Understanding *Brettanomyces* behaviour to optimise the use of alternatives to SO_2 in wines

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ABSTRACT

The wine world is constantly evolving, and the market is increasingly demanding with regard to the characteristics of the final product. Winegrowers must follow the trends that have been emerging in relation to winemaking methods, not only in terms of the final product, but also when talking about all the processes involved in obtaining it. As far as wine defects are concerned, one of the producers' greatest focus is *Brettanomyces* yeast, which is considered to have the greatest capacity to cause wine spoilage. It has been, in the last decades, a reason for great attention, since it causes great economic losses when the conditions for its establishment in the winery are met, especially when we talk about higher quality red wines that have been submitted to expensive ageing processes in wooden barrels. This yeast has the capacity to produce ethylphenols which, above certain quantities, cause highly undesirable changes in the wine's organoleptic characteristics. To date, the most used and efficient approach to dealing with *Brettanomyces* is the use of sulphites to prevent its growth. SO_2 is the most widely used additive in wineries for the control of this yeast. However, in recent years there has been growing concern from a number of health and food industry stakeholders about the presence of sulphites in various foods. In addition to the fact that they can be harmful to human health above certain ingested values, there is now an increasing trend towards the reduction of all chemical additives in food. The current trend has led the consumer to prefer all products that are related to organic, sustainable, natural production, words that are increasingly referred to throughout the industry. As a food product, wine has also been following this trend, which is becoming increasingly demanding and challenging.

The aim of this review was to analyse most of the available alternative methods to the use of sulphites for the reduction of *Brettanomyces* in wine, in an attempt to minimise the amount of SO_2 to be added to the final product. Knowing how this yeast behaves, which factors influence its growth and at which stages of the winemaking process it is most likely to develop, are some of the topics. In this way, it is intended to make a synthesis of alternative methods to reduce its incidence, to understand which are the most advantageous and what still has to be done in the future to achieve the desired objectives.

Keywords: *Brettanomyces*, ethylphenols, off-flavours, SO_2, wine.

RESUMO

O mundo dos vinhos tem vindo a evoluir no sentido que, o consumidor e o mercado são cada vez mais exigentes no que toca a características do produto. Os enólogos têm de acompanhar as tendências que surgem no que diz respeito a métodos de vinificação, não só em relação ao produto final mas também a todos os processos envolvidos na sua obtenção. Relativamente aos defeitos do vinho, uma das grandes preocupações dos produtores é a levedura *Brettanomyces,* considerada a que tem maior capacidade para provocar estragos derivados de defeitos que poderá causar. Tem sido, nas últimas décadas, motivo de grande atenção pois é causadora de grandes perdas económicas quando se reúnem condições para a sua instalação na adega, principalmente quando falamos de vinhos tintos de maior qualidade que passaram por processos dispendiosos de envelhecimento em barricas de madeira. Até à data, a abordagem mais utilizada e com maior eficiência no combate à *Brettanomyces* é a utilização de sulfitos para impedir o seu crescimento. O SO_2 é o aditivo mais utilizado nas adegas para o controlo desta levedura, no entanto, nos últimos anos tem existido uma preocupação crescente por parte de várias entidades relacionadas com a saúde e a indústria alimentar, relativamente à presença de sulfitos em vários alimentos. Para além de que poderão ser prejudiciais para a saúde humana, a partir de determinados valores ingeridos, existe agora e cada vez mais uma tendência para a redução de todos os aditivos de origem química nos alimentos. A tendência e a 'moda' atual, por uma ou várias razões, levaram o consumidor a preferir todos os produtos que estejam relacionados com produções orgânicas, sustentáveis, biológicas, naturais, palavras estas que são cada vez mais referidas em toda a indústria. Ora, o vinho, como produto alimentar, tem vindo também ele a seguir esta tendência que cada vez se torna mais exigente e desafiante.

O objetivo desta revisão foi analisar a maioria dos métodos alternativos existentes ao uso de sulfitos no combate à *Brettanomyces* no vinho numa ótica que pretende diminuir a quantidade de SO_2 a adicionar ao produto final. Conhecer como se comporta esta levedura, quais os fatores que influenciam o seu crescimento e em que fases do processo de vinificação é mais propício ao seu desenvolvimento são alguns dos temas abordados com o objetivo de melhor conhecer a ecologia da *Brettanomyces*.

Palavras-chave: *Brettanomyces*, defeitos, etilfenóis, vinho, SO_2.

RESUMO ALARGADO

O mundo dos vinhos tem vindo a evoluir no sentido que, o consumidor e o mercado têm padrões cada vez mais altos no que toca a características do produto, tendência esta que se regista em toda a indústria alimentar. Como tal, também o setor vitivinícola terá de acompanhar as exigências de qualidade que se fazem notar com a evolução dos tempos. Os métodos de vinificação para obtenção do produto final evoluíram e observa-se uma tendência para que assim continue, sendo que o enólogo é testado todos os dias de modo a acompanhar a evolução que se nota no resto desta indústria. Relativamente ao vinho, os padrões de qualidade são cada vez mais exigentes pelo mundo fora, e no que diz respeito aos defeitos do vinho, uma das grandes preocupações dos produtores são os estragos causados pela levedura *Brettanomyces,* considerada a que tem maior capacidade para alterar as qualidades organoléticas do vinho. Esta levedura provoca alterações no vinho a nível sensorial altamente indesejadas quando presente em níveis acima do razoável e tem sido nas últimas décadas motivo de grande atenção pois é causadora de grandes perdas económicas quando se reúnem condições para a sua instalação na adega, principalmente quando falamos de vinhos tintos de maior qualidade que passaram por processos dispendiosos de envelhecimento em barricas de madeira. A *Brettanomyces* tem a capacidade de produzir etilfenóis no vinho que, a partir de certos valores estão associados a defeitos descritos como 'Brett character', 'stable', 'horse sweat' entre muitos outros. A luta contra esta levedura tem sido feito ao longo do tempo pelos enólogos de modo a limitar ao máximo possível os estragos causados pela mesma, no entanto, até à data, a abordagem mais utilizada e com maior eficiência é a utilização de sulfitos para impedir o seu crescimento. O SO_2 é o aditivo mais utilizado nas adegas em todo o mundo para o controlo desta levedura sendo que a sua fácil utilização e o seu custo razoavelmente acessível tornam-no um candidato difícil de superar. No entanto, nos últimos anos tem existido uma preocupação crescente por parte de várias entidades relacionadas com a saúde e a indústria alimentar, relativamente à presença de sulfitos em vários alimentos. Para além de que poderão ser prejudiciais para a saúde humana a partir de determinados valores ingeridos, existe agora e cada vez mais uma tendência para a redução de todos os aditivos de origem química nos alimentos. A tendência e a 'moda' atual, por uma ou várias razões, levaram o consumidor a preferir todos os produtos que estejam relacionados com produções orgânicas, sustentáveis, biológicas, naturais, palavras estas que são cada vez mais referidas em toda a indústria alimentar. Ora, o vinho, como produto alimentar, tem vindo também ele a seguir esta tendência que cada vez se torna mais exigente e desafiante. Vários trabalhos realizados na última década têm vindo a estudar alternativas ao uso de sulfitos no vinho no que diz respeito ao controlo dos danos causados por esta levedura. Estas alternativas têm como objetivo a utilização de abordagens que possam ser associadas a modos de produção com o recurso mínimo a aditivos químicos e máximo a todas as técnicas associadas à

produção ‘natural’. O objetivo desta revisão foi analisar a maioria dos métodos alternativos existentes até à data ao uso de sulfitos no combate à *Brettanomyces* no vinho numa ótica que pretende diminuir a quantidade de SO_2 a adicionar ao produto final. Conhecer como se comporta esta levedura, quais os fatores que influenciam o seu crescimento e em que fases do processo de vinificação é mais propício o seu desenvolvimento são alguns dos temas abordados com o objetivo de melhor conhecer a ecologia da *Brettanomyces*. Deste modo, pretende-se fazer uma síntese de métodos alternativos estudados para a redução da sua incidência, perceber quais são os mais vantajosos, quais os que realmente são viáveis a níveis práticos e o que ainda terá de ser feito no futuro para se alcançar os objetivos pretendidos num caminho que tende para a produção de vinho com recurso mínimo à utilização de SO_2.

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1. Introduction

With regard to yeasts that have the capacity to cause spoilage in wine, *Brettanomyces* have earned high importance in recent years worldwide. The damage caused by these yeasts is essentially associated with their capacity to produce ethylphenols which, depending on the values produced, can severely affect the organoleptic qualities of wine. These values vary according to people's preferences, but above certain limits they are considered highly undesirable and seriously affect the quality of the wine, especially when we talk about high quality red wines that undergo expensive aging processes in oak barrels and can therefore incur serious economic losses for companies.

The wine industry largely uses SO_2 as an additive for microbiological stabilisation in wine and also to control *Brettanomyces.* It is a very efficient, easy-to-use and affordable additive, however, the trend in recent years is to reduce the number of sulphites in the whole food industry and the situation with wine is no different. Due to human health aspects and perhaps recent trends, the aim of several researchers has been to look for suitable alternatives to this additive. There are already a great number of works that refer to physical and chemical processes as an alternative to the use of SO_2, many of which, even if they do not completely replace it, aim to reduce it. For these reasons, the concentrations of SO_2 allowed in wine has been gradually reduced and it is today strictly defined, according to the International Organisation of Vine and Wine (OIV) and the European Commission (EC) No. 606/2009 as we can observe in table 1.

The reduction or even elimination of SO_2 in wine is even more critical when talking about organic wines. There has been in the wine industry, along with all the other food industries, a tendency towards organic products in replacing the common ones. According to the Commission Implementing Regulation (EU) No. 203/2012 (accessed 13th July 2020) the legal limits of maximum sulphur dioxide to be added to wine in organic wines are also represented in table 1. What is more, 'free sulphite wines' have been gaining expression in the market. These wines, although not a properly regulated category in the EU, are all those with a total sulphite content of less than 10 mg/L. That is, according to Part E of annex II EC No 1333/2008, all wines containing more than 10 mg/L must present the information 'contain sulphites'. All others are considered sulphite free.

To find suitable alternatives to the use of sulphites it is necessary to study and understand the behaviour and ecology of this yeast in the winery environment and how they perform throughout the entire winemaking process.

Table 1: Legal limits of total sulphur dioxide to be added to wine according to the OIV and the European Commission (EC) No. 606/2009 for normal wines and for organic wines according to the Commission Implementing Regulation (EU) No. 203/2012 (accessed 13th July 2020).

	Conventional Wines				Organic Wines	
	OIV		EC		EU	
Red Wines	Residual Sugar	Sulphur Dioxide	Residual Sugar	Sulphur Dioxide	Residual Sugar	Sulphur Dioxide
	≤ 4 g/L	150 mg/L	< 5 g/L	150 mg/L	< 2 g/L	100 mg/L
	> 4 g/L	300 mg/L	≥ 5 g/L	200 mg/L	2 - 5 g/L	120 mg/L
					≥ 5 g/L	170 mg/L
White and Rosé Wines	≤ 4 g/L	200 mg/L	< 5 g/L	200 mg/L	< 2 g/L	150 mg/L
	> 4 g/L	300 mg/L	≥ 5 g/L	250 mg/L	2 - 5 g/L	170 mg/L
					≥ 5 g/L	220 mg/L

This review aims to better understand the behaviour and ecology of *Brettanomyces*, its metabolic pathways in the production of ethylphenols and what are the most appropriate ways to prevent the damage caused by these yeasts in wine. An analysis of several well-known methods has been made in order to understand which the best alternatives for its control are.

2. Wine spoilage by *Brettanomyces bruxellensis*

2.1. The genus *Brettanomyces/Dekkera*

2.1.1. Taxonomy and Morphology

The earliest reference to the genus *Brettanomyces* dates back to 1904 in a paper to the Institute of Brewing, where N. Hjelte Claussen describes the contribution of *Brettanomyces* for the secondary fermentation of beers and for its characteristic English flavour (Gilliland 1961). It is not until 1904 that M.T.J Custers conducted the first systematic study, where he associates *Brettanomyces* with the wine industry (Custers 1940).

The yeast *Dekkera/Brettanomyces* is present in two forms: *Dekkera*, the sexual sporulating form and *Brettanomyces*, the asexual non-sporulating form (Coulter et al. 2003). Today there are five species considered to belong to the genera *Brettanomyces/Dekkera*: *Brettanomyces custerianus*,

Brettanomyces naardenensis, *Brettanomyces nanus*, *Brettanomyces anomalus* and *Brettanomyces bruxellensis*. Their teleomorphs are known for the species *Dekkera anomala* and *Dekkera bruxellensis* (Kurtzman and Fell 1998; Cocolin et al. 2004;).

Brettanomyces bruxellensis is regarded as one of the yeasts with the greatest capacity to provoke wine spoilage. *B. bruxellensis* has an oval to an ellipsoidal shape and it reproduces by budding. After just a few months of incubation, its cell morphology changes from elliptic to branched (Wedral et al., 2010).

2.1.2. Spoilage Effects

2.1.2.1. Volatile Phenols

Brettanomyces presence in wine can cause different damages associated with cloudiness, however, the reason why it has become so important the past years and the biggest oenological concern worldwide is due to the fact that this yeast is responsible for a range of off-flavours, mainly due to the production of volatile phenols that are particularly notorious in high quality red wines aged in costly oak barrels (Chatonnet 1995; Oelofse et al. 2008; Wedral et al. 2010; Chandra et al. 2016; Malfeito-Ferreira 2018). These damages could lead to major problems in the wine industry as they can cause enormous economic losses (Oelofse et al. 2008). According to Yap *et al.* (2007), the spoilage caused by these yeasts has also gained importance in the past decade due to new winemaking styles and practices trends, such as wines with higher pH and residual sugar levels and the reduced use of filtration and SO_2. What is more, the author also considers the poor hygiene and insufficient sanitisation of barrels (a critical source of *Brettanomyces/Dekkera* contamination) and the use of barrels purchased second hand from contaminated wineries.

In small concentrations, *Brettanomyces/Dekkera* yeasts can contribute to the complexity of wines, however, above certain values they became undesirable (Schumaker et al. 2017), being responsible for the development of phenolic character in red wines (Chatonnet 1995) through the production of ethylphenols, namely 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG). These yeasts multiply through the fermentation of small amounts of residual sugars such as glucose, fructose, galactose and trehalose in wines (Chatonnet 1995). They have the ability to transform (50-60%) substrate into 4-EP when in the presence of hydroxycinnamic acids (Chatonnet et al. 1992), thus changing the organoleptic qualities of wine (Chatonnet et al. 1993). Through the consumption of only 300 mg/L of fermentative sugars, which is less than what we normally find in young red wines, *Brettanomyces/Dekkera* are able to produce enough ethylphenols to cause aroma changes (Chatonnet 1995). Spoilage caused by *B. bruxellensis* occurs mainly during wine aging in oak barrels (Chatonnet et al. 1993), however this yeast

may also interfere with alcoholic fermentation (AF) and malolactic fermentation (MLF) according to Renouf et al., 2006. *B. bruxellensis* has slow growth, fermentative and oxidative metabolism, high tolerance to ethanol, acetic acid production under aerobic conditions and the ability to persist through the winemaking process (Chatonnet et al. 1992; Jolly et al. 2003; Renouf et al. 2006; Oelofse et al. 2008).

2.1.2.2. Other Faults: Mousiness and Biogenic Amines

Brettanomyces has also the capacity to produce other off-flavours which, although sporadic, could cause disastrous problems. This off-flavour is known as the 'mousy' character. It was first reported and isolated in 1986 by Heresztyn who also characterized the compounds responsible for the emergence of this aroma. This descriptor appears in wines where *Lactobacillus* and *Brettanomyces* are to be found (Oelofse et al. 2008). The 'mousy' character appears when production of these three compounds, 2-acetyltetrahydropyridine (ATHP), 2-ethyltetrahydropyridine (ETHP) and 2-acetylpyroline (APY) (Heresztyn, 1986b) occurs, being that *Brettanomyces* is only able to produce the first two. In order to produce these two compounds, the presence of the amino acid L-Lysine and ethanol are obligatory, whereby oxygen also promotes their development, probably by increasing the growth rate of *Brettanomyces* (Heresztyn 1986b; Grbin 1998; Snowdon et al. 2006). Although we are aware of these conditions, it is not yet known why this off-flavour occurs very infrequently (Oelofse et al. 2008).
We know that the microorganisms involved in the fermentation of wine, as in other fermented products, may form undesirable compounds. Biogenic amines are substances formed through the decarboxylation of amino acids that are necessary for the human body, but that in higher concentrations than would be ideal can cause undesirable effects in more sensitive people, especially in the presence of alcohol and acetaldehyde (Caruso et al. 2002; Oelofse et al. 2008). Work done by Caruso et al. 2002 and Granchi et al. 2005 showed that *B. bruxellensis* is responsible for a large part of the production of biogenic amines in wines and thus responsible for the damage caused by these compounds.

2.2. Volatile phenols production

2.2.1. Metabolic Pathways

Ethylphenols are formed by *Brettanomyces/Dekkera* in the presence of hydroxycinnamic acids in wine due to the sequential activity of two enzymes. The first one is a cinnamate decarboxylase (CD) that ensures the transformation of cinnamic acids into their corresponding vinylphenols. The second one is

a vinylphenol reductase that catalyzes the reduction of vinylphenols into their respectively ethylphenols as shown in Figure 1. Unlike *Saccharomyces cerevisiae*, the cinnamate decarboxylase activity of *B. bruxellensis* is not inhibited by phenolic compounds in grapes, making it possible for it to produce several milligrams of ethylphenols per liter of wine, the amount produced being directly proportional to its population (Chatonnet et al. 1992; Chatonnet 1995).

The hydroxycinnmaic acids (p-coumaric, ferulic, caffeic and sinapic) are present in grape juices and wines esterified to other molecules. P-coumaric acid and ferulic acid (4-hydroxycinnamic and 4-hydroxy-3-methoxy-cinnamic acid respectively) may be conjugated with tartaric acid (Chatonnet et al. 1992; Oelofse et al. 2008; Wedral et al. 2010) and may be hydrolysed either by enzymes from fungi or commercial pectolytic preparations. On the other hand, ethyl and glucose esters are directly metabolized by *Brettanomyces* (Malfeito-Ferreira 2018). The esterification to anthocianins is chemically hydrolysed during wine aging. Figure 1 summarizes all these mechanisms.

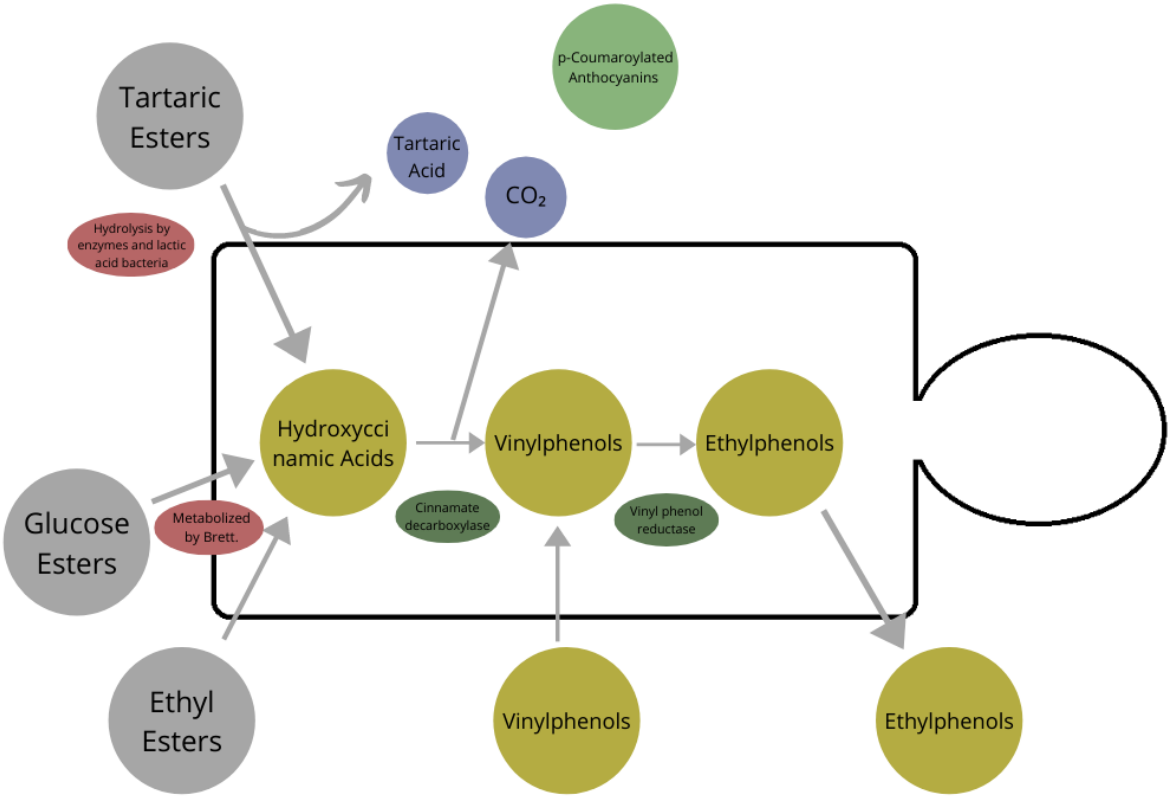

Figure 1: Formation of ethylphenols through several precursor molecules in *Brettanomyces*.

There are several microorganisms, such as bacteria, fungi and yeasts, present in wine that are capable of synthesizing 4-EP and 4-EG, meaning that they are capable of carrying out the decarboxylation phase (e.g. *Bacillus* and *Lactobacillus* bacteria and *Saccharomyces* yeasts), however, most of them are not

able to perform the reduction phase where vinylphenol reductase catalyzes the reduction of vinylphenols into their respectively ethylphenols (Chatonnet et al. 1992; Chatonnet 1995; Dias et al. 2003; Suárez et al. 2007; Ganga et al. 2011). *Brettanomyces/Dekkera* has the ability to use vinylphenols previously synthesized by other microorganisms, as a precursor for the formation of ethylphenol in the absence of *p*-coumaric acid (Loureiro and Malfeito Ferreira 2006; Suárez et al. 2007; Coterno et al. 2013).

The enrichment of wines in ethylphenols may take place at different stages in the production process, however it usually occurs during the ageing phase, particularly during summer months. It is more common to happen in used oak barrels, however, it is also possible for it to happen in tanks. This is due to the fact that *Brettanomyces/Dekkera* are capable of synthesizing 4-EP and 4-EG from both phenolic acids present in grapes and in oak wood (Chatonnet et al. 1992; Chatonnet, 1995).

Therefore, to prevent the problem and avoid spoilage, the understanding of *Brettanomyces/Dekkera* ecology and behaviour is a key point to reduce the conversion of hydroxycinnamic acids to volatile phenols, as the natural concentrations of this acids in wines are high enough to provide substrate for the production of this compounds (Malfeito-Ferreira 2018).

2.3. *Brettanomyces* inhibition and 4-EP production

Brettanomyces/Dekkera are adapted to poor nutritional environments, which makes them capable of surviving even under difficult conditions. This, together with the fact that they are adapted to high ethanol concentrations, has allowed them to became established in many wineries (Suárez et al. 2007). It has been shown that the formation of volatile phenols in wines depend mostly on the presence of precursors and on the proportion of the *Brettanomyces/Dekkera* population size. However, the production capacity of volatile phenols by different strains of *B.bruxellensis* depends on alcohol content, temperature, wine pH, dissolved oxygen and nutrient availability in the must (Suárez et al. 2007; Romano et al. 2008;). Nitrogen availability is considered by Conterno et al. (2007) more important as an influencing factor than the presence of residual sugars.

As it has been already established, *B. bruxellensis* is considered tolerant to high levels of ethanol and it has been demonstrated that it can produce 4-EP in synthetic media up to 13% alcohol (v/v) and up to 13.4% in red wine according to Dias et al. (2003) and Coulon et al. (2010). What is more, according to Conterno et al. (2006), 94% of the yeast from a global collection were able to grow at pH 2.0.

When speaking of nutritional requirements of *B. bruxellensis*, most wines contain sufficient nitrogen and carbon sources to support its growth. A considerable low concentration of 275 mg/L combined of either glucose, galactose, trehalose or fructose is enough for the production of ethylphenols above

there sensory threshold level by this yeast (Chatonnet 1995; Curtin et al. 2015) and so is 6 mg/L of yeast assimilable nitrogen (Childs et al. 2015).

A study conducted by Malfeito-Ferreira et al. (2001) showed that the production rate of 4-EP was more dependent on *Brettanomyces/Dekkera* growth rate than on other factors. By decreasing the yeast growth, we also decrease its production. The authors also concluded that oxygen levels up to about 7-8mg/L (saturation concentration) makes the production of 4-EP faster and ethanol may also be used as a substrate for growth and production of 4-EP if aerobic conditions are favoured. Oxygen is known to influence this yeasts metabolism due to the 'Custers effect', which may be described as the inhibition of alcoholic fermentation in anaerobisis (Conterno and Mach 2010).

Keeping wines at low cellar temperatures can be used as a prevention measure if cell growth is fully inhibited as it delays the process of 4-EP production. The effect of temperature on 4-EP production is related to the production rate and not the total amount produced (Malfeito-Ferreira et al. 2001). A study conducted by Brandam et al. (2008) observed that a maximum specific growth rate value for the population of *Brettanomyces/Dekkera* increased regularly with temperature up to a temperature threshold of 32°C, beyond which a decrease was observed.

There are several studies and several values obtained regarding the amount of SO_2 to be added to wine in order to control the growth of *Brettanomyces* populations. This shows that managing sulphites is not an easy task. *Brettanomyces* tolerance to sulphites is highly dependent on strain and parameters already observed such as temperature and pH (Zuehlke and Edwards 2013; Longin et al. 2016). According to Longin et al. (2016), one of the reasons for the difficulty in managing sulphite levels is that yeast cells enter a VBNC (viable but nonculturable) state and as soon as SO_2 levels decrease, they recover their growth, hence cells in this state are not always easy to detect.

2.4. Off-flavour perception

2.4.1. Sensory Thresholds

Phenol derivatives have been identified as part of the volatile compounds that, when present in small quantities may contribute to the aroma's complexity of wines (Chatonnet et al. 1992; Oelofse et al. 2008), however, above certain thresholds they are considered undesirable (Schumaker et al. 2017). The volatile phenols that have shown more relevance over several studies in the past years are 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG), as mentioned before and 4-ethylcatechol (4-EC) in red wines and both 4-vinylphenol (4-VP) and 4-vinylguaiacol (4-VG) in white wines (Chatonnet et al. 1992; Chatonnet 1995; Schumaker et al. 2017; Malfeito-Ferreira 2018). Thus, the production of 4-EP and 4-

EG is the main factor affecting wines sensory properties (Wedral et al. 2010). When this compounds are present in wine and exceed their combined perception threshold, they impart off-odours and can also affect wine flavour. Together, the sensory attributes that come from both 4-EP and 4-EG are known as 'Brett' character and *B. bruxellensis* is considered to spoil wine (Loureiro and Malfeito-Ferreira 2003; Lattey et al. 2010).

In general, red wines have high levels of ethylphenols and low levels of vinylphenols, contrary to what happens in white wines, as they have high levels of vinylphenols and low levels of ethylphenols (Chatonnet et al. 1992). Compared to white wines, red ones are particularly prone to *Brettanomyces* development and the subsequent production of ethylphenols (Romano et al. 2008) due to their lower acidity, higher polyphenol content and barrel aging. What is more, Vitis vinifera red varietals contain phenolic precursors (Monagas et al. 2006; Wedral et al. 2010). Vinylphenols are found at meaningless concentration in red wines as they incorporate into pyranoanthocyanins (Morata et al. 2007). According to Chatonnet et al. (1992) and Loureiro and Malfeito-Ferreira (2006), white wines do not seem to have the *Brettanomyces* aroma character due to the efficacy of sulphur dioxide ate low pH and the absence of precursor compounds.

There are thresholds from which these compounds are responsible for the production of off-flavours and off-odours in wine (Peña et al. 2019). Vinylphenols in white wines are responsible for 'pharmaceutical' odours (Chatonnet et al. 1993) while ethylphenols in red wines are accountable for odours described as 'phenolic', 'animal', 'stable', 'barn', 'horse sweat', 'leather', 'varnish' and 'medicinal' (Chatonnet et al. 1992; Suárez et al. 2007; Oelofse et al. 2008). Nevertheless, if present in concentrations below 400 µg/L, 4-EP are able to provide notes described as 'spices', 'leather' and 'smoke' and in this way contribute to the aromatic complexity of wine which are valued by most wine consumers (Oelofse et al. 2008). Over the past few years, several studies have been published by a variety of authors concerning the concentration of 4-EP and 4-EG in wines that are likely to cause sensory alterations. Some of these works are briefly summarized in table 2.

Table 2: Works conducted by several authors regarding the concentration of 4-EP and 4-EG in wines that are likely to cause sensory alterations in red wines

Significance	Concentration	Reference
Perception threshold	≥ 440 µg/L of 4-EP	Boidron et al. (1988)
	≥ 600 µg/L of 4-EP	Chatonnet (1995)
	≥ 390 µg/L of 4-EP	López et al. (2002)
	≥ 368 µg/L of 4-EP	Curtain et al. (2007)
Preference threshold (considered spoilage)	≥ 620 µg/L of 4-EP	Chatonnet et al. (1992)
Contributes favourably to the complexity of wine aroma with aromatic notes of spices, leather and smoke, appreciated by many consumers	< 400 µg/L of 4-EP	Oelofse et al. (2008)

There is an enormous amount of volatile compounds present in wine and for these to be perceptible they need to be volatilised from the complex organoleptic matrix of wine to reach the human olfactory perception (Cameleyre et al. 2018). Although there are several thresholds for these compounds that vary according to the environment in which they were determined (water, model wine, red wine), we can admit that they are highly dependent on factors such as wine (figure 2), judge effects (figure 3) and perceptual interactions on olfactory perception of taint compounds (figure 4).

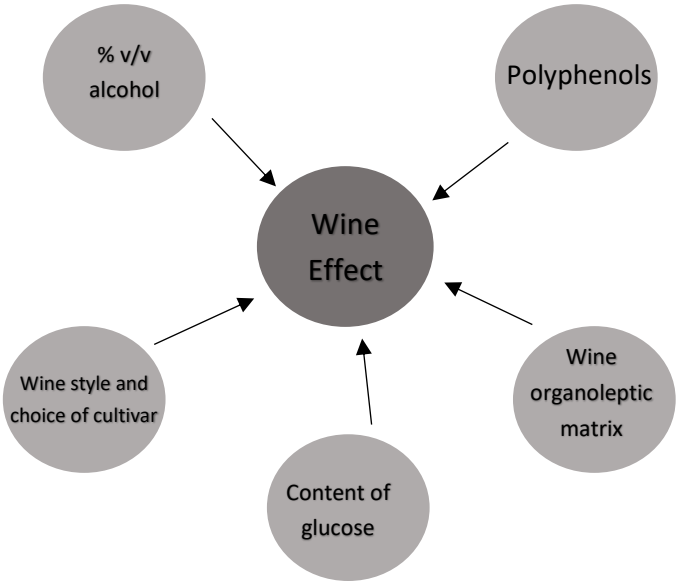

Figure 2: Effects related to wine, adapted from McKay and Buica (2019).

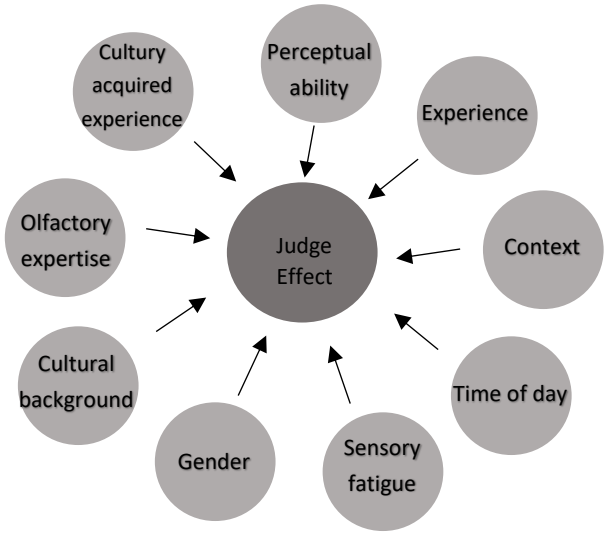

Figure 3: Effects related to judge, adapted from McKay and Buica (2019).

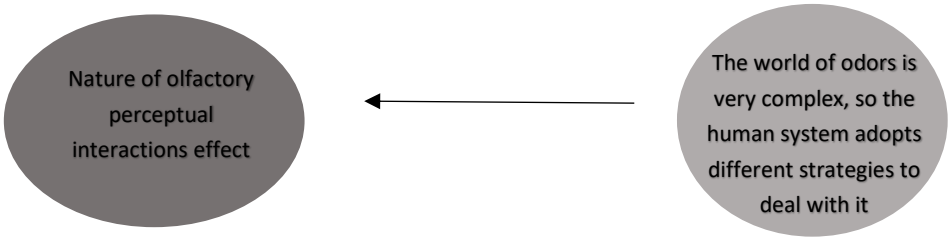

Figure 4: Effects related to perceptual interactions on olfactory perception of taint compounds adapted from McKay and Buica (2019).

Other related factor when talking about the detection of defects is the 'organic wines' element. According to Romano et al. (2020), tasters in general tend to 'accept' defects in wine more easily when they know they came from organic production.

3. Prevention of yeast growth

As it has been previously mentioned, the natural concentrations of hydroxycinnamic acids present in wine is sufficient to provide substrate for the production of volatile phenols well above the limits that would be preferable. Thus, the most sensible way to prevent this problem would be to understand the behaviour and ecology of *Brettanmyces/Dekkera* yeasts in order to apply appropriate control

measures to prevent and reduce the conversion of these acids into volatile phenols (Malfeito-Ferreira 2018). According to Oelofse et al. (2008) the phenomena related to spoilage caused by these yeasts are not simply solved by manipulating individual factors, but by creating a holistic approach. Several studies have identified factors that, individually or combined, minimize or delay the growth of *Brettanomyces/Dekkera* and their consequent production of ethylphenols (Chandra et al. 2014; Sturm et al. 2014). In order to prevent these yeasts growth in wine, it is necessary to pay attention to fruit quality and winery sanitation, control of oxygen levels and sulphite and the use of uncontaminated barrels (Wedral et al. 2010).

For years the wine industry has been looking for ways and tools to eradicate contaminating microorganisms in the fermentation and ageing process of wine in barrels. Therefore, it is necessary to reduce the number of cells and growth of *Brettanomyces/Dekkera* yeasts, in order to limit their development as much as possible since they are characterized by their ability to survive under adverse nutrient scarcity conditions, which allows them to persist throughout the winemaking and storage process (Malfeito-Ferreira 2018; Peña et al. 2019).

The most common and efficient way to control *Brettanomyces/Dekkera* yeasts is through the correct use of SO_2, while a high pH, a high temperature, high residual sugar and a low alcohol content are positively correlated to its growth (Dias et al. 2003). Spoilage caused by these yeasts can be prevented through the proper use of SO_2 and by reducing the available oxygen during the winemaking process (Suárez et al. 2007). SO_2 is one of the most widely used additives in the food industry nowadays, due to its microbial, antioxidant and stabilizing properties to the final product. This additive is particularly effective when it comes to preventing the proliferation of *Brettanomyces bruxellensis*, regardless the presence of residual sugars and concentrations of ethanol that promote this yeast growth (Vigentini et al. 2013; Lisanti et al. 2019; Pinto et al. 2020). According to Edwards and Oswald (2018), it was demonstrated a great efficiency in the use of SO_2 against *Brettanomyces* spp. in wines, even in the presence of high concentrations of ethanol and low storage temperatures. Sulphite in its free molecular form, has long been the number one choice for the preservation of wine and is considered by Coulter et al. (2003) to be the key point for any successful *Brettanomyces/Dekkera* control strategy. Moreover, in the wine industry, the addition of SO_2 to the must before alcoholic fermentation, shortens the fermentation time, repressing the growth of non-*Saccharomyces* species (Egli et al. 1998). Nowadays, within the authorized additives, SO_2 is considered to have the best proven efficiency in the microbiological stabilization of wine, as well as advantages such as the low economic cost of treatment and its easy use (Ribéreau-Gayon et al. 2006a).

Although there are several benefits that come from using SO_2 to obtain microbiological stabilization in wines, there are also some disadvantages. Bearing in mind that there are different tolerances for different strains to sulphites and that wine composition varies in terms of pH and ethanol

concentration, it is not always easy to obtain the correct amount of potassium metabissulfite that needs to be added to wine for proper control of *Brettanomyces/Dekkera* yeasts (Curtin et al. 2015). What is more, during the last decade, the use of SO_2 in the food industry has raised some concerns regarding consumer's health. There is a small percentage of wine consumers (about 1%) that are sensitive to sulphite intake, therefore the World Health Organization (WHO) has estimated an acceptable daily maximum intake limit of SO_2 per kg of body weight. Wine is responsible for part of the amount of sulphites ingested in adults, especially in countries where such a habit exists, that way, WHO encourages the study of alternative methods to SO_2 as part of wine preservation (Papazian 1996; Vally and Thompson 2003; Lisanti et al. 2019).

As SO_2 has been presented as a potentially harmful agent for human consumption, there is an increasing need to reduce the use of sulphites in the control strategies against *Brettanomyces/Dekkera* yeasts, and in recent years, wine research has been strongly oriented towards the study of alternative techniques and additives to the of SO_2 (Lisanti et al. 2019; Peña et al. 2019). Therefore, several emerging technologies have been proposed to control *Brettanomyces* spp. spoilage during the winemaking process: the winemaker can choose to use chemical preservatives (DMDC, chitosan) or physical methods to help controlling these yeasts spoilage (Malfeito-Ferreira 2018; Pinto et al. 2020).

According to Malfeito-Ferreira (2018), the concept of spoilage prevention can be described as the 'hurdle' concept used in food microbiology. The idea being to weaken the microbial populations by making them 'jump' several obstacles, which in the case of winemaking may be factors such as temperature or dissolved oxygen, or even processes such as filtration an fining. Thus, the more obstacles microbial populations have to face, the easier it will be to prevent microbial growth.

3.1. Removal of yeast cells

3.1.1. Fining Agents: Charcoal and PVPP

We have already seen that the way to solve *Brattanomyces/Dekkera* spp. yeasts spoilage has both a preventive and a curative dimension. A palliative solution may be the fining of red wines prior to their introduction in barrels (Suárez et al. 2007). According to Murat and Dumeau (2003), contamination by these yeasts may be reduced or even almost removed through the use of treatments with fining proteins. The greater the number of fining agents used, the greater the reduction in the initial populations of these yeasts. Some of the most used fining agents in winemaking to reduce ethylphenols levels are casein, potassium caseinate, liquid gelatine, polyvinylpolypyrrolidone (PVPP) and charcoal, some of which are more effective than others. Fining with casein, potassium caseinate

and liquid gelatine has been shown to reduce the populations of *Brettanomyces/Dekkera* spp. during settling (Suárez et al. 2007).

A study conducted by Lisanti et al. (2017), tested the fining agents PVPP and activated charcoal. Two levels of ethylphenols contamination were considered: the first one, a red wine with a naturally low contamination and the second, the same wine but containing higher levels of 4-EP and 4-EG. Both agents, PVPP and activated charcoal showed the ability to greatly decrease the contents of 4-EP and 4-EG in the naturally contaminated wine, however not equally efficiency was demonstrated in the case of the second contamination. In both treatments, the addition of charcoal and PVPP has negative impacts on the concentration of volatile aromas, polyphenols and colour intensity. Nevertheless, there was a decrease in the intensity of phenolic off-odours, and the outcome of both treatments turned out to be very positive.

According to Oelofse et al. (2008), the amount of PVPP and charcoal added to wine varies, depending on the intensity of off-flavours, between 60 and 480 mg/L for PVPP and between 15 and 20 mg/L for activated charcoal. However, treatment using PVPP is only allowed in red wines, while fining treatments using activated charcoal are allowed in musts, new wines still in fermentation and white wines (Lisanti et al. 2017).

Overall, an effective reduction of 4-EP and 4-EG levels can be obtained through the use of these fining agents, however, the colour and favourable aroma compounds are also affected, which means that a balance must be achieved between the benefits and losses of wine attributes (Malfeito-Ferreira 2018).

3.1.2. Filtration and Reverse Osmosis

In order to minimize chemical and thermal treatments during storage it is very important to ensure the correct management of operations such as filtration, clarification and fining. Winemakers may choose the type of filtering media they prefer to use, but the final concern is always to prevent microbial growth in bottled wines (Malfeito-Ferreira 2011).

The removal of particles in wine can be achieved through macro- or microfiltration. Macrofiltration is traditionally done through the use of diatomaceous earth or by pad filtration, while microfiltration uses the integrity-tested membrane. Other types of filtration also used depending on the final objective are the ultrafiltration and reverse osmosis as observed in table 3 (Fugelsang and Edwars 2007).

Table 3: Types of filtration according to size particles removal (Ribéreau-Gayon et al. 2006; Fugelsang and Edwars 2007; El Rayess et al. 2011).

		Removal of particles: size of pores
Ultrafiltration (Cross-flow)		0.001 – 0.2 µm
Microfiltration	Cross-flow	0.2 µm
	Perpendicular-flow	0.45 – 0.65 µm
Reverse Osmosis (Cross-flow)		Separates low molecular weight compounds as well as ions

Traditionally, filtration is done through the use of perpendicular-flow systems ('dead-end') in order to obtain wine clarity, where the wine flows perpendicular to the membrane surface. Depending on the phase of the process (cellaring vs. bottling) the systems used are depth ('nominal') or membrane ('absolute' or 'sterile'). However, ultrafiltration and reverse osmosis use cross-flow (tangential) designs, whereas the wine flow is tangential to the membrane (Ní Mhurchú and Foley, 2006; Fugelsang and Edwars 2007; El Rayess et al. 2011).

Microfiltration is one of the most common and effective physical method used to remove *Brettanomyces/Dekkera* spp. yeasts and other microorganisms from wine. According to El Rayess et al. (2001) there are several advantages from using cross-flow microfiltration, despite of its high cost, such as the possibility to combine clarification, stabilization and sterile filtration in only one single continuous operation, allowing to eliminate steps like previous filtrations.

Some studies regarding the removal of *Brettanomyces/Dekkera* spp. yeast cells have been carried out through wine filtration. According to Calderón et al. (2004), the filter size required for the removal of *Brettanomyces/Dekkera* is 0.45 µm. On the other hand, Renouf et al. (2007) states that a 1 µm filter has very positive effects as well.

What is more, according to Millet and Lonvaud-Funel (2000) who studied the VBNC state of wine microorganisms during storage, it was discovered that non-culturable cells have the ability to pass through pores of 0.45 µm and it is believed that the cell size of these yeasts decreases when they enter a VBNC state. This may explain why some wines are contaminated after being considered sterile by agar plate enumerations (Oelofse et al. 2008).

Nevertheless, wine microfiltration has been a controversial subject regarding possible negative effects on wine quality, especially when talking about high quality red wines (Waterhouse et al. 2016). According to Calderón et al. (2004) membranes with a pore size smaller than 0.45 µm should be used in order to be affective when removing *Brettanomyces/Dekkera* spp. cells, and this can cause reduction

of wine colour and aroma as well as the deterioration of the wine's colloidal structure (Suárez et al. 2007). The studies on this controversy are scarce, therefore results may not be generalized, especially when filtration effects can vary according to wine characteristics and the membrane filter media (Lisanti et al. 2017).

3.1.3. Hygiene and Biofilm Production

As previously mentioned, *Brettanomyces* has the ability to cause damage especially when we talk about higher quality red wines aged in oak barrels. As this yeast can be easily installed in wood, it is extremely important to maintain barrel hygiene and sanitization in order to avoid cross-contamination between different wines (Pinto et al. 2020). In addition, the formation of biofilm by *Brettanomyces* may not always be given due importance, as they may increase their persistence in the winery through adherence and colonization in inert materials (Dimopoulou et al. 2019). Biofilm production is extremely dependent on yeast strain, and some are more capable of producing it than others (Dimopoulou et al. 2019). Therefore, the hygienization of the winery also requires the removal of the biofilm formed by this yeast, in order to obtain further control of this microorganism (Pinto et al. 2020).

3.2. Growth inhibition

3.2.1. Physicochemical variables: pH, Temperature, Oxygen and Ethanol

The growth of *Brettanomyces/Dekkera* yeasts is favourable when variables such as temperature, pH, oxygen and alcohol content meet certain conditions. This way, controlling the variables that favour the growth of this yeast is a way to inhibit it (Suárez et al. 2007; Wedral et al. 2010). Keeping the oxygen levels and pH as low as possible and ensuring that the temperature of storage is low, is a way to control *Brettanomyces/Dekkera* spp. yeasts growth according to Suárez et al. (2007).

The impact of such variables on volatile phenol production has been studied by Gerbeaux et al. (2000) and this authors demonstrated that the capacity to produce volatile phenols vary depending on different strains of *B.bruxellensis,* however it is always greater at lower alcohol concentrations and higher temperature.

3.3. Cell inactivation

3.3.1. Additives

3.3.1.1. DMDC

Alternative additives have been investigated as growth inhibitors for *Brettanomyces/Dekkera* yeasts. Dimethyl dicarbonate (DMDC), also commercially known as Velcorin is an additive evaluated for this yeast inhibition (Delfini et al. 2002; Malfeito-Ferreira and Loureiro 2006; Suárez et al. 2007). DMDC is able to inhibit microorganisms by reacting irreversibly on active sites of cellular enzymes with the amino groups (Daudt and Ough 1980).

This product was first approved for use in winemaking in the United States but was only in 2006 that it was approved in the European Union, with the maximum application limit being 200 mg/L in wines with sugars contents greater than or equal to 5 g/L (Regulation EC No 606/2009). Its addition should be made shortly before bottling (Regulation EC No. 606/2009).

DMDC hydrolyses rapidly into carbon dioxide and methanol, this way its effect can be considered instantaneous and without residual activity. In theory 200 mg/L of DMDC produces about 96 mg/L of methanol. This compound should be monitored if excessive concentrations are suspected as it is a toxic substance (Ribéreau-Gayon et al. 2006; Costa et al. 2008; Malfeito-Ferreira 2018). Applying treatment to wine using DMDC has some implications regarding health and safety. Specific dosing equipment is required to allow its safe application, since the product is toxic by ingestion and inhalation, is skin and eye irritant and can be combustible when exposed to an open flame (Fugelsang and Edwars 2007; Curtin et al. 2015;).

Several studies have been carried out over the years regarding the quantity of DMDC that should be added to wine in order to prevent microorganism's growth. In the following table there are some results summarized from different works that allows us to conclude that there is not only one solution.

Table 4: Content of DMDC applied in treatment in works conducted by different authors.

Content of DMDC applied in treatment	Obtained results	Reference
250 – 400 mg/L	*B. bruxellensis* inhibition	Delfini et al. (2002)
100 mg/L	Completely sterilized wine	Ough et al. (1988)
50 mg/L DMDC + 25 mg/L Free SO_2	Provides an efficient microbial control	Divol et al. (2005) Ough et al. (1988b) Threlfall and Morris (2002)

What is more, according to several authors (Costa et al. 2008; Bartowsky 2009; Malfeito-Ferreira 2004, 2018), the effectiveness of DMDC against *Brettanomyces* growth depends on factors such as:

- Initial cell concentration
- Ethanol content
- Adequate DMDC homogenization
- Species and strains
- Wine Chemical Composition
- Temperature

It has also been shown that the application of DMDC alone, due to its maximum permitted limits, does not protect wine effectively against bacterial contamination. Moreover, it does not protect against oxidation. Therefore, its use alone does not completely replace the use of SO_2, but the combination of both can be a good solution for protection of wine, thus reducing the use of sulphites (Ough et al. 1988b; Terrel et al. 1993; Lisanti et al. 2019).

3.3.1.2. Chitosan

Chitosan is a linear heteropolysaccharide derived from chitin by deacetylation (Raafat and Sahl, 2009). Chitin and Chitosan are small polymers that have a unique structure of great interest due to the presence of amine groups, which are susceptible to modifications in order to achieve desirable characteristics. For the past years, the antimicrobial activity of chitosan has been investigated in areas such as medicine, agriculture and food industry (Gómez-Rivas et al. 2004).

In winemaking the use of chitosan has been authorized in Europe by the Organisation Internationale de la Vigne et du Vin (OIV) for prevention of cloudiness, heavy metals and contaminant removal and reduction of *Brettanomyces* spp. populations and cannot exceed 0.1 g/L (Regulation EC 53/2011).

Regarding the reduction of *Brettanomyces* spp. populations, the microbial effect of chitosan can act in two ways: the first through the generation of interaction between molecular groups of chitosan and the cell membrane leading to its denaturation and eventual death; the second implying the adsorption of chitosan through cell walls and causing a blockage of transfers which can also result in cell death (Gómez-Rivas et al. 2004). The effectiveness of chitosan as an agent against *Brettanomyces* growth depends, according to several authors (Vincent Renouf et al. 2007; Ferreira et al. 2013; Malfeito-Ferreira 2018), on:

- Cell numbers
- Strain
- Molecular weight of chitosan molecules
- pH
- Acetylation degree

According to Dutta et al. (2009) and Kong et al. (2010), chitosan performs better antimicrobial activity under conditions of low molecular weight, low pH and low degree of acetylation.

There are a few studies regarding the effect of chitosan in controlling the growth of *Brettanomyces* spp. yeasts populations. Some works conducted by different authors are summarized in table 5.

Table 5: Content of Chitosan applied in wine treatment.

Content of chitosan applied in treatment	**Obtained Results**	**Reference**
3 – 6 g/L	Drastically decreased the growth of *B. bruxellensis* and *B. intermedius*	Gómez-Rivas et al. (2004)
0.2 – 0.5 g/L	Inhibition of *Brettanomyces/Dekkera* growth	Ferreira et al. (2013)
0.08 g/L	Cultivability of a *B.bruxellensis* strain was greatly reduced	Petrova et al. (2016)
0.1 g/L	Chitosan decreased the headspace 4-EP and 4-EG concentrations	Filipe-Ribeiro et al. (2018)

Based on this results, chitosan should be considered a preventive instrument to be used together with SO_2 in reducing populations of *Brettanomyces/Dekkera* spp. yeasts, rather than substituting SO_2 action

(Filipe-Ribeiro et al. 2018; Lisanti et al. 2019). However, as the product is not exactly cheap (>0,05 €/L), a proper balance between costs and benefits should be done before application (Malfeito-Ferreira 2018).

3.3.1.3. Sorbic Acid

Sorbic acid is a short-chain unsaturated acid that has been used in the food industry as an antifungal agent. In winemaking its added in the form of soluble potassium sorbate salt and its limit of application in Europe is 200 mg/L (Regulation EC No 606/2009; Lisanti et al., 2019). According to Fugelsang and Edwars (2007) it acts by altering the cell membrane functions. It is used in wine to obtain biological stability, to prevent the re-fermentation of wines containing fermentable sugars and to prevent the development of undesirable yeasts (OIV, 2020). The inhibition of re-fermentation by *Saccharomyces cerevisiae* is its main application, contrary to *Brettanomyces* yeasts, which is not so effective (du Toit and Pretorius, 2000). The effectiveness of sorbic acid depends, according to Zoecklein et al. (1995), Millet and Lonvaud-Funel (2000), and Quintas et al. (2005) on:

- Wine pH
- Ethanol content
- Number and nature of yeasts
- Intracellular concentration of preservative used

Due to its fungicidal activity, sorbic acid is very effective when used together with SO_2 preventing bacterial activity and oxidative reactions, as long as a sufficient high dose of free SO_2 is used (Ribéreau-Gayon et al. 2006). What is more, by preventing oxidative reactions it is indirectly detrimental to the proliferation of *Brettanomyces/Dekkera* yeasts (Oelofse et al. 2008) inhibiting their growth (Suárez et al. 2007).

3.3.1.4. Yeast Killer Toxins

There are yeasts that produce antimicrobial proteinaceous compounds that are capable of inhibiting the activity of certain yeasts that are susceptible to it. These compounds are called 'yeast killer toxins' (Pinto et al. 2020). This phenomenon was first discovered in 1963 in *Saccharomyces cerevisiae* yeasts, however, later several non-*Saccharomyces* yeasts also showed their ability to produce killer yeasts (Berbegal et al. 2018). Since then, several studies have been carried out to reduce the populations of *B. bruxellensis* using these killer toxins where they have been isolated and have the specific killing capacity against this yeast. A few examples of toxins are found in table 6.

Table 6: Examples of killer toxins used in works to control *B. bruxellensis*, derived from non-*Saccharomyces* yeasts.

Yeast	Corresponding Killer Toxin	Reference
Pichia anomala	Pikt	Comitini et al. (2004)
Pichia membranifaciens	PMTK2	Belda et al. (2007)
Ustigo maydis	KP6	Santos et al. (2011)
Candida pyralidae	CpKT1 and CpKT2	Mehlomakulo et al. (2014)

The disadvantages of this method are the fact that no products are available yet, the relatively unstable nature of these toxins in the wine environment and their high economic cost (Mazzuco et al. 2019).

3.3.1.5. Antimicrobial Peptides

Antimicrobial Peptides (AMPs) can be of plant, animal or microbial origin and have been studied in recent years within the food industry for microbiological control (Lisanti et al. 2019). As far as *Brettanomyces* control is concerned, few studies have been performed and more work is needed, however, authors such as Enrique et al. (2008) have demonstrated the effect of a bovine peptide on reducing the viability of some *Dekkera* strains in white wines. In addition, Peña and Ganga (2019) found that *Candida intermedia* produces peptides effective against some *Brettanomyces* strains. According to Enrique et al. (2007), AMPs probably act by cracking the permeability functions of cell membranes.

3.3.2. Thermal processing

3.3.2.1. Heat Treatment

Heat treatments have long been used in killing microorganisms in the food industry, however they have lower application in winemaking (Malfeito-Ferreira 2011). According to Ribéreau-Gayon et al. (2006) the destruction of microorganisms through pasteurisation should contribute to wine stabilization and this way limit the SO_2 added. In the table below there are two treatments summarized according to the authors.

Table 7: Studies conducted by several authors applying heat treatments to obtain wine sterility.

Applied Treatment	Obtained Results	References
37.5 ºC for 6 min or 41 ºC for 0.6 min	Population of 1 million cells/mL of *B. bruxellensis* was inactivated	Couto et al. (2005)
45 – 48 ºC	Achieve sterility of both wine and bottle	Ribéreau-Gayon et al. (2006)

3.3.2.2. Microwave Treatment

Microwave technology application is winemaking has also been studied in recent years, although its main purpose being to accelerate the extraction of phenolic compounds in red wines (Carew et al. 2014). However, it has been shown by the authors that microwave treatment of musts reduces the opportunity for its colonisation by aerobic spoilage microorganisms and has a sort of pasteurizing effect, this way enabling the reduction of SO_2 addition at crushing (Carew et al. 2014).

Although heat conditions necessary for wine stabilization should be easy to achieve without compromising organoleptic characteristics of wine according to Ribéreau-Gayon et al. (2006), results obtained by Zheng et al. (2011) suggests that microwave heating could have negative effects on sensory quality.

3.3.3. Non-thermal processing

3.3.3.1. Ultrasound

Having an antimicrobial effect, ultrasound can be used in food processing as an alternative method to conventional thermal treatments as it has a large number of applications (Piyasena et al. 2003; Chemat et al. 2011). Pressure waves with frequencies of 20 KHz or more are considered ultrasound, although they can be divided in different frequency ranges (Gracin et al. 2016). In winemaking, ultrasound can be used for the reduction in load of spoilage organisms in musts, thus it has several other applications according to Jiranek et al. (2008). Gracin et al. (2016) tested a high power ultrasound in continuous flow treatment for the reduction of *Brettanomyces* yeasts cells in wine samples and had very satisfying results, despite of the heating of the wine samples caused by the application of the ultrasounds.

3.3.3.2. High pressure processing

When talking about high pressure processing in winemaking, there are two treatments that stand out. The high hydrostatic pressure (HHP) which consists of the application of pressures between 100 and 1000 MPa to the product, through the use of a liquid pressure-transmitting medium at mild or room temperature (Cao et al. 2011). By applying HHP treatment to wine, the microbial inactivation is achieved through the change of protein conformation and cellular structures leading to alteration of their functional and consequent cell leakage (Wuytack et al. 2002). The other treatment is the continuous high pressure homogenization (HPH) that was proposed for must treatment where the liquid is forced to pass, applying pressure, through a homogenization valve (Puig et al. 2008). There is a quick release of energy when the liquid is passing the valve which is responsible for the disruption of microbial cells (Donsì et al. 2013).
Several studies performed using this type of technology are summarized in table 8.

Table 8: Applied treatment through high pressure processing according to different authors.

Applied Treatment	Obtained Results	Reference
400 – 500 MPa for 5 min	99.99% reduction in the initial microbial population of *B. bruxellensis*, without modifying the chemical and organoleptic properties of wine.	Puig et al. (2003)
HPH: 200 MPa	Efficient decrease of microbial load of musts.	Puig et al. (2008)
100 MPa at 25 ºC for 24h	Highly effective at controlling the growth of *Brettanomyces* spp.	Morata et al. (2012)
200 – 300 MPa	Complete inactivation of *B. bruxellensis* regardless of winemaking conditions.	González-Arenzana et al. (2016)
400 MPa	Total inactivation of *B. bruxellensis* cells in wine, preventing the formation of 4-EP and 4-EG.	van Wyk et al. (2018)

The application of HHP is a promising technology regarding *Brettanomyces* spp. growth control in wine as it can be applied at refrigeration temperatures and it produces fewer sensorial modifications when comparing to other physical techniques (Morata et al. 2012, 2017). According to González-Arenzana et al. (2016) and van Wyk et al. (2018), *Brettanomyces* spp. inactivation rate resulting from HHP processing depends on factors such as:

- pH
- Ethanol content
- Duration of treatment
- Strain
- Wine type

What is more, when the pressure applied is too high, losses of phenol content and colour density may occur, as well as physicochemical and sensory properties of red wines may be affected (Tao et al. 2012; van Wyk et al. 2018).Thus, as chemical oxidation should also be ensured, the use of HHP treatment should be considered to use in association with SO_2 itself or other alternative methods (Pinto et al. 2020; Suárez et al. 2007).

3.3.3.3. Pulse electric fields

Pulsed electric field (PEF) is a non-thermal technology for pasteurization as it inactivates pathogenic and spoilage microorganisms without modifying food quality (Barbosa-Cánovas and Zhang 2001; Vega-Mercado et al. 1997). The PEF technology consists on the application of short duration pulses, normally for microseconds, of high electric fields strengths – 5 to 50 kv/cm (Pataro et al. 2010) to products, between two electrodes (Puértolas et al. 2009).

Studies describing the effect of PEF treatments on wine spoilage microorganisms are not so common when compared to other food industries, nevertheless, there are some works conclusions summarized in table 9.

Table 9: Studies conducted by several authors on the application of PEF treatments as a way to prevent spoilage by *Brettanomyces* yeasts.

Applied Treatment	Obtained Results	Reference
186 kJ/kg and 29 kV/cm	Reduction of 99.9% of the spoilage flora of wine and must	Puértolas et al. (2009)
1,44 J/kg for 60 days	Reduction of the viable cells of *D. bruxellensis*	Lustrato et al. (2010)
320 kJ/kg and 20kV	Total inactivation of *B. bruxellensis*	Delsart et al. (2015)
32 kV/cm and 250 Hz for 51,2 µs	Reduction of *B. bruxellensis* load	van Wyk et al. (2018)
50 kV/cm	Reduction of 3 orders of magnitude in the *B. bruxellensis* population	van Wyk et al. (2019)
95 kJ/kg and 23 kV/cm for 8 µs	Reduction of Brettanomyces populations, but spoilage yeasts recovered during the aging in barrels	González-Arenzana et al. (2019)

The efficacy of PEF inactivation depends on the treatment intensity, microbial species and temperature (Yang et al. 2016). What is more, according to Delsart et al. (2015) and Puértolas et al. (2009), this non-thermal alternative treatment has several advantages such as:

- Short time of treatment (a few microseconds)
- Low energy consumption
- Continuous process
- Low costs when compared to other treatments
- Easily integrated into existing industrial processes
- Minimal impact on quality

3.3.3.4. Ultraviolet radiation

The microbial inactivation of yeasts populations by ultraviolet radiation (UV-C), 254 nm, interferes directly with the capacity of microorganism to reproduce through the rearrangement of its nucleic acid (Bintsis et al. 2000; Tran and Farid 2004). Although this technology in treating wine spoilage microorganism is still considered unexplored, Fredericks et al. (2011) studied UV-C (254 nm) as an alternative technology to inactivate microorganisms in wine. The authors evaluated the effect of

different UV-C dosages on the *B. bruxellensis* inactivation, combined with 20 mg/L of SO_2. They showed that yeast inactivation increased with the UV-C dosage used and varied depending on the wine type.

4. Post-Spoilage Ethylphenols Removal

Research has been carried out more recently to explore the potential for extracting ethylphenols produced by *Brettanomyces* in wine. Some adsorbents have been presented in studies that, although positive results have been achieved, require a lot of studies and research in order to reach the reality of using them in the wine industry (Curtin et al. 2015). Some examples can be considered in table 10.

Table 10: Studies conducted by several authors on the application of different adsorbents for the removal of ethylphenols post-spoilage of wine.

Applied Treatment	Obtained Results	Reference
Yeast lees in the form of active dried yeast	Removal of 4-EP and 4-EG	Chassagne et al. (2005)
Other adsorbents combined with physical treatments	Lowered the concentration of 4-EP and 4-EG but also adsorbed other compounds	Ugarte et al. (2005)
Esterified cellulose polymers	Removal of 20-30% of 4-EP and 4-EG with modest impact on colour and anthocyanins	Larcher et al. (2012)
Molecularly imprinted polymers (MIPs)	Reduction of 4-EP and 4-EG, however sensorial changes occur	Teixeira et al. (2015)

5. Good Winery practices

In order to achieve the control of *Brettanomyces* occurrence it is necessary to apply good cellar practices. Effective and strict hygiene and cleaning, the correct use of sulphur dioxide, the general oxygen reduction and the correct use of non-contaminated barrels are some simple and efficient cellar practices that should be applied in the winery (Wedral et al. 2010).

5.1. Fermentation management

The must contains a large number of different microorganisms that coexist. *Saccharomyces cereviseae* yeast is normally dominant during alcoholic fermentation and is considered its main agent. However, other microorganisms are also present very regularly, some of which may cause wine spoilage. *Brettanomyces* are one of these microorganisms. *B. bruxellensis* has the capacity to convert glucose and fructose into ethanol inclusively and its metabolism can integrate *Saccharomyces*'s one, reducing the total time of sugar fermentation (Renouf et al. 2006). With the evolution of alcoholic fermentation, the microorganisms present also vary their concentrations according to their ability to adapt to the environment, being that *Saccharomyces* has great resistance capacity, so do *Brettanomyces* that can survive in difficult winemaking conditions (SO_2, low pH, high alcohol content, etc.) (Unknown 2011).

The use of selected yeasts during alcoholic fermentation has been demonstrated by Renouf et al. (2006) as an effective tool in *Brettanomyces* control. These authors obtained, through spontaneous fermentation, a population of *Brettanomyces* in the range of 6 x 10^3 CFU/mL and a production of 430 µg/L of 4-EP. In contrast, through an alcoholic fermentation with a strain of selected yeasts, a reduced *Brettanomyces* population of 6 x 10 CFU/mL and only 45 µg/L of 4-EP. Furthermore, the correct management of fermentation is crucial as slow or sluggish fermentation provides favourable conditions for the multiplication of *Brettanomyces.*

The end of alcoholic fermentation and the beginning of malolactic fermentation is a crucial period as it may result in an opportunity for the proliferation of *Brettanomyces* yeasts. According to Gerbaux et al. (2009), malolactic fermentation helps to preserve the quality of the wine as it helps to prevent the development of *Brettanomyces* yeasts: wines that have not completed malolactic fermentation are more susceptible to the development of these yeasts, and those that have been early inoculated with malolactic bacteria are considered more protected.

Furthermore, according to Pinto (2020), winemakers should ensure that *Saccharomyces* yeast depletes all fermentable sugars during alcoholic fermentation and malolactic fermentation also exhausts malic acid. This nutrient management may be an approach to reducing spoilage caused by *Brettanomyces* spp.

There are recommendations for interventions in the cellar regarding practices in order to avoid or limit contamination by *Brettanomyces* that are summarized in table 11.

Table 11: Recommendations according to the OIV (http://www.oiv.int/public/medias/4830/code-brett-oiv-oeno-462-2014-fr.pdf) for interventions in the cellar regarding practices in order to avoid or limit contamination by *Brettanomyces* during fermentations.

When	What to do	Why
During Alcoholic Fermentation	Inoculation of musts with selected yeasts	Achieve a more dependable alcoholic fermentation
	In case there is a stuck fermentation, use a restart process ASAP	The environment becomes more propitious to the multiplication of *Brettanomyces* in the eventuality of a stationary fermentation
	Bear in mind that residual sugars are substrates for the *Brettanomyces* growth	A concentration of 0.3 g/L of residual sugars is enough for the development of *Brettanomyces* capable of producing over 1000 µg/L of volatile phenols
	Nutrients for yeasts should be added only in cases where it is extremely necessary to prevent stuck fermentation	They can also enhance the growth of *Brettanomyces*
	Monitor *Brettanomyces* populations	After alcoholic fermentation is complete, the conditions promote the proliferation of *Brettanomyces*
Before Malolactic Fermentation	Control the temperature, micro-oxygenation and the release of sugars in case of uncrushed grapes	These factors are beneficial to the development of *Brettanomyces*
	Co-inoculation of selected yeasts	Can help reduce the lag phase between alcoholic and malolactic fermentation and hence the growth of *Brettanomyces*
During Malolactic Fermentation	Control pH, temperature and total SO_2	This physicochemical parameters affect the progress of malolactic fermentation
	Add SO_2 at the end of malolactic fermentation with perhaps DMDC	To eliminate microorganisms

5.2. Oxygen levels: micro-oxygenation

Oxygen has a very important role in the winemaking process and influences the composition and quality of both must and wine. As it is an essential element in the wine aging process, it is also considered a driving factor in the production of volatile phenols (Malfeito-Ferreira et al. 2001) The growth rate of *Brettanomyces* is favoured by the addition of O_2 (du Toit et al. 2005). Since, throughout the winemaking process, the must/wine is in contact with O_2 on several occasions, from crushing and pressing the grapes to operations such as pumping, transfers, filtration, racking, centrifugation, bottling, topping and micro-oxygenation, its careful monitoring and minimisation are essential in order to minimise the diffusion of O_2 into the wine (du Toit et al. 2006; Malfeito-Ferreira 2018).

Micro-oxygenation is a technique that has been developed to inject O_2 into wine in a controlled way by means of small additions. It is considered a process to accelerate the ageing of red wines due to the positive role that small additions of O_2 will play in the development of colour, aromas, flavours and phenolic compounds during the ageing of a red wine (du Toit 2010; Malfeito-Ferreira 2018). Micro-oxygenation may also be used as an alternative to the ageing of wines in wooden barrels when used together with other products derived from wood (du Toit 2010).

It has already been demonstrated that the growth of *Brettanomyces* yeasts is enhanced by the presence of O_2 (du Toit et al. 2005), therefore, according to Malfeito-Ferreira (2018), whenever the concentration of free sulphites is below 20 mg/L (at pH 3.5) there is an opening for the growth of the *Brettanomyces* population, and extreme attention should be given. The proper use of micro-oxygenation together with adequate monitoring of SO_2 is extremely important to prevent the development of *Brettanomyces* yeasts and their consequent production of unwanted odours (du Toit 2010).

5.3. General Hygiene

There are still limited studies on the ecological and infection routes of *Brettanomyces* spp. yeasts in wineries. It is known that rotten or damaged grapes may contain a high number of microorganisms, which may explain the higher occurrence of volatile phenols in vintages (Guerzoni and Marchetti, 1987). It is also known that contamination can occur by transferring contaminated blends to storage sites that have also become contaminated or through various winery equipment mainly those that have been in contact with wine residues, skins, juice, etc. (Henick-Kling et al. 2000; Connell et al. 2002; Malfeito-Ferreira 2018).

The maintenance of good hygiene in the winery is an important first step towards the control of the infectious route of *Brettanomyces* spp. Once settled in the winery, this population becomes more difficult to eradicate. Thus, the prevention of the growth of this yeast requires attention to the quality of the fruit entering the winery and its sanitization, control of contaminated barrels, levels of sulphites and oxygen and effective cleaning of the equipment used. Only through efficient hygiene and adequate SO_2 control, particularly during aging in wooden barrels, can we proceed towards a more effective prevention against *Brettanomyces* (Chatonnet 1995; Henick-Kling et al. 2000; Wedral et al. 2010; Malfeito-Ferreira 2018).

5.4. Barrels management

The wooden barrels used in winemaking are well known as an ecological niche that is quite prone to the development of microorganisms capable of causing wine spoilage, especially when it comes to *Brettanomyces/Dekkera* yeasts (Oelofse et al. 2008). Sanitization of barrels plays a crucial role regarding measures to avoid cross-contamination between different blends of wines and also in the installation of these yeasts on barrels, since they are capable of penetrating up to 8 mm deep into the wood through the penetrative capacity of wine, thus making them very difficult to eradicate (Barata et al. 2013; Pinto et al. 2020).

The barrel age is a factor which has an influence on the growth and settlement of *Brettanomyces* populations - old barrels tend to favour *Brettanomyces* contamination. Due to the oak porosity these yeasts become difficult to eradicate (Chatonnet et al. 1999). Nevertheless, some authors have reported that new barrels also favour the appearance of contamination by *Brettanomyces* due to high levels of oxygen and sugars (Lonvaud and Renauf 2005). Moreover, the frequent re-use of barrels and the use of the micro-oxygenation technique to accelerate the wine's maturation results in the proliferation of this yeast (Ciani et al. 2003).

In order to achieve adequate barrel sanitization several treatments have been tested and evaluated to obtain control of *Brettanomyces* spp. yeasts. Some of these treatments include aqueous steam, hot water, UV irradiation, ultrasounds, microwaves and gaseous and aqueous ozone (Breniaux et al. 2019; Edwards and Cartwright 2019). According to Pinto et al. (2020) the most effective technologies being hot water, high power ultrasounds and gaseous ozone. Over the last few years several authors have reported different alternatives on this subject, which are summarized in the table 12.

Table 12: Resume of several works performed by different authors regarding barrel sanitation.

Applied Treatment	Obtained Results	Reference
At least 9 g of SO_2 gas per barrel	Sanitation of barrel wood	Chatonnet et al. (1992)
30 – 35 mg/L of free SO_2 during summer months	Sanitation of barrels	Henick-Kling et al. (2000)
Treatment with ozone gas followed by hot water (82 ºC for 20 min)	Reduction of *Brettanomyces* population	Cantacuzene et al. (2003)
Treatment with ozonated water	*Brettanomyces* population where reduced up to 99 %	Coggan (2003)
Cold water rinse follower by a hot water rinse (70 ºC) and low pressure steam for 10 min	Most effective of the treatments performed by the author who recommends isolation of *Brettanomyces* intentioned barrels to reduce the contamination of others	Malfeito-Ferreira (2005)
Ultrasound power at 50 W for 90 – 120 s	More than 97 % of *D. bruxellensis* population were destroyed	Oelofse et al. (2008)
Microwave treatment (3000 W power for 3 min, maximum temperature reached on the wood surface 48 ºC)	Reduction of 35 – 67 % of *Brettanomyces*	González-Arenzana et al. (2013)
Gaseous ozone treatment	Reduction of two orders of magnitude *Brett.* spp. with no relevant changes on the phenolic profile and sensory properties of aged wine	Guzzon et al. (2017)
High power ultrasound treatment combined with hot water (60 º C) for 6 min	Reduction of *B. bruxellensis* population at 5-9 mm depths	Breniaux et al. (2019)
Hot water treatment (80 ºC for 20 min or 70 ºC for 30 min	Prevented the yeast recovery at 5 – 9 mm depth from oak barrels	Edwards and Cartwrigh (2019)

It is the opinion of several authors that oak barrels that are contaminated by these yeasts are not possible to be sterilized effectively, greatly due to their large internal volume and the enormous natural porosity of oak (Pollnitz et al. 2000). However, a good sanitization of the barrels is of extreme importance in order to avoid the installation of *Brettanomyces* spp. (Pinto et al. 2020).

6. Yeast Detection: Microbiological Control

When compared to other food industries, the wine industry still has a rather conservative attitude towards the microbial characterisation of wine, which is perhaps undervalued. However, inadequate microbiological control, especially during bulk wine storage, is extremely inadvisable. A good prevention of *Brettanomyces* activity requires its detection through microbiological analysis (Morata 2013; Malfeito-Ferreira 2018; Pinto et al. 2020)

6.1. Microbial Guidelines

According to Malfeito-Ferreira (2013), microbiological criteria have been established for many Portuguese wineries that have been used as precise guidelines for used barrels that are known as ecological niches well suited to the development of *B. bruxellensis*. These cases are summarised in Figure 5 for bulk stored wines (where monitoring of *D. bruxellensis* should be made once a month, every two months or every three months, according to the type of wine) and figure 6 for wines before bottling.

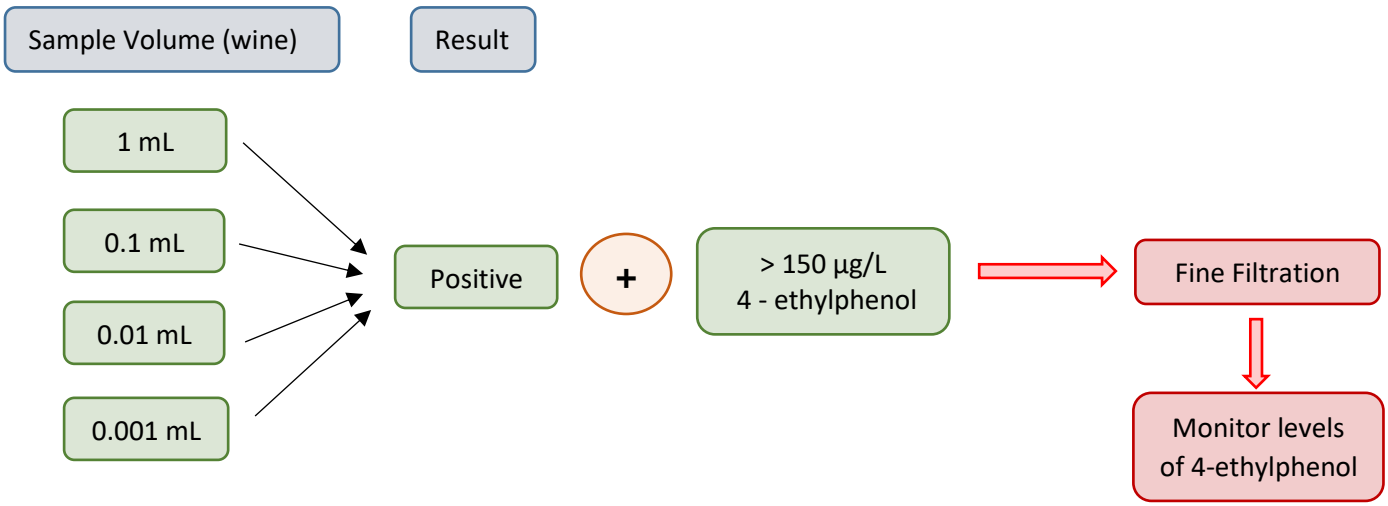

Figure 5: Detection of *D. bruxellensis* for bulk stored wines that have been used as guidelines for used barrels that are known as ecological niches. Adapted from Malfeito-Ferreira (2013).

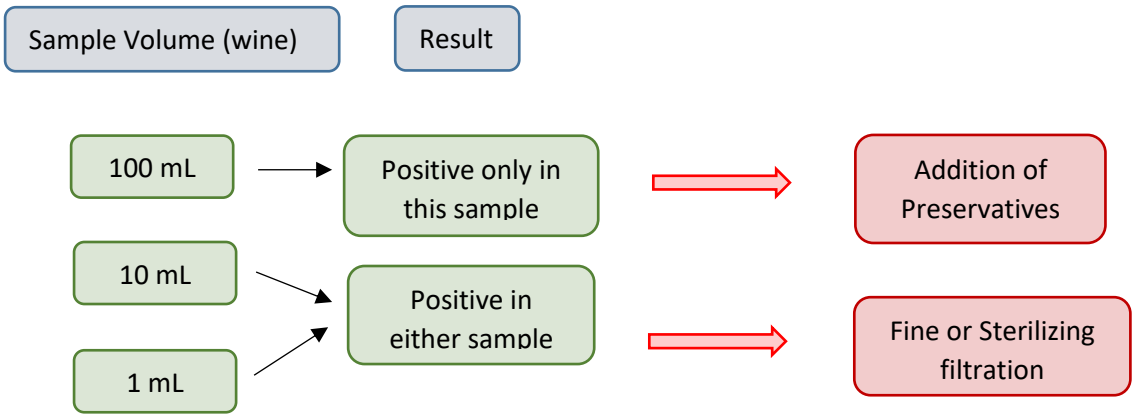

Figure 6: Detection of *D. bruxellensis* for wines before bottling that have been used as guidelines. Adapted from Malfeito-Ferreira (2013).

6.2. Standard Detection Methods for *Brettanomyces*

Nowadays, there are several microbiological methods for the monitoring of *B. bruxellensis* periodically, the most common technique being plate counting. However, most wineries have to resort to external laboratories to carry out this control due to the high economic cost of the necessary laboratory materials and equipment and also due to the need for specialist technicians. For the smaller wineries, these procedures are very costly, becoming an enormous economic burden and therefore leads them to adopt specific monitoring plans accompanied by the determination of 4-ethylphenol (Malfeito-Ferreira 2018; Pinto et al. 2020).

Brettanomyces in wine can be detected directly or indirectly (table 13), through traditional methods by the direct detection of yeast or based on the detection of microbial metabolic products, respectively, being the most widely used due to their relative simplicity. However, both methods are costly, require specialised technicians, have long incubation periods and can even lead to incorrect results according to Tubia et al. (2018). More studies are therefore needed for the development of other, faster and more economical techniques.

Table 13: Standard Detection Methods for *Brettanomyces* according to Tubia et al. (2018)

		Examples	References
Traditional Methods **- The most widely used for the detection of Brettanomyces due to their ease of use and the fact that they are reasonably affordable**	**Direct Methods** - Yeast detection through direct yeast cell observation	- Plating methods - Microscopy - Molecular Detection - Flow Cytometry	Cocolin et al. (2004); Vincent Renouf et al. (2007); Ibeas et al. (1996)
	Indirect Methods - Detection of metabolic yeast products - Analysis of metabolised molecules and changes in the chemical characteristics of wine - Usually are combined with direct methods in order to be more accurate - Used to assess the quality of the product and not the presence of contaminants	- Gas chromatography and mass spectrometry	Tubia et al. (2018)
Experimental Methods		- Biosensors - Microfluidic devices	Tubia et al. (2018)

7. Conclusions

After intensive research on the topic of *Brettanomyces* in wine, it was possible to review the main problems and approaches to address them that are currently known. The installation of *Brettanomyces* in the winery is still a real concern of all winemakers due to the high damage they may cause. In recent years it has been the subject of several studies and work aimed at alternatives for its control beyond the use of sulphites. The known and already performed treatments include their removal, inhibition of growth and inactivation of viable cells and there are several positive results that allow the effective reduction of *Brettanomyces* populations in contaminated wines. In table 14 it is listed a general summary of the alternative methods discussed throughout this review, their practical effects and their great disadvantages. We then concluded that, despite the numerous alternatives described, none is yet in a position to compete with the use of SO_2, which due to the fact that it is easy and quick to use and relatively affordable, becomes a candidate with characteristics that are very difficult to overcome. There are, however, real alternatives that make it possible to reduce its application and that, used together with SO_2, allow the achievement of quite positive results. The insistence on future studies

and work on this subject is very important if we are ever to discover approaches that completely replace the application of sulphites in wines, in order to follow the trends of the rest of the food industry. The viable alternatives in practical terms are quite expensive and not all wineries have the economic structure to apply them. It was also possible to conclude that the most efficient approach to *Brettanomyces* control is to avoid its first installation in the winery through good hygiene and sanitization practices. If, from the beginning, all barrels, all vats and all instruments and surfaces that are in contact with the wine during the winemaking process have been properly sterilised and disinfected as often as necessary, it is possible to reduce the risk of contamination and thus avoid post-spoilage treatments. Also, an adequate control of factors such as temperature, pH, oxygen and ethanol allow, in the different phases of vinification, to avoid or hinder its installation. Consideration must be given to the most propitious times during the process for its development and during these a more rigid control must be carried out. Adequate control of fermentation is another measure that allows us to prevent and control the growth of this yeast and thus avoid future damage. The barrels, which are considered to be suitable environments for the development of *Brettanomyces*, will have to be specially cleaned and there are several effective methods for this.

An extensive knowledge of *Brettanomyces*' behaviour in this environment is imperative if proper control of this yeast, which could be responsible for catastrophic damage to wine, is to be achieved. Knowing at which moments its growth is most propitious, in which environments and under which conditions are extremely important aspects in combating this problem. Nowadays, its control still goes a long way towards prevention that can be achieved through adequate hygiene and sanitization of the entire winery environment. After installation, dealing with the problem becomes more complicated, more demanding and certainly more expensive. Creating conditions that hinder its development both in the wine and in the cellar during the different phases of the winemaking process is an efficient strategy in reducing the incidence of this yeast, however it is still necessary to create new more effective approaches.

Table 14: General summary of the alternative treatments to sulfites in controlling *Brettanomyces* in wine

Mode of Action	Treatment	Effect on Brett. Population	Disadvantages
Removal of yeast Cells	**Fining Agents: PVPP and Charcoal**	- Effective reduction of 4-EP and 4-EG	- Colour and favourable aroma compounds can be affected
	Filtration and Reverse Osmosis	- Removal of Brett. yeasts	- Colour and favourable aroma compounds can be affected
Growth inhibition	**pH, Temperature, O_2 and Ethanol**	- Controlling this factors is a way to control Brett. yeasts growths	
	DMDC	- Inhibits Brett. growth	- Excessive concentrations are considered toxic - Its use alone does not completely replace the use of SO_2
	Chitosan	- Inhibits Brett. growth	- High economic cost - Its use alone does not completely replace the use of SO_2
Cell inactavation	**Sorbic Acid**	- Used together with SO_2 can be very effective - Prevents oxidative reactions which inderectly prevents Brett. Growth	- Has to be used combined with SO_2
	Yeast Killer Toxins	- Reduces Brett. Population	- High economic cost - Relatively unstable nature of toxins in wine environment
	Antimicrobial Peptides	- Reduces the viability of some Brett. strains	- Few studies have been perfomed, more studies are needed
Thermal Processing	**Heat Treatment**	- By contributing to wine stability, reduces the SO_2 to be added	- Has to be used combined with SO_2
	Microwave Treatment	- By contributing to wine stability, reduces the SO_2 to be added	- Could have negative effect on sensory quality of wine
Non-Thermal Processing	**Ultrasound**	- Reduces Brett. Population	- Could have negative effect on sensory quality of wine
	HPP	- Inactivation of viable Brett. Cells	- Could have negative effect on sensory quality of wine
	PEF	- Inactivation of viable Brett. Cells	- Few studies have been perfomed, more studies are needed
	UV Radiation	- Inactivation of viable Brett. Cells	- Has to be used combined with SO_2

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