicitation: Medicinal Plant Biotechnology. *CBS Publisher*, 270 – 293.

Villangó, Sz., Pásti, Gy., Kállay, M., Leskó, A., Balga, I., Donkó, A., Ladányi, M., Pálfi, Z., Zsófi, Z., 2015. Enhancing Phenolic Maturity of Syrah with the Application of a New Foliar Spray. *South African Journal of Enology and Viticulture* **36**, 304 – 315.

Vink, N., Deloire, A., Bonnardot, V., Ewert, J., 2012. Climate change and the future south African's wine industry. *International Journal of Climate Change Strategies and Management* **4**, 420 – 441.

Webb, L.B., Whetton, P.H. and Barlow, E.W.R., 2007. Modelled impact of future climate change on the phenology of winegrapes in Australia. *Australian Journal of Grape and Wine Research* **13**, 165 – 175.

Winterhalter, P. & Schreier, P., 1994. C_{13}-Norisoprenoid glycosides in plant tissues: an overview on their occurrence, composition and role as flavour precursors. *Flavour and Fragrance Journal* **9**, 281 – 287.

White, M.A., Diffenbaugh, N.S., Jones, G.V., Pal, J.S., Giorgi, F., 2006. Extreme heat reduces and shifts United States premium wine production in the 21st century. *Proceedings of the National Academy of Sciences* **103**, 11217 – 11222.

Wolfe, D.W., Schwartz, M.D., Lakso, A.N., Otsuki, Y., Pool, R.M. and Shaulis, N.J., 2005. Climate change and shifts in spring phenology of three horticultural woody perennials in northeastern USA. *International Journal of Biometeorology* **49**, 303 – 309.

Zhao, J., Davis, L.C., Verpoorte, R., 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advance* **23**, 283–333.