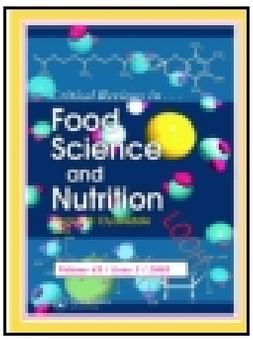

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4-Ethylphenol, 4-ethylguaiacol and 4-ethylcatechol in red wines: Microbial formation, prevention, remediation and overview of analytical approaches

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ABSTRACT

The presence of 4-ethylphenol, 4-ethylguaiacol and 4-ethylcatechol in red wines affect negatively their aroma conferring horsy, barnyard, smoky and medicinal aromatic notes. These volatile phenols formed from free hydroxycinnamic acids and their ethyl esters by *Dekkera/Brettanomyces* yeasts, can contaminate wines. Their formation can cause serious negative economic impact to the wine industry worldwide as consumers tend to reject these wines. For these reasons various preventive and remedial treatments have been studied. This review summarises the wine microbial volatile phenols formation, preventive measures during winemaking and remedial treatments in finished wines along with their advantages and limitations for dealing with this sensory defect and impact on wine quality. Also it is important to control the levels of volatile phenols in wines using fast and convenient analytical methods namely with a detection limit below their olfactory perception threshold. The analytical methods available for quality control and performance characteristics as well their advantages and disadvantages when dealing with a complex matrix like wine are discussed in detail.

Abbreviations: 4-EC: 4-ethylcatechol; 4-EG: 4-ethylguaiacol; 4-EP: 4-ethylphenol; *D/B*: *Dekkera/Brettanomyces*; VP: volatile phenols

KEYWORDS

Wine; volatile phenols; microbial formation; prevention; remediation; analytical methods

1. Introduction

Volatile phenols (VPs) namely 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG) and 4-ethylcatechol (4-EC) when present in wines above a certain concentration confer an aromatic defect that has serious implications on wine quality, considered negative by professionals, consumers and wine industry (Chatonnet et al. 1992; Oelofse et al. 2008; Wedral et al. 2010; Šućur et al. 2016; Tempère et al. 2016; Schumaker et al. 2017). The impact of these ethylphenols on wine quality is due to their unpleasant sensory attributes, namely phenolic, animal, and stable aromatic notes, and additionally low olfactory detection threshold, although the values reported are dependent on the wine matrix and if they are present together or separately (Table 1) (Botha 2010; Petrozziello et al. 2014). The VPs olfactory detection threshold is in average lower for 4-EG, followed by 4-EP and 4-EC (Table 1). Nevertheless, there are fewer studies concerning 4-EC and its impact on the wine sensory profile and also the concentrations of 4-EC when determined are lower (Larcher et al. 2008; Botha 2010; Diako et al. 2017). Their presence has been detected in several wine styles around the world (Table 2), making this a global problem in winemaking. In wine their presence is mainly due, although not exclusively, to the activity of yeast *Dekkera/Brettanomyces bruxellensis* (Heresztyn 1986; Chatonnet et al. 1997), that can decarboxylate the hydroxycinnamic acid precursors, *p*-coumaric, ferulic and caffeic acids, respectively, to the correspondent vinylphenols by the hydroxycinnamate decarboxylase (Figure 1). Through reduction by vinylphenol reductase, vinylphenols are converted to the correspondent ethylphenols (Figure 1) (Chatonnet et al. 1997). These yeasts can convert free caffeic, ferulic, *p*-coumaric and sinapic acids (Heresztyn 1986), their ethyl esters when *Dekkera/Brettanomyces* present ethyl esterase activity (Hixson et al. 2012) but not the tartaric acid esters like caftaric, fertaric and coutaric acids (Schopp et al. 2013), the most abundant phenolic acids present in wine. Red wines always present higher values of VPs than rosé and white wines (Table 2). Red wines are particularly susceptible to *Dekkera/Brettanomyces bruxellensis* contamination and proliferation due to their lower acidity and frequent ageing in wood barrels. *Vitis vinifera* red grape varieties with higher content of polyphenol precursors are the most susceptible to the *Dekkera/Brettanomyces* defects (Chatonnet et al. 1997). Nevertheless, when white wines are produced with prolonged maceration and aged in wood barrels the VPs levels increase (Lukić et al. 2015). When present in wines the levels of 4-EP are always higher than that of 4-EC and 4-EG (Table 2), and although some variations in their proportion have been found that seem to be dependent on the grape variety or *Dekkera/Brettanomyces* strains and geographical location,

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Table 1. Olfactory detection thresholds, physicochemical and spectroscopic characteristics of 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG) and 4-ethylcatechol (4-EC).

	Molecular weight (g/mol)	Boiling point (°C)	Log K_{OW}	pKa	Solubility in water (mg/L, 25°C)	Maximum absorption wavelength (nm)	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Olfactory detection threshold (mg/L)				Reference
									Water	Model	White wine	Red wine	
4-EP	122.16	218–219	2.47	10.2	4900	280	260	305	0.13	0.44	1.1	1.2	Boidron and Chatonnet (1988)
									0.13	0.44		0.605	Chatonnet et al. (1992)
												0.368–0.569	Curtin et al. (2008)
												0.221	Botha (2010)
4-EG	152.19	234–236	2.18	10.3	693.8	280	260	305	0.025	0.047	0.07	0.15	Boidron and Chatonnet (1988)
									0.025	0.047		0.110	Chatonnet et al. (1992)
												0.033	Ferreira et al. (2000)
												0.158–0.373	Curtin et al. (2008)
												0.107	Botha (2010)
4-EC	138.16	273–274	1.87	9.7	5520	280	260	305				0.030–0.060	Hesford and Schneider (2004)
											0.100–0.400	0.100–0.400	Larcher et al. (2008)
												0.774–1.528	Curtin et al. (2008)
												0.442	Botha (2010)
												0.823	Diako et al. (2017)

ratios of 4-EP to 4-EG from 40:1 to 3:1 have been reported (Chatonnet et al. 1992; Pollnitz et al. 2000; Rayne & Eggers 2007). On average, they are present in a 10:1 ratio, respectively (Chatonnet et al. 1992; Romano et al. 2008). The ratio of 4-EP to 4-EC was found to be 3.7:1 and that of 4-EG to 4-EC of 0.7:1 (Larcher et al. 2008). In dry wines, the production of VPs by *B. bruxellensis* may be accompanied by the synthesis of acetyl-tetrahydropyridine with a roasted, caramel, bready odour, carboxylic acids, and some ethyl-esters, which contributed to their "*Brett character*" (Romano et al. 2008). It can also occur a masking effect by isobutyric and isovaleric acids (cheesy type odour/flavour) on the detection of ethylphenols making it difficult to be sensory perceived in wines (Romano et al. 2009). Tempère et al. (2011) showed that due to complex aromatic composition of wines, considerable variations in experts' olfactory sensitivity to key wine compounds can arise, including ethylphenols (Tempère et al. 2014). It has been demonstrated that above their olfactory perception threshold these compounds result in a decrease or even elimination of fruity and varietal aromas in wine (Chatonnet et al. 1990; Gerbaux & Vincent 2001; San-Juan et al. 2011; Tempère et al. 2016; Filipe-Ribeiro et al. 2017a; Filipe-Ribeiro et al. 2018) and it has been shown that even subliminal concentrations affected the wine fruity notes (Tempère et al. 2016). The presence of VPs in wines also seems to affect the bitterness and astringency perception of wines (Filipe-Ribeiro et al. 2018). The correlation of bitterness and astringency, both unpleasant wine sensory attributes, with the abundance of 4-ethylphenol and 4-ethylguaiacol, responsible for the unpleasant sensory phenolic attribute, can be explained by central cognitive interactions, where one strong taste or aroma reduces perception of the other in the brain (Coupland & Hayes 2014). The interaction of aroma with the taste perception of food and wine has been described (Sáenz-Navajas et al. 2010; Coupland & Hayes 2014; Ferrer-Gallego et al. 2014).

In order to eliminate or minimize this serious wine sensory defect, the knowledge of the contamination sources, conditions favouring *Dekkera/Brettanomyces* growth and VPs production are important to produce high quality wines. The adoption of preventive actions during winemaking, many of them already allowed, if implemented during the winemaking process, could decrease the contamination of wine and also the formation of VPs in already contaminated wines. These preventive measures should be favoured over remediation measures designed to remove the already formed VPs in wines, as these remediation treatments have in general a higher impact on wine quality, although with a careful selection of the treatment their impact can be greatly reduced. This review summarises the wine microbial ethylphenol formation preventive actions in winemaking known to reduce *Dekkera/Brettanomyces* contamination and ethylphenols production and remedial treatments to remove ethylphenols from already contaminated wines. Also, the analytical methods developed and some of them validated currently available for the determination of 4-EP, 4-EG and 4-EC in wines are discussed regarding their most important aspects including performance characteristics, advantages and disadvantages.

2. Microbial formation, preventive actions and remedial treatments of volatile phenols in wine

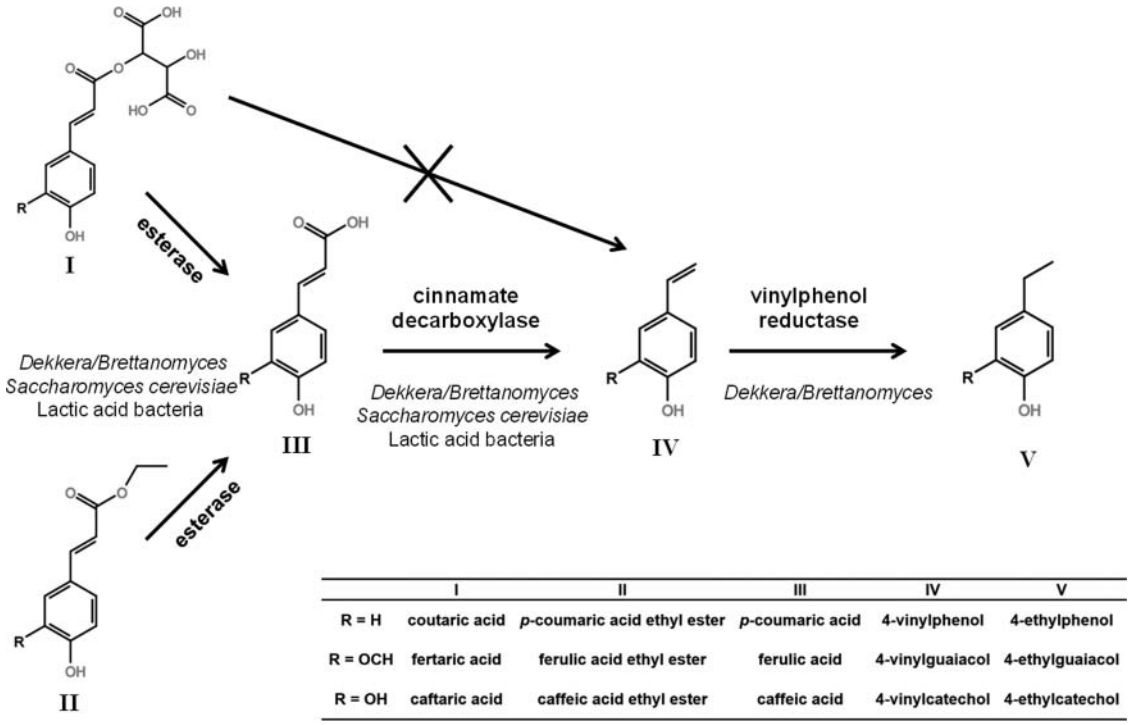
2.1. Microbial formation of volatile phenols

Yeasts classified as *Dekkera/Brettanomyces* spp. are probably the most important organisms responsible for the production of VPs in wines (Chatonnet et al. 1992). Although *Saccharomyces cerevisiae* and lactic acid bacteria can produce vinylphenols precursors that can be used by *Dekkera/Brettanomyces* for producing the correspondent ethylphenols, in wine *Dekkera/Brettanomyces* were the only yeasts with the ability to produce significant amounts of ethylphenols (Chatonnet et al. 1995). Excellent reviews on the microbiology and ecology of these yeasts have

Table 2. Concentration ranges reported in different countries and wine styles of 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG) and 4-ethylcatechol (4-EC).

	Country	Concentration range in wines (mg/L)	Wine style	Sampling strategy	Reference
4-EP	Australia	0.002–2.660 (n = 61)	Red	Random	Pollnitz et al. (2000)
		n.d. - 3.21 (n = 213)	Red	Random	Cynkar et al. (2007)
	Brazil	n.d.-3.820 (n = 126)	Red	Random	Ávila and Ayub (2013)
	Canada	<0.001–0.586 (n = 54)	Red	Random	Rayne and Eggers (2007)
	France	n.d.-0.028 (n = 54)	White	Random	Chatonnet et al. (1992)
		n.d.-0.075 (n = 12)	Rosé	Random	
		0.001–6.047 (n = 83)	Red	Random	
	Germany	n.d-2.583 (n = 589)	Red	Random	Nikfardjam et al. (2009)
		n.d.-0.291 (n = 548)	White	Random	
		n.d.-0.093 (n = 163)	Rosé	Random	
	Italy	n.d.-1.80 (n = 153)	Red	Random	Larcher et al. (2007)
		n.d.-0.476 (n = 65)	White	Random	
		n.d.-0.071 (n = 32)	Raisin	Random	
	Portugal	n.d. - 0.403 (n = 29)	White	Random	Rodrigues et al. (2001)
		n.d. - 4.43 (n = 88)	Red	Random	
	Slovenia	6–953 (n = 82)	Red	Random	Česnik and Lisjak (2016)
	Spain	n.d. - 9.73 (n = 510)	Red	Random	Garde-Cerdán et al. (2010)
		n.d. - 5.78 (n = 267)	Red	Random	Garde-Cerdán et al. (2008)
		0.394–1.368 (n = 10)	Red	Random	Carrillo and Tena (2007)
		n.d.-2.708 (n = 10)	Red	Random	Carpinteiro et al. (2010)
		0.009–1.50 (n = 57)	Red	Random	López et al. (2002)
		0.006–2.265 (n = 10)	Red	Random	Carpinteiro et al. (2012)
	Switzerland	0.170–3.78 (n = 15)	Red	Wines with "*Brett character*"	Hesford and Schneider (2004)
4-EG	Australia	0.001–0.437 (n = 61)	Red	Random	Pollnitz et al. (2000)
	Brazil	n.d-0.260 (n = 126)	Red	Random	Ávila and Ayub (2013)
	Canada	<0.001–0.411 (n = 54)	Red	Random	Rayne and Eggers (2007)
	France	n.d.-0.007 (n = 54)	White	Random	Chatonnet et al. (1992)
		n.d.-0.015 (n = 12)	Rosé	Random	
		0.001–1.561 (n = 83)	Red	Random	
	Germany	n.d.-0.517 (n = 589)	Red	Random	Nikfardjam et al. (2009)
		n.d. - 0.215 (n = 548)	White	Random	
		n.d. - 0.156 (n = 163)	Rosé	Random	
	Italy	n.d. - 0.430 (n = 153)	Red	Random	Larcher et al. (2007)
		n.d.-0.253 (n = 65)	White	Random	
		n.d.-0.035 (n = 32)	Raisin	Random	
	Slovenia	6–479 (n = 82)	Red	Random	Česnik and Lisjak (2016)
	Spain	n.d. - 3.23 (n = 510)	Red	Random	Garde-Cerdán et al. (2010)
		n.d. - 3.23 (n = 267)	Red	Random	Garde-Cerdán et al. (2008)
		0.031–0.137 (n = 10)	Red	Random	Carrillo and Tena (2007)
		n.d-0.204 (n = 10)	Red	Random	Carpinteiro et al. (2010)
		0.001–0.420 (n = 57)	Red	Random	Lopéz et al. (2002)
		0.001–0.251 (n = 10)	Red	Random	Carpinteiro et al. (2012)
		n.d.- 0.116 (n = 52)	Red	Random	Ferreira et al. (2000)
	Switzerland	<0.050–0.329 (n = 15)	Red	Wines with "*Brett character*"	Hesford and Schneider (2004)
4-EC	Italy	n.d. −1.610 (n = 153)	Red	Random	Larcher et al. (2008)
		n.d.-0.777 (n = 65)	White	Random	
		n.d.-0.073 (n = 32)	Raisin	Random	
	Spain	n.d-0.230 (n = 10)	Red	Random	Carrillo and Tena (2007)
		n.d-0.344 (n = 10)	Red	Random	Carpinteiro et al. (2010)
		n.d.-0.158 (n = 10)	Red	Random	Carpinteiro et al. (2012)
	Switzerland	0.027–0.427 (n = 15)	Red	Wines with "*Brett character*"	Hesford and Schneider (2004)

been produced in the last few years, from spoilage organisms to valuable contributors to industrial fermentations (du Toit & Pretorius 2000; Renouf et al. 2007b; Suárez et al. 2007; Oelofse et al. 2008; Wedral et al. 2010; Kheir et al. 2013; Zuehlke et al. 2013; Steensels et al. 2015). These yeasts are distributed worldwide, and may arrive at the winery on grape skins, be present on winery surfaces or wood barrels (Chatonnet et al. 1990; Chatonnet et al. 1992; Gerbaux & Vincent 2001). The *Brettanomyces* genus is known since 1904. This yeast was isolated in the end of the fermentation of an English beer (Ale) (Zuehlke et al. 2013). Among their physiological and morphological traits are acetic acid production, slow growth on malt extract agar, short survival and frequency of lancet-shaped cells with absence of ascosporous (Zuehlke et al. 2013). The genus *Dekkera* was only designated in 1964 when spore formation was observed. Currently there are recognised five species belonging to *Brettanomyces* and *Dekkera*: *B. custersianus, B. naardenensis, B. nanus, B. anomalus* and *B. bruxellensis*; of these, the perfect form (teleomorph) is known for the last two species, called *D. anomala* and *D. bruxellensis*, respectively (Oelofse et al. 2008). *Dekkera/Brettanomyces* alcoholic fermentation is detained by anaerobiosis - the so-called Custer effect (negative Pasteur effect) (Smith 2011). Although its growth is detained by anaerobiose, this can be reverted by oxygen. The tolerance of *B. bruxellensis* to increasing ethanol concentrations varies dependent on the strain, although ethanol above 9% (v/v) slows growth (Rodrigues et al. 2001; Barata et al. 2008a), nevertheless it can tolerate concentration as higher as 15% (v/v) of ethanol depending on the strain and conditions (Rodrigues et al. 2001; Barata et al. 2008a; Childs et al. 2015). The time for adaptation to ethanol is strain dependent, resulting in either enhanced or reduced sensitivity (Vigentini et al. 2008). The optimal temperature range for *B. bruxellensis* is 25–28 °C



	I	II	III	IV	V
R = H	coutaric acid	*p*-coumaric acid ethyl ester	*p*-coumaric acid	4-vinylphenol	4-ethylphenol
R = OCH	fertaric acid	ferulic acid ethyl ester	ferulic acid	4-vinylguaiacol	4-ethylguaiacol
R = OH	caftaric acid	caffeic acid ethyl ester	caffeic acid	4-vinylcatechol	4-ethylcatechol

Figure 1. Formation of volatile phenols from hydroxycinnamate precursors or their degradation products (vinylphenols) in wines by *Dekkera/Brettanomyces*.

(Smith 2011), although it has been observed spoilage of red wines at lower temperatures (10 °C, 15 °C, 20 °C and 25 °C) (Barata et al. 2008; Zuehlke et al. 2013). Nevertheless, the exposure to higher temperatures (36 °C) during 12 h resulted in a complete loss of viability (Barata et al. 2008b). Ethanol and ferulic acid seems to increase *B. bruxellensis* sensitivity to thermal inactivation (Couto et al. 2005). The effect of pH on *B. bruxellensis* growth has been less investigated in wine, nevertheless growth in enrichment media was observed at pH 2.0 (Conterno et al. 2006). There is a growing evidence that environmental stress can induce a viable but non-culturable (VBNC) state in *Brettanomyces* (Fleet 1999; Serpaggi et al. 2012). In this dormant physiological state, cells remain alive but cannot be cultured using synthetic media without some form of stimulation (Palkova & Vachova 2006). In wine *B. bruxellensis* have been reported to enter VBNC states following sulphur dioxide additions (Divol & Lonvaud-Funel 2005; du Toit et al. 2005; Agnolucci et al. 2010). Therefore in winemaking if low free sulphur dioxide (molecular SO_2 <0.5 mg/L) levels are conjugated with high wine pH (> 3.80) and temperatures higher than 15 °C then *Dekkera/Brettanomyces* can grow and problems may arise (Benito et al. 2009). Furthermore these yeasts can also multiply after wine bottling if the wine contains residual fermentable sugars, a situation more likely if the wine was minimally filtered (du Toit & Pretorius 2000). The low wine alcohol degree was found to determine the accumulation of VPs after bottling. In the light of this results modern tendency in reducing alcohol degree of wines represents an additional risk factor (Lisanti et al. 2013). Wine wood barrel maturation is particularly favourable to *Brettanomyces* growth, being a slow growing yeast that does not compete well against other microorganisms, as during alcoholic fermentation yeast *Saccharomyces* easily out-competes it. These wines usually have also lower levels of SO_2 (Garde-Cerdán et al. 2010). Furthermore, the necessary processes of racking off lees and regularly topping up barrels ensures that there are always reasonable levels of dissolved oxygen in the wine. Although wood barrels can promote the sensory improvement of wines they are porous and difficult to clean and sterilize. Therefore, they provide an environment in which undesirable microorganisms can survive and be transferred from one wine to another. Although cleaning methods are becoming more sophisticated, the shape and microstructure of wooden barrels afford undesirable microorganisms a great degree of protection (Suárez et al. 2007).

2.2. Measures for preventing volatile phenols formation in wines

Due to the serious impact on wine quality resulting from the spoilage by *Dekkera/Brettanomyces*, a series of preventive actions have been developed and implemented during winemaking. Most of the preventive actions are of hygienic nature such as properly sanitised front-end processing equipment, hoses, pumps, valves, drains, barrels and bottling equipment. These procedures minimise the spread of *Dekkera/Brettanomyces*. Insects such as the fruit fly are also potential vectors of infection (Licker et al. 1997). In the pre-fermentative operations, a range of actions can be taken in order to decrease red wine contamination with *Dekkera/Brettanomyces*, as well as decreasing the amount of precursors present and hence the potential of VPs formation. Low maceration temperatures (Gerbaux & Vincent 2001) and avoidance of the use of pectinolytic enzymes contaminated with cinnamoyl esterase activity are two measures aiming to decrease the extraction and formation of precursors (Dugelay et al. 1993; Romano et al. 2008). To date, sulphiting is the most efficient preventive measure to avoid the development of *Dekkera/Brettanomyces,* therefore the occurrence of fast and complete fermentations is desirable to quickly sulphite wine. Nevertheless sulphite addition induced

loss of cultivability, but maintenance of viability and more importantly production of VPs could persist in VBNC cells because they still retain part of their enzymatic activity (Laforgue & Lonvaud-Funel 2012). Therefore, continuous wines sulphiting can prevent the detection of *B. bruxellensis* when methods based on culture growth media are used. Morata and co-workers (2013) proposed an alternative procedure to deal with *Dekkera/Brettanomyces* contamination and VPs formation by using a cinnamoyl esterase before fermentation to release cinnamic acid precursors and fermentation with hydroxycinnamate decarboxylase positive (HCDC+) *Saccharomyces cerevisiae* in order to produce vinylphenols that will react with anthocyanins, and therefore decrease the amount of precursors (free hydroxycinnamic acids) for formation of VPs, nevertheless the final impact on VPs formation and appearance of sensory phenolic notes in wines are difficult to predict as vinylphenols are also VPs precursors (Figure 1, Table 3).

The wine pH manipulation for *Dekkera/Brettanomyces* control is not viable as their growth is only inhibited at pH<2.62, a value not usually found in wines. The absence of oxygen is very difficult to mantain during normal winemaking operation like racking and transfer and the amount of ethanol needed to impair their development is much higher than those found in regular red wines (Table 3). Chemical alternatives to SO_2 have also been explored due to the possible allergenic reactions to this additive and its decrease during wine ageing. Dimethyldicarbonate (DMDC) is a preservative which shows remarkable antimicrobial activity and inhibitory action with a concentration within the legal limits (200 mg/L). Nevertheless, its action is transitory since it is quickly hydrolysed in wine into methanol and carbon dioxide (Delfini et al. 2002; Costa et al. 2008), thus, it cannot completely replace SO_2. Another anti-microbial agent recently introduced in the market is fungal chitosan (Table 3) that shows inhibitory and biocidal action within the legal limit (10 g/hL). Although sorbic acid efficiency against *Dekkera/Brettanomyces* has been studied, its inhibitory concentration was four times higher than the legal limit (200 mg/L). Other alternatives to these anti-microbial agents have been tested like benzoic acid, hydroxycinnamic acids, oenological tannins, killer toxins, antimicrobial peptides and β-glucanase enzymes (Table 3). Benzoic acid has been shown to be efficient for *Dekkera/Brettanomyces* control, nevertheless is not authorised to use in wines. This is also the case of killer toxins isolated from different microorganisms and peptides either synthetic or obtained from enzymatic hydrolysis of lactoferrin. The efficiency of oenological tannins and hydroxycinnamic acid, namely ferulic acid, were dependent of the *Dekkera/Brettanomyces* strains, and the amount of ferulic acid need was higher than concentrations normally present in wines (Godoy et al. 2013; Campolongo et al. 2014).

Regular rackings, which eliminate the sedimented cells, can contribute to lower yeast populations and in particular *B. bruxellensis* (Renouf & Lonvaud-Funel 2004). Thus, the reincorporation of lees during ageing can sometimes bring *B. bruxellensis* to wines. Therefore, this practice should be considered only after a microbial analysis showing the absence or a low level of *B. bruxellensis* in lees. Wine fining has also been shown to be beneficial because it helps *B. bruxellensis* flocculation and their elimination with sediments (Millet & Lonvaud-Funel 2000; Murat & Dumeau 2003). More radical treatments like heat treatments (Couto et al. 2005) or wine filtration (Ubeda & Briones 1999; Renouf et al. 2007c) are effective against *B. bruxellensis*. These microbial stabilisation methods should be considered only before bottling, the probability of recontamination being then lower. Nevertheless, heat treatment and microfiltration are not generally applied by winemaker's due to claimed deleterious effect on wine sensory quality and longevity and in the latter case due to deterioration of wines colloidal structure and reduction of colour and aroma (Table 3). To cope with this problem alternative technologies have been evaluated, with success. High pressure processing (HPP), pulsed electric field (PEF) and low electric current (LEC) are, currently, new technological approaches (Table 3). These treatments didn't result in wine aromatic defects when compared to control wines (Puig et al. 2003; Lustrato et al. 2006; Puértolas et al. 2009; Lustrato et al. 2010; Puértolas et al. 2010; Morata et al. 2012). UV-C has also been tested and its use showed more technical constrains and cause a small decrease in anthocyanins (Fredericks et al. 2011; Pala & Toklucu 2013).

From the literature survey it can be concluded that there are presently several efficient tools for dealing with the *Dekkera/Brettanomyces* contamination, their growth and preventive inhibition of VPs formation (Table 3), nevertheless as they are still commercialised worldwide wines with high levels of these VPs (Table 2) therefore it can be concluded that probably these preventive procedures are not adequately implemented and/or their efficiency properly monitored during the winemaking process. The new technologies that have been tested to deal preventively with this problem, HPP, PEF, LEC and UV-C are very promising concerning their efficiency, nevertheless further studies are needed in order to evaluate more deeply their impact on the wine quality and the maintenance of its sensory quality until its consumption.

2.3. Measures for removing or reducing the aroma impact of volatile phenols

A series of remediation treatments have also been developed to remove from wines the already formed VPs and decrease or eliminate their negative sensory impact. The remediation treatments proposed can be divided into two main groups, those intended to decrease the headspace contents by decreasing their partition coefficients to the gas phase without changing the total VPs contents of wines (non-subtractive techniques), and those directed to the removal of the 4-EP and 4-EG from wines, therefore decreasing their content and their headspace concentration (subtractive techniques). The impact of these techniques on wine quality has been only studied in some works, but this ultimately will dictate the suitability and the choice by winemakers. Most of the proposed treatments are not yet authorised by the International Organisation of Vine and Wine (OIV) and are therefore not allowed in winemaking (Table 4). Some treatments were only tested in model wine solutions (yeast cell walls, oak wood, cork) being difficult to predict their efficiency in a more complex matrix like red wine. Also, their efficiency is dependent on the levels of these contaminants (activated carbons, fine yeast lees, potassium caseinate, suberin adsorbed on glass beads, chitosan) and on the wine matrix (yeast cell walls,

Table 3. Preventive actions and treatments during winemaking and storage to prevent the contamination and/or production of volatile phenols (VPs) in wines.

	Objectives/ Reasoning	Disadvantages and legal constrains	Reference
Pre-fermentative operations			
Low maceration temperature	Prevents solubilisation of hydroxycinnamic acids; cold maceration temperatures ($\leq$10°C) prevents proliferation	May cause loss of colour and aroma; subsequent contamination and microorganisms development is possible	Ferreira et al. (2000)
Avoidance of pectinolytic enzymes with cinnamoyl esterase activity	Cinnamoyl esterase activity increases the levels of free hydroxycinnamic acids precursors in must and wine		Dugelay et al. (1993) Romano et al. (2008)
Fermentation			
Fermentation	Fast and complete fermentations should be favoured in order to quickly sulphite the wine once the fermentative species decline.		Vigentini et al. (2008)
Vinylphenol polymerisation with anthocyanins	Use of cinnamoyl esterases before fermentation and of HCDC$^+$ *Saccharomyces cerevisiae* strains transform hydroxycinnamic acids into vinylphenols that react chemically with grape anthocyanins yielding vinylphenolic pyranoanthocyanins, reducing globally the precursors for VPs.	Not able to eliminate completely 4-EP formation. Impact on aroma difficult to predict	Morata et al. (2013)
Post-fermentative operations			
Low pH	Inhibitory effect 2.17–2.62 Preventive effect 1.75–2.17	Too low pH values for oenological use	Benito et al. (2009)
Absence of oxygen	Even a small ingress of oxygen strongly increases growth rate and biomass and VPs formation in wines.	Difficult to exclude oxygen during a wine transfer operations between barrels and tanks	Malfeito-Ferreira et al. (2001)
High alcohol levels	Maximum ethanol resistance <15% (v/v): Inhibition of hydroxycinnamoyl decarboxylase and vinylphenol reductase at ethanol ~15% (v/v)	Too high alcohol content for most wine styles	Benito et al. (2009)
Anti-microbial Agents			
Sulphur dioxide (SO_2)	Only free SO_2 is effective. Inhibition of growth between 0.5 and 0.8 mg/L of molecular SO_2. SO_2 levels decrease during storage and regular SO_2 check should be done.	Different strains of *D/B* possess different SO_2 sensitivities.	du Toit et al. (2005)
	Treatment more efficient combined with racking.	SO_2 is a chemical stressor inducing VBNC state in *B. bruxellensis* grown in synthetic wine medium.	Agnolucci et al. (2010)
		WHO recommended reducing SO_2 use because of negative health effects to consumers.	Rubio et al. (2015)
		Maximum legal dose: 150 mg/L of total SO_2 for red wine[a]	Chatonnet et al. (1993) Portugal et al. (2014) Conterno et al. (2006)
Dimethyldicarbonate (DMDC)	150 mg/L was sufficient in almost all situations to stop *B. bruxellensis* growth or to reduce significantly its population, after alcoholic fermentation.	Cost of dosing equipment, proper safety, operator training and maintaining a constant rate of addition.	Costa et al. (2008)
		DMDC has a short half-life, hydrolysing to methanol and carbon dioxide within hours. Its fungicidal impact is transient. DMDC is less effective in unfinished red wine.	Delfini et al. (2002)
		Maximum legal dose: 200 mg/L, without detectable residues in finished wines[a].	Portugal et al. (2014) Renouf et al. (2008)
Chitosan	Fungal chitosan. Minimal inhibitory concentration that inhibited 90% of *B. bruxellensis* of 6.2 g/hL. Efficiency was not dependent on ethanol concentration 12.5% (v/v).	The currently allowed chitosan for oenological practices should be exclusively of fungal origin[a].	Portugal et al. (2014)
	Minimal biocidal concentration that kill 50% of *B. bruxellensis* of 6.2 g/hL. Efficiency was not dependent on ethanol concentration 12.5% (v/v)	Chitosan affected some physicochemical characteristics of wine, particularly the hue and colour intensity.	Gómez-Rivas et al. (2004)
	Crab shell chitosan (deacetylation degree 91%) - 6 g/L was required to inhibit *Brettanomyces* growth.	Maximum legal dose for *D/B* control is 10 g/hL[a]	Ferreira et al. (2013) Bağder et al. (2015)
Sorbic acid	Inhibitory effect of hidroxycinammate decarboxylase and vinylphenol reductase >900 mg/L; no activity >1100 mg/L (pH 3.6)	Maximum legal limit of 200 mg/L[a]; Unstable in the presence of lactic acid bacteria (yielding 1,3-pentadiene)	Benito et al. (2009)
Benzoic acid	Inhibits the hidroxycinammate decarboxylate and vinylphenol reductase between 150–200 mg/L at pH 3.6.	Not allowed to use in wines[a]; Concentration dependent on the species	Benito et al. (2009)
Hydroxycinnamic acids	Ferulic acid was more active than *p*-hydroxycinnamic acid and caffeic acids.	Need to supplement wines as concentrations normally don't found in wines.	Harris et al. (2010)
	Fungistatic effect at >2 mM ferulic acid (388 mg/L). With a positive interaction with ethanol concentration.	MIC depends on the strain.	Campolongo et al. (2014) Godoy et al. (2013)

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Table 3. (*Continued*)

	Objectives/ Reasoning	Disadvantages and legal constrains	Reference
Oenological tannins	Minimum inhibitory concentration of 0.0061 g/hL to 0.0488 g/hL of tannic acid equivalents Ethanol at 12.5% (v/v) increase inhibitory action of 0.00020 g/hL to 0.0061 g/hL of tannic acid equivalents	Efficiency was dependent on the strain	Portugal et al. (2014)
Oenological woods extracts	Toasted American oak wood pressurised liquid extract (IC50 0.77–1.36 mg/mL) and cherry wood pressurised liquid extracts (IC50 0.54–0.98 mg/mL) exhibited growth inhibition of *D/B*	Efficiency not tested in wines	Alañón et al. (2015)
Killer toxins	KwKt and PiKt (*Kluyveromyces wickerhamii* and *Pichia anomala*, respectively) were active during 10 days at pH 3 to 5, below 35 °C, and production of VPs was reduced.	KwKT was not efficient to 28% of the South African strains;	Comitini et al. (2004)
	PMKT2 (*Pichia membranafaciens*) showed best killer action at pH 3.5–4.5 and 20°C. *S. cerevisiae* was resistant.	Not allowed to use in wines[a]	Santos et al. (2009)
	A killer toxin isolated from *Ustillago maydis* with optimal killer conditions pH 3.0-4.5, temp. 15–20°C. Decrease of 4-EP production with increasing killer activity with no 4-EP production from 400 AU/mL.		Santos et al. (2011)
	CpKT1 and CpKT2 (*Candida pyralidae*) were active and stable at pH 3.5–4.5, and temperatures between 15 and 25°C. No effect of ethanol and sugar concentrations found in grape juice and wine. No inhibition of *S. cerevisiae* or lactic acid bacteria strains.		Mehlomakulu et al. (2014)
Lactoferrin derived peptides and synthetic peptides	Peptides obtained by pepsin hydrolysis and synthetic peptide LfcinB17–31 showed fungicidal properties against *D/B* at micro-molar concentration range in white wines.	Fungicidal activity was dependent on the strain and also on the matrix. Not allowed to use in wines[a]	Enrique et al. (2007) Enrique et al. (2008)
β-glucanase	GlucanexTM presented a minimum inhibitory concentration of 30 g/hL.	IC50 and MIC values approximately 3 to 4-fold greater than the recommended dose for improving wine filtration.	Enrique et al. (2010)
Clarification/Stabilisation			
Racking	Very efficient in reducing *D/B* populations as they are eliminated with the lees.	Difficult to avoid oxygen incorporation during racking	Renouf and Lonvaud-Funel (2004) Rubio et al. (2015) du Toit et al. (2005) Uscanga et al. (2003)
Gelatine/egg albumin/potassium caseinate/casein	Reduces by 40 to 2000 fold *D/B* populations by flocculation	Loss of colour and aroma	Murat and Dumeau (2003)
Avidin	Use of avidin to remove biotin to levels <0.2 μg/L to inhibit *D/B* growth in wines	No allowed to use in wines[a].	von Cosmos and Edwards (2016)
Microfiltration	Using membranes of 0.45 μm or ultrafiltration reduces *D/B* population by physical separations	Cells which remained in wine after several weeks could pass through a 0.45 μm membrane and can re-enter a growing phase Deterioration of the wine's colloidal structure and can reduce colour and aroma intensity.	Calderón et al. (2004) Millet and Lonvaud-Funel (2000) Renouf et al. (2007c) Ubeda and Briones (1999)
Ageing and storage			
D/B free lees	Ageing on lees should only be considered after a microbial analysis showing the absence or a low level of *B. bruxellensis* in lees.		Renouf et al. (2007a)
Low temperature storage	Achieve stable levels of 4-EP during barrel storage at low temperatures (6–8 °C).	No inactivation or removal of the *D/B*.	Barata et al. (2008a)
Pre-bottling			
Thermal technologies			
Flash pasteurisation	*D. bruxellensis* decimal reduction time (D) of 14.8–23.3 min at 32.5 °C and 0.7 to 1.0 min at 37.5 °C. and Z value 3.3–4.3	Winemakers are generally reluctant to use heat treatments due to claimed deleterious effects on wine sensory quality and longevity.	Couto et al. (2005b)
	D. anomala D value 7.9–8.9 min at 32.5°C and 0.4–0.6 at 37.5°C and Z values 3.8–4.2		Barata et al. (2008b)
	At 36 °C various *D/B* strains within 24 h of incubation, and at 44°C within 12 h.		
Non Thermal Technologies			
High Pressure Processing (HPP)	The use of 400 or 500 MPa, 4 °C or 20 °C, 5 or 15 min in white and red wines, resulted in the complete inactivation of yeasts, lactic and acetic acid bacteria	No sensory differences between untreated and HHP-treated wines.	Puig et al. (2003) Morata et al. (2012)
Pulsed Electric Field (PEF)	*B. bruxellensis* populations were reduced by 99.9% in grape must and wine when treated with 186 kJ/kg at 29 kV/cm.	Treatment did not change the sensory profile of wines when compared to control	Puértolas et al. (2009)

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Table 3. (*Continued*)

	Objectives/ Reasoning	Disadvantages and legal constrains	Reference
	D. anomala was the most PEF resistant independently of the medium.		Puértolas et al. (2010)
Low Electric Current (LEC)	200 mA treatment over a 60-day interval reduced *B. bruxellensis* populations by over six logs. Efficiency of LEC was similar to that of SO_2 addition.	Final wine was similar to those wines produced with SO_2 addition	Lustrato et al. (2006) Lustrato et al. (2010)
Ultraviolet C light (UV-C)	Highest microbial reduction was obtained after 3672 J/L.	Efficiency depends largely on the physical appearance of the liquid such as turbidity, colour and initial microbial load. May cause small loses of anthocyanins	Fredericks et al. (2011) Pala and Toklucu (2013)

[a]OIV (2016).

suberin adsorbed on glass beads). In other cases, the doses needed are very high (yeast cell walls, fine yeast lees, oak wood, cork, polyaniline based compounds in base form) that they do not seem to be technically and/or economically feasible.

For the subtractive techniques various adsorbents have been used with different applied doses (Table 4). Of those oenological products already authorised by the OIV and tested in red wines, like activated carbons, yeast cell walls, fine yeast lees, potassium caseinate, egg albumin and oak chips; those that prove to be efficient within the legal dose were activated carbons, egg albumin, potassium caseinate and oak chips. Activated carbons at 100 g/hL with high surface area and micropore volume, were the most efficient in removal of VPs (74% for 4-EP and 79% for 4-EG) and the negative sensory "*Brett character*" removal efficiency was validated by sensory analysis (Filipe-Ribeiro et al. 2017a). Potassium caseinate (200 g/hL) was also efficient in the removal of these two VPs (33% for 4-EP and 42% for 4-EG) although its efficiency in the sensory defect removal was not evaluated (Barbosa et al. 2012). Egg albumin (10g/hL) and oak chips (300 g/hL) were less efficient (in average 19% and 15% VPs, removal) (Barbosa et al. 2012; Milheiro et al. 2017), but also their sensory impact were not evaluated. Although with a promising VPs removal efficiency, the use of potassium caseinate and egg albumin have the drawback of the potential allergenicity of these fining agents and, therefore, are of mandatory labelling in wines if residual concentration is higher than 0.25 mg/L (Regulation EU 579/2012). On the other hand, yeast cell walls and fine yeast lees were efficient at 300 g/hL (in average 28% of VPs removal) and at 3000 g/hL (in average 40% of VPs removal), respectively (Barbosa et al. 2012) but these high doses can have serious impact on the other wine aroma compounds. Other adsorbents were also assayed: esterified cellulose (200g/hL); suberin adsorbed on glass beads (dose not supplied) and polyaniline (PANI) based adsorbent in base form (1000 g/hL), all of them allowed a significant removal of 4-EP and 4-EG from red wines (43% and 39%; 49% and 45% and 68% and 50% for 4-EP and 4-EG, respectively) (Larcher et al. 2012; Marican et al. 2014; Carrasco-Sánchez et al. 2015; Gallardo-Chacón & Karbowiak 2015). Esterified cellulose only caused a small decrease in colour (Larcher et al. 2012) and the effect of PANI was much more detrimental showing a 44% decrease in total phenols (Marican et al. 2014). To increase the specificity of adsorbents for 4-EP and 4-EG without impacting on the other wine components, molecular imprinted polymers (MIPs) have been synthesised and applied to wines (Garde-Cerdán et al. 2008; Teixeira et al. 2015). The only monomer tested for MIP production was vinylpyridine in both works, although with different cross-linkers, porogens as well as different templates (EDMA cross-linker and 4-EP and 4-EG, Teixeira et al. 2015) and divinylbenzene-80 and penthachlorophenol (Garde-Cerdán et al. 2008), respectively. Their application doses were only supplied in one work and used at levels of 200 g/hL (Teixeira et al. 2015). Although in both works, the non-imprinted polymers showed already high adsorption for the two VPs - in average 45% (Teixeira et al. 2015) and 59% (Garde-Cerdán et al. 2008), the MIP showed increased adsorption values - 55% (Teixeira et al. 2015) and 91% (Garde-Cerdán et al. 2008). In both works significant amounts of volatile compounds and anthocyanins were also removed. Nevertheless, the sensory analysis of the wines treated with MIP in the work of Teixeira et al. (2015) indicated an improvement of the wine flavour. Only two works in the literature described the application of a non-subtractive approach to deal with the impact of VPs in wine (Filipe-Ribeiro et al. 2018; Milheiro et al. 2017). The reasoning of this approach is that by modifying the partition coefficients of the VPs for the gas phase in equilibrium with wine by adding substances that can bind these VPs, their sensory perception will be lower (Petrozziello et al. 2014). Several allowed oenological products like isinglass, carboxymethylcellulose (CMC) and chitosan were shown to decrease significantly the headspace aroma abundance of volatile phenols in red wines without changing their total concentration in wines. Nevertheless, no sensory analyses of the wines were performed to access the impact of the treatments on wine aroma (Milheiro et al. 2017). Filipe-Ribeiro et al. (2018), using chitins and chitosans with different structural features (deacetylation degree and molecular weight) as well different origins (crustacean *vs* fungal), observed that chitosans with a high deacetylation degree decreased significantly the headspace abundance of VPs, and these decreases resulted in a significant decrease in the sensory phenolic attribute and increased the floral and fruity wine sensory attributes. Crustacean chitosan was more efficient than fungal chitosan and this difference was attributed to the different structural features of fungal chitosans when compared to crustacean chitosans, namely the fact that fungal chitosan are chitosan-glucan covalent complexes. The solubility of crustacean chitosans was higher than that of fungal chitosan and this was related to the different impact in the headspace abundance reduction as well as in the sensory impact of wines.

From the different approaches for reducing the sensory impact of VPs on wine aroma some conclusions can be made: reduction of the total concentration of VPs in wines is efficient when the unintended removal of the other aroma compounds

Table 4. Remedial treatments of *Dekkera/Brettanomyces* contaminated red wines for decreasing the volatile phenol concentration or sensory impact of volatile phenols in contaminated wines.

Additive	Efficiency, dose used and matrix tested	Impact on wine quality and legal constrains	Reference
Removal (subtractive techniques)			
Activated carbon (AC)	0% (1969 μg/L 4-EP) and 0% (126 μg/L 4-EG); Dose 20 g/hL. 12% (539 μg/L 4-EP) and 0% (46 μg/L 4-EG); Dose 20 g/hL. Matrix: Red wine.	Efficiency was dependent on ACs S_{BET}, V_{micro}. ACs also reduce headspace aroma compounds, nevertheless volatile phenols reduction was more important for the positive fruity attribute perception than the abundance of headspace aroma compounds. A higher surface area of mesopores and total pore volume were detrimental for anthocyanins and colour intensity, while a higher surface area and micropores volume were important for removing phenolic acids. Activated carbon is an oenological product used in oenology for many years in grape must and white wine (Commission Regulation (EC) No 606/2009)	Lisanti et al. (2017)
	58% (1500 μg/L 4-EP) and 56% (300 μg/L 4-EG); Dose 80 g/hL. VPs Headspace abundance reduction 74%(1500 μg/L 4-EP) and 77% (300 μg/L 4-EG); Dose 80 g/hL. Matrix: Red wine.	Maximum Legal Dose 100g/hL[a]	Milheiro et al. (2017)
	71% (1500 μg/L 4-EP) and 68% (300 μg/L 4-EG); Dose 100 g/hL.		Filipe-Ribeiro et al. (2017a)
	74% (750 μg/L 4-EP) and 79% (150 μg/L 4-EG); Dose 100 g/hL. VPs Headspace abundance reduction 86% (750 μg/L 4- EP) and 91% (300 μg/L 4-EG); Dose 100 g/hL. Matrix: Red wine.		Filipe-Ribeiro et al. (2017b)
Yeast cell walls	15–20% (500 μg/L 4-EG) and 10–81% (1000 μg/L 4-EP); Dose 400–600 g/hL. Matrix: model wine solution.		Chassagne et al. (2005)
	Isolated compounds 41% (610 μg/L 4-EP) and 34% (142 μg/L 4-EG); Dose 500 g/hL. Competitive assay 22% (610 μg/L 4-EP) and 48% (760 μg/L 4-EG); Dose 500 g/hL. Matrix: model wine solution.		Nieto-Rojo et al. (2014)
	Yeast Cell walls 5–20% (1256–2029 μg/L 4-EP) and 9–14% (241–334 μg/L 4-EG); Dose 100g/hL.	The presence of other wine constituents, namely ethanol, sorbed by yeast influenced the sorption of both volatile phenols. Temperature and pH also have a negative effect	Barbosa et al. (2012)
	7–16% (1256–2029 μg/L 4-EP) and 3–39% (241–334 μg/L 4-EG); Dose 300 g/hL. Matrix: Red wine.	Maximum Legal Dose 40 g/hL[a]	
	~30% (10 mg/L 4-EP). Dose 5000 g/hL (fresh biomass). Matrix: Model wine solution.		Pradelles et al. (2008) Pradelles et al. (2009)
Fine yeast lees	28–36% (1256–2029 μg/L 4-EP) and 24–44% (241–334 μg/L 4-EG); Dose 1000 g/hL. 25–30% (1256–2029 μg/L 4-EP) and 10–42% (241–334 μg/L 4-EG); Dose 3000 g/hL. Matrix: Red wine.	Maximum Legal Dose 5000 g/hL[a]	Barbosa et al. (2012)
Potassium caseinate	18–33% (1256–2029 μg/L 4-EP) and 10–42% (241–334 μg/L 4-EG); Dose 200 g/hL. Matrix: Red wine.	Can cause a significant loses of anthocyanins at 40 g/hL Allergen labelling mandatory[b]	Barbosa et al. (2012)
	0% (1500 μg/L 4-EP) and 0% (300 μg/L 4-EG); Dose 60 g/hL. Matrix: Red wine.		Milheiro et al. (2017) Gambuti et al. (2012) Cosme et al. (2007)
Egg albumin	1% (3179 μg/L 4-EP) and 11% (377 μg/L 4-EG); Dose 40 g/hL. Matrix: Red wine.	Can cause a significant loss in colour. Allergen labelling mandatory[b]	Barbosa et al. (2012)
	20% (1500 μg/L 4-EP) and 17% (300 μg/L 4-EG); Dose 10 g/hL.		Milheiro et al. (2017) Cosme et al. (2007)
Oak wood	21% (4-EP)[$] and 14% (4-EG)[$]; Dose 1000 g/hL. Matrix: Model wine solution.		Barrera-García et al. (2006)
Oak chips	0–10% (1256–2029 μg/L 4-EP) and 0–14% (241–334 μg/L 4-EG); Dose 100 g/hL. 10–14% (1256–2029 μg/L 4-EP) and 4–15% (241–334 μg/L 4-EG); Dose 300 g/hL. Matrix: Red wine.		Barbosa et al. (2012)
Cork	39% (4-EP)[$] and 32% (4-EG)[$]; Dose 3333 g/hL. Matrix: Model wine solution.		Karbowiak et al. (2010)

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Table 4. (*Continued*)

Additive	Efficiency, dose used and matrix tested	Impact on wine quality and legal constrains	Reference
Suberin (Cork)	49% (1700 μg/L 4-EP), 44.6% (210 μg/L 4-EG) and 67% (370 μg/L 4-EP) 71% (110 μg/L 4-EG). Dose not shown. Matrix: Red wine	Volatile phenols sorption by suberin depends on the wine.	Gallardo-Chacón and Karbowiak (2015)
Polyaniline based compounds in base form	68% (5 mg/L 4-EP) and 50% (5 mg/L 4-EG). Dose 1000 g/hL. 100% (5 mg/L 4-EP) and 94% (5 mg/L 4-EG). Dose 10000 g/hL Matrix: Red wine	Reduction of total phenols in wine (41% dose 1000 g/hL and 99.99% dose 10000g/hL)	Marican et al. (2014) Carrasco-Sánchez et al. (2015)
Esterified cellulose	Cellulose acetate 22–26% (0.5–2 mg/L 4-EP) and 23–25% (0.5–2 mg/L 4-EG); Dose 400 g/hL. Cellulose acetate propionate 34–43% (0.5–2 mg/L 4-EP) and 32–39% (0.5–2 mg/L 4-EG); Dose 400 g/hL. Cellulose acetate butyrate 30–34% (0.5–2 mg/L 4-EP) and 27–30% (0.5–2 mg/L 4-EG); Dose 400 g/hL. Cellulose proprionate 33–43% (0.5–2 mg/L 4-EP) and 34–39% (0.5–2 mg/L 4-EG); Dose 400 g/hL. Matrix: Red wine.	Small decreases in colour	Larcher et al. (2012)
Molecular imprinted polymers (MIP)	4-vinylpiridine-divinylbenzene MIP	Production: 4-vinylpiridine (monomer, 0.66 mmol)-divinylbenezen-80 (cross-linker, 3.30 mmol)-pentachlorophenol (template 0.16 mmol)-acetonitrile:tolune (progen 75:25 v/v, 12.5 mL)-AIBN (radical initiator, 0.24 mmol) - batch production 60°C, 24 h.	Garde-Cerdán et al. (2008)
	MIP 92% (4-EP level not supplied) and 89% (4-EG level not supplied); Dose not supplied. NIP 55% (4-EP level not supplied) and 62% (4-EG level not supplied); Dose not supplied. Matrix: Red wine.	Also remove significant amounts of aroma compounds.	
	4-vinylpiridine-EDMA MIP	Production: 4-vinylpiridine (monomer, 4 mmol)-EDMA (cross-linker, 20 mmol) - 4-EP or 4-EG (Template 1 mmol)-acetonitrile:chloroform, (porogen 3:1 v/v, 40 mL) - AIBN (radical initiator, 0.5 mmol) - batch production 60 °C, 24 h.	Teixeira et al. (2015)
	MIP-4-EP 56% (1.659 mg/L 4-EP); Dose 200 g/hL. MIP-4-EP 54% (0.149 mg/L 4-EG); Dose 200 g/hL. MIP-4-EG 55% (1.659 mg/L 4-EP); Dose 200 g/hL. MIP-4-EG 40% (0.149 mg/L 4-EG); Dose 200 g/hL. NIP 46% (1.659 mg/L 4-EP); Dose 200 g/hL. NIP 43% (0.149 mg/L 4-EG); Dose 200 g/hL. Matrix: Red wine.	There was observed a significant decrease in the phenolic compounds, anthocyanins, volatile compounds. Sensory analysis indicates an improvement of wine flavour	
Reversed osmosis (RO) with a polymeric membranes	RO with a hydrophobic adsorptive resin reduced the volatile phenols to acceptable levels.	Significant loss of other aroma compounds	Ugarte et al. (2005)
Decrease of headspace abundance of volatile phenols (non-subtractive techniques)			
Isinglass	31% (1500 μg/L 4-EP) and 24% (300 μg/L 4-EG); Dose 4 g/hL. Matrix: Red wine.		Milheiro et al. (2017)
CMC	20% (1500 μg/L 4-EP) and 10% (300 μg/L 4-EG); Dose 10 g/hL. Matrix: Red wine.	Small decrease in colour and anthocyanins of red wine by CMC at the applied dose. Maximum legal dose 100 g/hL[a]	Milheiro et al. (2017)
Chitosan	36% (1500 μg/L 4-EP) and 17% (300 μg/L 4-EG); Dose 10 g/hL. From crustacean origin. Deacetylation degree: 66%	No sensory analysis Only chitosan of fungal origin allowed- until 500 g/hL depending on the intended use[a].	Milheiro et al. (2017)
	29% (750 μg/L 4-EP) and 30% (150 μg/L 4-EG); Dose 10 g/hL. From crustacean origin. Deacetylation degree: 85%	Decrease of phenolic acids and anthocyanins with increasing application doses. 500 g/hL resulted in a 32% decrease in colour intensity of red wine.	Filipe-Ribeiro et al. (2018)
	36% (750 μg/L 4-EP) and 35% (150 μg/L 4-EG); Dose 100 g/hL. From crustacean origin. Deacetylation degree: 85%	The reduction of headspace abundance of VPs by chitosan without changing the total amount of VPs in wine allowed to decrease the negative sensory phenolic and bitterness attributes and to increase the positive fruity and floral attributes in contaminated wines.	
	39% (750 μg/L 4-EP) and 39% (150 μg/L 4-EG); Dose 500 g/hL. From crustacean origin. Deacetylation degree: 85%	Efficiency of chitosans for reducing VPs headspace abundance is dependent on the deacetylation degree and origin. Crustacean chitosans more efficient than fungal chitosans, apparently related to the lower solubility or different structural features.	
	15% (750 μg/L 4-EP) and 11% (150 μg/L 4-EG); Dose 10 g/hL. From fungal origin. Deacetylation degree: 91%		
	28% (750 μg/L 4-EP) and 23% (150 μg/L 4-EG); Dose 100 g/hL. From fungal origin. Deacetylation degree: 91%		
	30% (750 μg/L 4-EP) and 27% (150 μg/L 4-EG); Dose 500 g/hL. From fungal origin. Deacetylation degree: 91% Matrix: Red wine.		

[$]estimated from K_{ww} calculated in the work cited; [a]OIV (2016); [b]OIV (2015).

is not also very high (Filipe-Ribeiro et al. 2017a). When the impact on anthocyanins is not very significant, the sensory colour intensity perception is not changed. The presence of VPs in wines also affect negatively the sensory bitterness and astringency, therefore their removal decreases these negative sensory attributes increasing the balance and persistence of wines if the treatment does not impact negatively on the wine phenolic composition (Filipe-Ribeiro et al. 2018). These points should be taken into account when selecting or designing new approaches for red wine treatment for removing VPs and their sensory impact on wine.

3. Detection and quantification of 4-ethylphenol, 4-ethylguaiacol and 4-ethylcatechol in wines

In the literature, there are described several methods for quantification of VPs in wines, with one exception (*e_nose*), they all use chromatography for separation of these VPs from other substances present in the complex wine matrix, either gas-chromatography or liquid chromatography (Tables 5 and 6). The studies concerning 4-EC quantification in wines are less common (Hesford & Schneider 2004; Carrillo & Tena 2007; Larcher et al. 2008; Carpinteiro et al. 2010; Carpinteiro et al. 2012). Methods for 4-EC involving gas-chromatography are more troublesome than those for 4-EP and 4-EG because of the high polarity of this VP and consequently problems associated to peak tailing in GC separation on polar columns (Hesford & Schneider 2004; Larcher et al. 2008), and therefore derivatisation before analysis is usually performed when analysed by GC (Carrillo & Tena 2007; Carpinteiro et al. 2010; Carpinteiro et al. 2012) nevertheless as this procedure increases the number of steps and therefore the time spent in the analytical procedure, its quantification has been performed preferably by liquid chromatography.

3.1. Gas chromatography

For the quantification of 4-EP and 4-EG due to their volatility the gas-chromatographic methods are the most widely used (Table 5). The preferred detection method has been mass spectrometry, mostly in the selected ion monitoring mode using fragment ions *m/z* 107 for 4-EP, *m/z* 137 for 4-EG (Pollnitz et al. 2000; López et al. 2002; Díez et al. 2004; Hesford & Schneider 2004; Marín et al. 2005; Fariña et al. 2007; Carpinteiro et al. 2010; Carpinteiro et al. 2012; Zhou et al. 2015; Milheiro et al. 2017), although tandem mass spectrometry (MS/MS) has also been employed using precursor ions at *m/z* 107 for 4-EP and *m/z* 137 for 4-EG and for quantification ions at *m/z* 91 for 4-EP and *m/z* 94 for 4-EG (Pizarro et al. 2007; Pizarro et al. 2011; Pizarro et al. 2012), and fewer methods have employed flame ionisation detection (Chatonnet & Boidron 1988; Domínguez et al. 2002; Martorell et al. 2002; Monje et al. 2002; Mejías et al. 2003; Sun et al. 2016) (Table 5). All methods use the internal standard method with calibration curve for quantification, usually employing 3,4-dimethylphenol as internal standard, except for Pollinitz et al. (2000), Rayne and Eggers (2007) and Boutou and Chatonnet (2007) that used deuterium labelled 4-EP and 4-EG as internal standards and López et al. (2002) that used 4-hydroxy-4-methyl-2-pentanone and 2-octanol. Also except for one work, all methods doesn't use derivatisation procedures. Carrillo and Tena (2007) used an acetylation procedure for the simultaneous quantification of 4-EP, 4-EG and 4-EC, because this last VP did not yield good peaks when analysed underivatised. Most of the methods used polar column based on polyethylene glycol (Domínguez et al. 2002; López et al. 2002; Martorell et al. 2002; Monje et al. 2002; Mejías et al. 2003; Díez et al. 2004; Carrillo & Tena 2007; Fariña et al. 2007; Pizarro et al. 2007; Pizarro et al. 2011; Pizarro et al. 2012; Zhou et al. 2015), nitroterephthalic acid treated polyethyleneglycol (Marín et al. 2005) and 14% cyanopropyl-phenyl-methylpolysiloxane (Pollnitz et al. 2000), but also the less polar 5% phenyl-methylpolysiloxane stationary phase (Boutou & Chatonnet 2007; Carpinteiro et al. 2010; Carpinteiro et al. 2012; Milheiro et al. 2017) has been used. For the gas-chromatography analysis, the VPs are usually extracted from wine matrix using different approaches: liquid-liquid extraction (LLE) (Chatonnet & Boidron 1988; Pollnitz et al. 2000; Monje et al. 2002; Carrillo & Tena 2007; Milheiro et al. 2017), dispersive liquid-liquid microextraction (DLLME) (Fariña et al. 2007; Pizarro et al. 2011; Carpinteiro et al. 2012), solid-phase extraction (SPE) (López et al. 2002; Carpinteiro et al. 2012), solid-phase microextraction (SPME) (Martorell et al. 2002; Monje et al. 2002; Mejías et al. 2003; Boutou & Chatonnet 2007; Carrillo & Tena 2007) or stir bar sorptive extraction (SBSE) (Díez et al. 2004; Marín et al. 2005; Zhou et al. 2015). Therefore, to clarify their strengths and weaknesses, a comparison between the gas chromatography methods described in the literature for determination of 4-EP, 4-EG and to a lesser extent 4-EC in wines are presented in Table 5.

3.1.1. Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) is among the oldest and most widely used procedure in sample preparation (pre-concentration and matrix isolation) for VPs analysis. It involves two liquid phases: the aqueous phase (containing the analyte) and the organic extractant phase. A step of vigorous mixing is needed to ensure the proper contact between the two phases. LLE allows different compounds to be separated based on their solubilities in the organic solvent used, with a short method development time and low cost (Cantwell & Losier 2002). However, LLE generally requires large amounts of organic solvent and it is difficult to automate (Rezaee et al. 2006).

The liquid-liquid extraction has been used for the direct extraction of 4-EP and 4-EG without derivatisation (Chatonnet & Boidron 1988; Pollnitz et al. 2000; Monje et al. 2002; Rayne & Eggers 2007; Milheiro et al. 2017) and 4-EC (Carrillo & Tena 2007) and after derivatisation for the simultaneous quantification of 4-EP, 4EG and 4-EC (Carrillo & Tena 2007). For the former LLE methods, it was usually involved more than one extraction step: sometimes re-extraction, agitation, and centrifugation (Pollnitz et al. 2000; Milheiro et al. 2017) and in some cases also a concentration step by evaporation under nitrogen gas flow (Chatonnet & Boidron 1988; Monje et al. 2002), being less prone to automation than other extraction techniques. Although, all these steps are usually quickly performed, so less time in sample preparation is needed, LLE methods are also usually cheaper because they only use solvents, mostly pentane: ether (2:1 v/v) (Pollnitz et al. 2000; Milheiro et al. 2017), but

Table 5. Gas chromatography methods for detection and quantification of volatile phenols in red wine.

	Wine vol. (mL)	Range (μg/L)	LOD (μg/L)	LOQ (μg/L)	Repeatability (%)	Recovery (%)	Observations	Advantage/Disadvantage	Reference
Liquid-liquid extraction (LLE)									
IS-FID	100						Extraction: 3x dichloromethane (20, 10 and 5 mL) followed by re-extraction of organic phase 2x with $NaHCO_3$ 5% pH 8.5 (50 mL) and 2x with NaOH 0.5% (50 and 25 mL). After acidification with HCl 20% to pH 1.5, extraction with 2x diethyl-ether (10 and 5 mL) Internal standard:3,4-dimethylphenol (100 mg/L, 1 mL addition) Injection volume 1 μL splitless	Long sample preparation time, use of large volumes of toxic organic solvents. No precision, recovery and LOD or LOQ reported.	Chatonnet and Boidron (1988)
Isotopic IS-MS	5	0–5000 (4-EP) 0–5000 (4-EG)			1.35 (4-EP) 1.31 (4-EG)		Extraction: 2 mL of pentane: diethyl ether (2:1), Internal standard: [$^2H_4$]-ethylphenol (23.4 mg/L, 100 μL) Injection volume: 2 μL in splitless mode	Need to synthetize the internal standard. Short time of sample preparation, use of toxic solvents, Good repeatability, difficult to automation.	Pollnitz et al. (2000)
Isotopic IS-MS	5	0–3000 (4-EP)		0.5 (4-EP)	4 (100 μg/L)		Extraction: 2 mL diethylether+2 g NaCl, mixed during 20s, centrifuged.	Short analysis time. Need to synthetize the two internal standards used. Use of toxic solvents, low LOQ, good repeatability, difficult to automation.	Rayne and Eggers (2007)
		0–666 (4-EG)		0.1 (4-EG)	7–8 (5 μg/L)		Internal standard 4-ethylphenol-d_3 (101.5 mg/L ethanol, 20 μL) 4-ethylguaiacol-d_3 (118 mg/L ethanol, 20 μL) Injection volume: 2 μL in splitless mode		
IS-FID	50	25–10000			15 (4-EP) 12 (4-EG) 2 (4-EP) 5 (4-EG)		Extraction method according to Chatonnet and Boidron (1988)	Wider linear range, lowest repeatatibility	Monje et al. (2002)
IS-MS	10	40–1550 (4-EP)	3 (4-EP)		1 (4-EP)		Derivatisation: 2 mL of K_2CO_3 at 25%, 400 μL of acetic anhydride (2 min agitation).	Allows to obtain good chromatographic peaks of 4-EC. Derivatisation followed by extractions and concentration too time consuming. No automation possible, uses of large volumes of toxic organic solvents. Good repeatability and LOD, nevertheless standard addition method needed for accurate quantification of 4-EP	Carrillo and Tena (2007)
		10–450 (4-EG)	2 (4-EG)		3 (4-EG)		Extraction: 2 $\times$ 10 mL of n-hexane (agitation 2 min), followed by centrifugation and drying over anhydrous sodium sulphate and concentrated to 1 mL at 40°C under a nitrogen stream.		
		15–450 (4-EC)	6 (4-EC)		5 (4-EC)		Internal standard 3,4-dimethylphenol (final of 500 μg/L in wine samples) Injection volume of 1 μL in splitless mode		
IS-MS	500	6.4–6435 (4-EP)	0.71 (4-EP)	2.35 (4-EP)	8.9 (4-EP)	122–156 (4-EP)	Extraction: 3x *n*-pentane (20, 10, 10 mL under magnetic stirring during 10 min); anhydrous sodium sulphate, concentration to 200 μL under nitrogen flow (0.5 mL/min)	Higher wine volume used, low recoveries of 4-EG and high recoveries of 4-EP. Use of toxic solvents, low LOQ and repeatability, difficult to automation.	Lisanti et al. (2010)
		0.49–490 (4-EG)	0.08 (4-EG)	0.27 (4-EG)	7.9 (4-EG)	63–76 (4-EG)	Internal standard 2-octanol (100 mg/L ethanol, 50 μL) Injection volume 1.2 μL in splitless mode		
IS-MS	20	25 - 1000 (4-EP)	7.94 (4-EP)	24.1 (4-EP)	4.72 (4-EP)	100	Extraction: 2 mL pentane: diethyl ether (2:1); NaCl 0.5 g; shaken during 15 min at 400 rpm	Good recoveries, fast and simple method; Use of toxic solvents, low LOQ, good repeatability, difficult to automation	Milheiro et al. (2017)
		10 - 1000 (4-EG)	3.58 (4-EG)	10.8 (4-EG)	18.3 $<$ 25 μg/L (4-EG) 7.46$>$ 25 μg/L (4-EG)		Internal standard: 3,4-dimethylphenol (0.1 mg/L, 2 mL) Injection volume 5 μL in splitless mode		
Ultrasound-assisted emulsification microextraction combined with solidification of floating organic drop (USAEME- SFOD)									
IS-MS/MS	5	1–2000	47.8 (4-EP)	159.3 (4-EP)	9.4 (4-EP)	91.8±5.7 (4-EP)	Extraction: Ultrasonic bath at 40 kHz and 100 W; 5% w/v NaCl, 70°C, 6 min; 425 μL of cyclohexanol; Cooling on an ice bath for solvent solidification.	Fast to perform, low use of less contaminant solvent. High LOQ. Good repeatability and difficult to automation	Pizarro et al. (2012)
			43.1 (4-EG)	143.7 (4-EG)	10.3 (4-EP) 5.0 (4-EP) 9.0 (4-EG) 6.0 (4-EG) 6.1 (4-EG)	91.2 $\pm$ 7.0 (4-EP) 93.0 $\pm$ 7.5 (4-EG) 96.5 $\pm$ 6.5 (4-EG)	Internal standard *p*-cresol (400 mg/L; not supplied) Injection volume not supplied in splitless mode		

Dispersive liquid-liquid microextraction (DLLME)								
IS-MS	5	50–1500	44 (4-EP)	147 (4-EP)	2.7 (4-EP)		Extraction; 1 mL of acetone (disperser solvent) containing 50 μL of CCl_4 (extraction solvent), shaken using 1 mL syringe, followed by centrifugation 5 min at 3000 rpm.	Simple and economical method. Reduced analysis time. Low solvent use. Use of toxic solvents High LOQ. Good repeatability and difficult to automation	Fariña et al. (2007)
			28 (4-EG)	95 (4-EG)	3.8 (4-EG)		Internal standard: 1-heptanol (final concentration 274 μg/L) Injection volume: 2 μL in splitless mode		
IS-MS/ MS	5	1–1800	0.051 (4-EP) 0.068 (4-EG)	0.169 (4-EP)	10.3 (4-EP)	81.4 ± 8.1 (4-EP)	Extraction: Disperser solvent 1.43 mL of acetone (disperser solvent) containing 173 μL of $CHCl_3$ (extraction solvent).	Simple and economical method. Reduced analysis time. Low solvent use. Use of toxic solvents. Low LOQ. Good repeatability and difficult to automation	Pizarro et al. (2011)
				0.169 (4-EG)	9.5 (4-EP) 4.9 (4-EP) 8.9 (4-EG) 11.6 (4-EG) 7.1 (4-EG)	82.0 ± 7.6 (4-EP) 87.0 ± 8.6 (4-EG) 92.5 ± 6.7 (4-EG)	Internal standard: *p*-cresol (400 mg/L; not supplied) Injection volume: not supplied in splitless mode		
Solid phase extraction (SPE)									
IS-FID	10	0.275–22 mg/L (4-EG)	1130 (4-EG)	3770 (4-EG)	6.21 (4-EG)	80.8 (4-EG)	Extraction: SPE sorbent - 200 mg Lichrolut EN resin; Conditioning - 5 mL methanol + 3 mL water; Sample loading - 10 mL of wine+IS; Washing - 0.6 mL water, dried with helium 150 s; Elution - 2.5 mL of dichloromethane	LOQ above the limit of olfactory perception of 4-EP and 4-EG. Use of toxic solvents	Domínguez et al. (2002)
		0.287–22.96 mg/L (4-EP)	480 (4-EP)	1600 (4-EP)	2.92 (4-EP)	87.1 (4-EP)	Internal standard: 3,4-dimethylphenol (100 μL, 100 mg/L) Injection volume: 1 μL in split mode 1:30 ratio		
IS-MS	50	9–588 μg/L (EP)	0.54 (4-EP)		1.3 (4-EP)	106 (4-EP)	Extraction: SPE sorbent - 200 mg Lichrolut EN resin; Conditioning - 4 mL dichlorometane, 4 mL methanol and 4 mL of ethanol:water (12% v/v). Sample loading - 50 mL of wine with 25 μL of BHA (10 mg/g of ethanol) at 2mL/min and dried at −0.6 bar 10 min; Elution - 1.3 mL of dichloromethane	Good LOQ below limit of olfactory perception of 4-EP and 4-EG. Use of toxic solvents. Good precision and recovery.	López et al. (2002)
		11–290 μg/L (4-EG)	0.035 (4-EG)		2.6 (4-EG)	107 (4-EG)	Internal standards: 4-hydroxy-4-methyl-2-pentanone and 2-octanol (300 μg/g of dichloromethane, 25 μL added to the elution solution). Injection volume: 3 μL in a temperature programmed vaporizer from 40°C during 5 min raised until 230°C at 2°C/min.		
Solid phase extraction/ Dispersive liquid-liquid microextraction (SPE/ DLLME)									
IS-MS	5	1–5		0.0004 (4-EP)	2.2 (4-EP)	92–108 (4-EP)	1st step SPE - SPE sorbent - OASIS MAX 60 mg;. Conditioning - 2 mL methanol + 2 mL water (pH adjusted to pH 3.5); Sample loading - Wine diluted with the same amount of water, drying under nitrogen stream. Elution - 1 mL acetone. Derivatisation: 8 mL of 5% (w/v) K_2HPO_4, 0.05 mL of acetic anhydride, shaken 1 min.	Acetylation of 4-EC allows to obtain good chromatographic peaks. Too many steps. Use of toxic solvents. Very low LOQ. Good repeatability. Difficult to automation	Carpinteiro et al. (2012)
				0.0003 (4-EG)	4.1 (4-EP)	89–106 (4-EG)	2nd Step: DLLE - 0.06 mL of 1,1,1 trichloroethane in 1.5 mL of solvent acetone, centrifuges at 3000 rpm, 3 min		
				0.0008 (4-EC)	7.8 (4-EG)	89–98 (4-EC)	Internal standard: 3,4-dimethylphenol (1000 μg/mL in ethanol)		
					3.7 (4-EG) 2.0 (4-EC) 6.8 (4-EC)		Injection volume: 2 μL in splitless mode		

(*Continued on next page*)

Table 5. (*Continued*)

	Wine vol. (mL)	Range (μg/L)	LOD (μg/L)	LOQ (μg/L)	Repeatability (%)	Recovery (%)	Observations	Advantage/Disadvantage	Reference
Headspace solid phase microextraction (HS-SPME)									
IS-FID	25	200–1800 (4-EP)	2 (4-EP)	5	10		50 mL vial, magnetic stirring, NaCl (final concentration 6M), 15 min at 25°C, 300 rpm (pre-conditioning), 60 min (extraction); PDMS fibre	Simple and fast methodology, good linearity, repeatability and LOD. Matrix interferences were observed and the standard addition method should be used	Martorell et al. (2002)
		40–400 (4-EG)	1 (4-EG)				Calibration: wine model solution (3.5 g tartaric acid, 120 mL ethanol, water to 1L, pH adjust to 3.5 with NaOH 1M) Internal standard: *p*-cresol (100 mg/L in ethanol)		
IS-FID	2	5–5000			6.0 (4-EP)		5 mL vial, 1 g NaCl, magnetic stirring, 55°C during 40 min (extraction); polyacrylarte fibre;	Solventless, easy of automation. Matrix effect possible. Good repeatability	Monje et al. (2002)
					5.0 (4-EG)		Calibration: wine model solution (11% (v/v) ethanol, 6 g/L glycerine, 2.5 g/L tartaric acid; 3g/L lactic acid; 1g/L K_3PO_4, pH = 3.0)		
					3 (4-EP) 2 (4-EG)		Internal standard: 3,4 dimethylphenol (1000 mg/L in model wine solution, 5 mg/L final concentration)		
IS-FID	12	15–3011 (4-EG)	18 (4-EG)	80 (4-EG)	4–16	112–109.3 (4-EG)	50 mL vials, 2.81 g NaCl, 5 min 60°C (pre-conditioning), 50 min of fibre exposure with magnetic stirring (extraction); CW/DVB filbre	Solventless, easy of automation. Matrix effect possible. Good quantification limit. Good repeatability	Mejías et al. (2003)
		17–3041 (4-EP)	19 (4-EP)	81 (4-EG)		82.1–91.6 (4-EP)	Calibration: wine model (15% (v/v) ethanol 3 g/L tartaric acid) Internal standard: 3,4-dimetilphenol (1.53 g/L, 14 μL)		
Isotopic IS-MS	5	11.5–1100 (4-EP)		25.1 (4-EP)	3.0 (4-EP)		20 mL vial, Sample pretreatment: 15 mL of wine pH adjusted to 7. 5 mL of sample is diluted with 5 mL of water, 3.0 g NaCl, 3 min at 45°C at 250 rpm (pre-conditioning), 60 min at 45°C, 250 rpm (extraction).; DVB/CAR/PDMS fibre	Solventless, easy of automation. Low Good quantification limit. Good repeatability	Boutou and Chatonnet (2007)
		3.8–260 (4-EG)		9.1 (4-EG)	3.9 (4-EG)		Calibration: wine model (6% (v/v) ethanol, 5g/L of tartaric acid adjusted to pH 7 with 0.5 M of NaOH) Internal standard: $^2H_{10}$E4P (1 g/L,10 μL)		
IS-MS	4	51–2125 (4-EP)	17 (4-EP)	30 (4-EP)	3.5 (4-EP)	98–105 (4-EP)	20 mL vial, 1 mL of 5.5% K_2CO_3, 140 μL of anhydride acetic, 0.9 g NaCl (70°C, 1 min) (derivatisation). SPME extraction 70°C, 70 min; DVB/CAR/PDMS fibre	Acetylation of 4-EC allows to obtain good chromatographic peaks. A matrix effect was observed for 4-EG; Solventless, easy of automation. Good quantification limit. Good repeatability	Carrillo and Tena (2007)
		38–554 (4-EG)	2 (4-EG)	3 (4-EG)	1.0 (4-EG)	99–100 (4-EG)	Calibration: wine model (6g/L tartaric acid, 12% (v/v) ethanol adjusted to pH 3.5)		
		12–986 (4-EC)	4 (4-EC)	6 (4-EC)	1.7 (4-EC)	97–101 (4-EC)	Internal standard: 3,4- dimethylphenol (final concentration 500 μg/L)		
IS-FID	10		3 (4-EP)	40 (4-EP)	4.76–6.10 (4-EP)	88.73 −93.50 (4-EP)	15 mL vials, 2.5 g NaCl, 10 min 55°C (pre-conditioning) magnetic stirring 800 rpm, 55°C 45 min (extraction) DVB/CAR/PDMS	Solventless, easy of automation. Minimal matrix effect. Good quantification limit. Good repeatability	Sun et al. (2016)
			12 (4-EG)	10 (4-EG)	3.11–3.94 (4-EG)	93.51–107.30 (4-EG)	Calibration: methanol Internal standard *p*-cresol (100 μL, 1000 mg/L)		
MHS MS-MS	5	LOD-1800 (4-EP)	0.05 (4-EP)	0.18 (4-EP)	10.52 (4-EP)	96.39 ± 8.150 (4-EP)	20 mL vials, 70°C 10 min (pre-conditioning), 70°C 60 min (extraction), 250 rpm agitation tray (extraction). No salt addition. DVB/CAR/PDMS fibre	MHS-SPME could be an alternative to the standard addition method for avoiding matrix effect.	Pizarro et al. (2007)
		LOD-1000 (4-EG)	0.06 (4-EG)	0.20 (4-EG)	5.54 (4-EP)	99.71 ± 5.85 (4-EG)	Calibration: wine model solution (tartaric acid 5g/L, 13% (v/v) ethanol, pH adjusted to 3.5).		
					7.55 (4-EP) 5.69 (4-EG) 8.57 (4-EG) 3.07 (4-EG)		No internal standard		

Stir bar sorptive extraction (SBSE)									
IS-MS	25	50–1080 (4-EP)	6 (4-EP)	21 (4-EP)	3.77 (4-EP)	101 (4-EP)	Wine sample dilute with water 1:4, 15 mL of dilute wine were analysed; PDMS coated stir bar (60 min, 900 rpm); Thermal desorption with PTV (20° C to 280 °C at 60°C/min, 280°C 5 min); Cryofocusing at −100°C (cryogenic nitrogen)	Easy, quantification limit of 4-EG above its olfactory detection threshold. Thermal desporption needs cryofocusing.	Díez et al. (2004)
		570–5790 (4-EG)	159 (4-EG)	529 (4-EG)	4.67 (4-EG)	97 (4-EG)	Internal standard: 3,4-dimethylphenol (125 μL, 1000 mg/L)		
IS-MS	25	25–1000	0.00012 (4-EG)	0.00013 (4-EG)	0.82 (4-EG)	>98	0.5 mm film thickness, 10 mm length PDMS coated stir bar (90 min, 700 rpm, room temperature); Thermal desorption 290°C/4 min, cold trap at −30°C	Easy to perform, LOD and LOQ below its olfactory detection threshold. Thermal desorption needs cold-trap	Marín et al. (2005)
			21 (4-EP)	20.99 (4-EP)	0.093 (4-EP)		Internal standard: γ-hexalactone (1 mg/L in ethanol, 250 μL)		
IS-MS	4	4.92–492 (4-EP)	0.10 (4-EP)	0.25 (4-EP)	2.5 (4-EP)	96.4 (4-EP)	Wine sample dilute with 16 mL phosphate buffer 1 M pH = 7; 0.5 mm film thickness 10 mm length EG/PDMS coated stir bar (180 min, 1000 rpm, room temperature); Thermal desorption in TDU (30°C to 220°C rate 120°C/min, 220°C, 3 min), re-cryofucosed in a TPV with a Tenax 60/80 packed liner at −80°C with liquid nitrogen.	Easy to perform, good LOD and LOQ below its olfactory detection threshold. Thermal desorption needs cryofocusing	Zhou et al. (2015)
		4.73–473 (4-EG)	0.24 (4-EG)	0.47 (4-EG)	2.9 (4-EG)	97.6 (4-EG)	Internal standard: 3,4-dimethylphenol (59.6 mg/L in ethanol, 20 μL)		
Disposable silicon sorbents (DSS)									
ES-MS	2	LOQ-5		5 (4-EP)	3.8 (4-EP)	105 - 108 (4-EP)	Silicon sorbent disc, 5 mm of diameter and 0.5 mm thickness; Desorption with 0.2 mL of ethyl acetate/ 15 min at room temperature; Derivatisation: diluted in 15 mL of aqueous solution of potassium bicarbonate 5% (w/v) with 2 g of sodium chloride, 90 μL of acetic anhydride; Extraction 2 h at room temperature	Allows the determination of 4-EC. Inexpensive silicon discs, low cost method. LOQ below the olfactory detection threshold. Uses low amounts toxic organic solvents. Avoids cross-contamination problems	Carpinteiro et al. (2010)
				5 (4-EG)	6.0 (4-EP)	92 - 116 (4-EG)	Internal standard: 3,4-dimethylphenol (1 mg/mL in methanol)		
				15 (4-EC)	6.6 (4-EC) 4.2 (4-EG) 5.3 (4-EG) 7.5 (4-EC)	91 - 112 (4-EC)	Splitless injection		
e-nose-HS									
PLS	5						Head space sampler; No chromatographic column	Good calibration results; Poor validation results	Cynkar et al. (2007)

LOD - limit of detection; LOQ - limit of quantification; IS - internal standard; ES - external standard; PLS - partial least squares; MS - mass spectrometry; FID - flame ionisation detector; 4-EP - 4-ethylphenol; 4-EG - 4-ethylguaiacol; 4-EC - 4-ethylcatechol.

Table 6. Liquid chromatography methods for detection and quantification of volatile phenols in red wine.

	Wine vol. (mL)	Range (μg/L)	LOD (μg/L)	LOQ (μg/L)	Repeatability (%)	Recovery (%)	Observations	Advantages/Disadvantages	
Direct Injection									
CoulArray	2	50–2000 (4-EP and 4-EG)	2.59 (4-EP)	8.6 (4-EP)	<3		Injection volume: not supplied	Fast, precise, accurate, sensitive, without sample preparation besides filtration	Larcher et al. (2007)
		1–1520 (4-EC)	3.13 (4-EG) 0.3 (4-EC)	10.4 (4-EG) 1.1 (4-EC)	<10		Column: LiChroCART RP-C18 Purospher (125 mm×3 mm× 5 μm) Temperature: 25°C, Flow 0.6 mL/min Isocratic: 50 mM NaH_2PO_4 pH 3.40 with phosphoric acid:ACN: MeOH 65:30:5		Larcher et al. (2008)
FLD	2	50–2000	4.0 (4-EP)	8 (4-EP)	<10		Injection volume: 10 μL Column Zorbax Eclipse Plus C-18 (4.6 mm×50 mm×1.8 μm) Temperature 25°C, flow rate 1.5 mL/min Isocratic: 50 mM NaH_2PO_4 pH 3.40 with phosphoric acid:ACN:MeOH 65:30:5	Rapid method (5 min analysis), no sample preparation besides filtration, Results comparable to CoulArray	Nicolini et al. (2007)
ESI - MS-MS		10–5000	10	50	0.93–4.37		Phenomenex Synergy MAX-RP (4.6 mm, 150 mm, 4 μm) Flow rate 0.3 mL/min, injection volume 10 μL Methanol/ H_2O (70:30) to 82:18 in 12 min, plus 3 min	Uses large amount of toxic solvents	Caboni et al. (2007)
IS - DAD		10–5000	10	50	0.3–5.4		Water Spherisorb ODS-2 250 mm×4.6 mm, 5 μm. Temperature 20°C ACN/ aqueous orthophosphoric acid 0.1% (10:90) to (90:10) in 25 min. Reconditionated 10 min; injection volume 100μL; Flow rate 1 mL/min	Uses large amount of toxic solvents	Caboni et al. (2007)
FLD		10–10000	1 (4-EP)	5 (4-EP)	6.88 (4-EP)		Water Spherisorb ODS-2 250 mm×4.6 mm, 5 μm. Temperature 20°C	Uses large amount of toxic solvents	Caboni et al. (2007)
			10 (4-EG)	50 (4-EG)	0.74 (4-EG)		ACN/ aqueous orthophosphoric acid 0.1% (10:90) to (90:10) in 25 min. Reconditionated 10 min; injection volume 100μL; Flow rate 1 mL/min		
ES - UV-FLD		20–2000	16 (4-EP)				Flow rate 1.5 mL/min	Uses large amount of toxic solvents;	Nikfardjam et al. (2009)
			7 (4-EG)				LiChroCART 250–4 LiChrospher 100 RP 18 of 5 μm; Temperature 40°C, injection volume 10 μL. Isocratic: 50 mM NaH_2PO_4 pH 3.40 with phosphoric acid:ACN:MeOH 65:30:5		
Quick Easy Cheap Effective Rugged Safe (QuEChERS)									
ES - UV-FLD	5	20–2200	1 (4-EP)	48 (4-EP)	6.2 (4-EP)	92 ± 6 (4-EP)	Temperature: room temperature; Eurospher 100–5C18(250 mm, 4 mm, 5 μm)	More time consuming	Valente et al. (2013)
			3 (4-EG)	92 (4-EG)	4.3 (4-EG)	108 ± 9 (4-EG)	Acetonitrile/ Acetate buffer 10 mM pH 4.7 (30:70) to 35:65 in 25 min; 50:50 in more 5 min. Recondioning in 5 min. Flow 0.7 mL/min, Injection volume 20 μL		
MWD	5	LOQ-5000	9 (4-EP)	31 (4-EP)	7–13%	100–111 (4-EP)	Filter-vial dispersive solid phase extraction (100 mg CaCl2 and 25 mg PSA)50 μL NaOH (12.5 M)/5 mL wine/ACN/in-vial d-SPE	More time consuming Matrix Effects observed and Matrix-mateched calibration used	Fontana and Bottini (2017)
			12 (4-EG)	40 (4-EG)		74–116 (4-EG)	Accucore C-18 (2.1 mm×100 mm×2.6 μm) 0.05 M H_2PO_4 pH 3.15/ MeCN (80:20 to 50:50 in 6 min; Reconditioning 3.5 min); flow 0.7 mL/min, Column Temperature 35°C, injection volume 20 μL.		

Solid phase extraction - molecularly imprinted polymers (SPE-MIP)									
DAD	5	10–10000	2.63 (4-EP)	8.77 (4-EP)	5.3 (100 μg/L (4-EP))	95.2–104.6	MIP-SPE sample preparation: 500 mg in 6 mL tubes; preconditioning 5 mL of methanol + 5 mL water 12% (v/v) ethanol. 5 mL of wine; Washed 5 mL 12% (v/v) ethanol solution; Sorbent dried under vacuum, 15 min, Elution 4 mL de dichloromethane for cleaning elution 5 mL Acetonitrile: methanol (8:2)	Sample preparation time consuming. Class selective sorbent; with minimal matrix effect.	Garcia et al. (2015)
					1.9 (500 μg/L (4-EP))		Molecular imprinted polymer packed in 250 mm×4.6 mm.		
							ACN/ methanol 100:0 during 5 min; 98:2 over 15 min; 90:10, 30 min. Flow rate 1 mL/min		

LOD - limit of detection; LOQ - limit of quantification; IS - internal standard; ES - external standard; FLD - fluorescence detector; MS - mass spectrometry; DAD - diode array detector; 4-EP - 4-ethylphenol; 4-EG - 4-ethylguaiacol; 4-EC - 4-ethylcatechol; ACN - acetonitrile; MeOH - methanol.

also pentane alone (Lisanti et al. 2010) and dichloromethane (Chatonnet & Boidron 1988; Monje et al. 2002) have been used. In this last case, the extraction procedure was followed by an alkaline solution re-extraction and again an extraction with diethyl ether after acidification to pH 1 and concentration of the extracts by a stream of nitrogen. Using pentane as extraction solvent, three consecutive extractions were used and the extraction solution containing the VPs needed to be concentrated under a stream of nitrogen before analysis (Lisanti et al. 2010). When pentane:ether was used as extraction solvent, only an extraction procedure step was performed and as small volumes were applied for extraction (2 mL) the extracts were analysed directly without the need for concentration (Pollnitz et al. 2000; Milheiro et al. 2017). When the analytes are derivatised by acetylation the extraction involves the use of hexane twice (Carrillo & Tena 2007) and concentration of the extracts by a stream of nitrogen. All methods showed good repeatability for 4-EP and for 4-EG, although for lower concentrations the precision, as expected, was worst (Table 5). In general, the developed methods showed an acceptable linear range and only three methods reported the limit of detection (Carrillo & Tena 2007; Lisanti et al. 2010; Milheiro et al. 2017) that was well below the most accepted olfactory detection threshold reported for these compounds (Chattonet et al. 1992). Only two methods reported the quantification limits and recoveries (Lisanti et al. 2010; Milheiro et al. 2017).

3.1.2. Liquid-phase microextraction (LPME)

To overcome the known limitations of liquid-liquid extraction, recently the miniaturisation of this extraction method has been performed with success and termed liquid-phase microextraction (LPME) or solvent microextraction (SME). This approach is based on the analyte partitioning between a drop of organic extracting phase and the sample matrix of aqueous nature (Sarafraz-Yazd & Amiri 2010). Recently different configurations of this technique have emerged including dispersive liquid-liquid microextraction (DLLME) and ultrasound assisted emulsification (USAE) among many others. The advantages of these techniques are simple experimental setup, short analysis time and minimum use of solvents. Nevertheless, they also present some disadvantages as instability of microdrop and relative low precision (Regueiro et al. 2008).

3.1.2.1. Dispersive liquid-liquid microextraction (DLLME). In this technique, the appropriate mixture of extraction solvent (high-density, non-polar and water-immiscible) and disperser solvent (polar and water-miscible) are injected rapidly into the aqueous sample. Only a short contact time is needed and then the dispersed phase can be separated by centrifugation and the extracted target analytes can be determined by conventional analytical techniques (Rezaee et al. 2006; Zgoła-Grześkowiak & Grześkowiak 2011; Fontana 2012). DLLME has great pre-concentration capabilities in a short time with little solvent utilisation, being a simple, low cost and environmental friendly technique (Rezaee et al. 2006; Zgoła-Grześkowiak & Grześkowiak 2011).

Two methods have been developed and validated for their application in wine, both using acetone as disperser solvent and one using chloroform (Pizarro et al. 2011) and the other using carbon tetrachloride (Fariña et al. 2007) as extraction solvents. Additionally, a mixed-mode solid-phase extraction (SPE) followed by dispersive liquid-liquid microextraction (DLLME) method has been developed (Carpinteiro et al. 2012) and used also acetone as disperser solvent and 1,1,1-trichloroethane as extraction solvent. All methods showed good repeatability for 4-EP, 4-EG and 4-EC, although again for lower concentrations the precisions were worst (Table 5). The methods showed an acceptable linear range and quantification limits, although the methods of Pizarro et al. (2011) and Carpinteiro et al. (2012) presented lower limits than that obtained for the LLE methods described. Also for the recovery, only one method (Pizarro et al. 2011) presented these values that were inside the acceptable range although presenting lower recoveries than that reported for the LLE methods (Table 5).

3.1.2.2. Ultrasound-assisted emulsification microextraction solidification floating organic drop (USAEME-SFOD). USAEME-SFOD was first introduced by Kamarei et al. (2011) and this technique brings together the advantages of ultrasound-assisted emulsification microextraction (USAEME) and solidification of floating organic drop (SFOD). The application of ultrasound radiation in an emulsion of immiscible solvents (USAEME) results in a high extraction efficiency in a short time. In a heterogeneous system of two immiscible phases the ultrasound radiation results in the fragmentation of one of the phases to form emulsions with submicron droplet size (Regueiro et al. 2008) that enormously increases the contact surface between the phases and the inverse effect (coalescence) occurring in certain conditions (Antonov et al. 2006).

On the other hand, solidification of floating organic drop (SFOD) involves the use of solvents with near room temperature melting points facilitating the collection of the extract by its solidification in an ice bath (Pizarro et al. 2012). While USAEME usually involves the use of toxic chlorinated solvents (Guo & Lee 2012), SFOD uses less contaminant solvents (greener extractants) being less toxic (Pizarro et al. 2012) and USAEME-SFOD brings together the advantages of both methods, very efficient extraction due to the high superficial area between aqueous and organic phases and the use of low volumes of nontoxic solvents (Pérez-Outeiral et al. 2015). Pizzaro et al. (2012) used USAEME-SFOD for the extraction of VPs from red wine, using ciclohexanol as the extraction solvent. The detection limits obtained were generally higher than those found for the LLE, and the quantification limit for the 4-EG was above the limit of olfactory detection threshold of this VP. The main advantage of this method seems to be the easier collection of the organic phase.

3.1.3. Solid phase extraction (SPE)

Solid-phase extraction (SPE) is a well-established method, in which a liquid sample is passed through a specific sorbent where the target analytes are retained. Then, the analytes are recovered by elution with a suitable solvent. SPE allows to perform in a single step the concentration and sample clean-up, presenting very good effectiveness and selectivity in a variety of matrixes (Poole 2003). SPE generally uses less solvent than LLE but it is more expensive due to the adsorbent and cartridges costs (Rezaee et al. 2006). However, SPE techniques have some problems associated to method development (Hennion 1999)

and the sorption properties of solid phases are not as reproducible as solvent properties (Poole 2003).

Two methods based on SPE sample clean up and concentration using polystirene-divinylbenzene adsorbent have been described by Domínguez et al. (2002) and Lopéz et al. (2002). The use of MS detector, when compared to FID detector, allowed a significant reduction in the detection limit of the method (López et al. 2002). The detection limit presented by the method of Domínguez et al. (2002) is above the olfactory detection threshold in red wine (Chatonnet et al. 1992). Both methods showed good precision, and the use of a higher wine volume increased the recovery (Table 5).

3.1.4. Solid phase microextraction (SPME)

SPME is a well-established extraction technique that eliminates the use of organic solvents. SPME sample preparation procedure consists in two steps: after exposure of the fibre to became in contact with the sample, either headspace or direct immersion into the solution, the analytes are absorbed or adsorbed by the fibre coating phase (depending on the nature of the coating) and in the second step, the analytes are thermally desorbed in the GC injection port for separation and quantitation (Zhang et al. 1994). It has several advantages over other techniques: it is solvent free and can be easily automated. Nevertheless, as the extraction is in general not exhaustive, SPME has in most cases higher detection limits than SPE (Prosen & Zupančič-Kralj 1999), also observed for the VPs (Table 5). One of the main disadvantage described for this method are the matrix effects in complex samples, nevertheless quantitation can be performed with different calibration procedures in order to eliminate the matrix interference like standard addition (Carrillo & Tena 2007; Martorell et al. 2002; Mejías et al. 2003), external standard method with matrix match standards (Carpinteiro et al. 2010; Carpinteiro et al. 2012; Sun et al. 2016), internal standard (Chatonnet & Boidron 1988; Domínguez et al. 2002; López et al. 2002; Martorell et al. 2002; Monje et al. 2002; Mejías et al. 2003; Díez et al. 2004; Carrillo & Tena 2007; Fariña et al. 2007; Pizarro et al. 2011; Carpinteiro et al. 2012; Pizarro et al. 2012; Zhou et al. 2015; Sun et al. 2016; Milheiro et al. 2017) or isotopic dilution analysis (Boutou & Chatonnet 2007; Rayne & Eggers 2007; Pollnitz et al. 2000). However in some cases matrix effects can't be removed completely. To avoid some of these matrix effects in quantitative analysis, a modification of SPME was developed and applied for VPs analysis: multiple headspace solid phase microextraction (MHS-SPME) (Pizarro et al. 2007). MHS-SPME implies consecutive extractions (3 or 4) from the same sample and the extrapolation of the decreasing analyte areas obtained to an infinite number of extractions. MHS-SPME can be carried out under non-equilibrium and under equilibrium conditions. The advantage of this technique is the elimination of matrix effects in complex samples (Carrillo & Tena 2007; Pizarro et al. 2007) due to analyte depletion from the sample (Kolb 1982). Some disadvantages associated to this technique are the increase of the analysis time, a shorter linear range and lack of depletion linearity comparatively to one-step SPME (Tena & Carrillo 2007). Other general limitations of SPME for sample preparation include cross-contamination problems between samples and the high cost and fragility of fibres (Carpinteiro et al. 2010). Also, there are several and important parameters in sample preparation that can affect the SPME performance that need prior optimisation such as salt concentration, stirring conditions, sample volume, the type of fibre coating, extraction temperature and time (Mejías et al. 2003; Pizarro et al. 2007). Many authors performed the optimisation of the SPME extraction of VPs in wines. Almost all authors selected DVB/CAR/PDMS fibre coating due to its efficient extraction (Boutou & Chatonnet 2007; Carrillo & Tena 2007; Pizarro et al. 2007; Sun et al. 2016), although Mejías et al. (2003) selected CW-DVB fibre coating, Martorell et al. (2002) performed the extractions with a PDMS fibre coating and Monje et al. (2002) selected a PA fibre coating. The time and the temperature of extraction differ (Table 5). Pizarro et al. (2007), Carrillo and Tena (2007), Sun et al. (2016) and Mejías et al. (2003) and Sun et al. (2016) showed lower than optimal recovery values for 4-EP (Table 5). Pizarro et al. (2007) performed MHS-SPME coupled to GC-MS-MS and presented the best results in the LODs and LOQs for both compounds, nevertheless the analysis time was increased in comparison to the other methods. However, all methods, when determined, presented LOD and LOQ below the most accepted olfactory detection threshold for the two compounds (Chatonnet et al. 1992).

The higher weakness of HS-SPME, the matrix effect, on the other hand has been used advantageously to measure the headspace abundance of wine aroma components (Petrozziello et al. 2014; Milheiro et al. 2017). Petroziello et al. (2014) used HS-SPME with a short sampling time (<1 min) for determination of the "true headspace" concentration of VPs at equilibrium between headspace and water (Roberts et al. 2000). HS-SPME abundance of volatile compounds is influenced by the partition coefficient of the analyte between the headspace and the matrix as well as between the fibre coating and the headspace, the use of short exposure times minimizes the disruption caused by the fibre coating/headspace partition, reflecting the volatile compounds in the air space at equilibrium between the headspace and sample solution (Jung & Ebeler 2003). On the other hand Milheiro et al. (2017) used higher extraction times with agitation at room temperature, simulating the tasting conditions, to measure the effect of fining agents in the 4-EP and 4-EG headspace abundance.

3.1.5. Mass spectrometry electronic nose (MS e_nose)

Cynkar et al. (2007) performed a feasibility study on the use of electron impact mass spectrometry (m/z 50–180) for the analysis of wine headspace directly without previous chromatographic separation and quantification of 4-EP by multivariate partial least squares regression (PLS). One claimed benefit of this method is its ability to analyse the entire vapour phase rather than analysing the individual components of the wine matrix. Despite some misclassified results of some of the wines analysed, this qualitative method might be used as a screening tool by the wineries, and it offers the possibility of reducing the amount of wines to be tested by either sensory or by GC-MS analysis, resulting in reduced cost of analysis (excluding the equipment) and higher throughput of samples under commercial conditions. Although promising results have been obtained it requires further development with considerably more commercial samples of different varieties before its

full potential can be assessed prior to adoption by the wine industry.

3.1.6. Sorptive extraction

Stir bar sorptive extraction (SBSE) is a solventless sample preparation technique where the analytes are extracted from an aqueous matrix into a magnetic stir-bar coated with a polymeric phase. After extraction, the desorption of the analyte(s) can be performed by thermal desorption (TD) or liquid desorption (LD). In comparison to SPME, SBSE has better sensitivity (Fontana 2012; David & Sandra 2007). The main advantage of this method is its simplicity and possibility of automation, but the cost of instrumentation is high. Díez et al. (2004) used a PDMS coated stir-bar and a dilution of the wine by a factor of 1:4, to reduce matrix interference, and obtained a LOD above the olfactory detection threshold of 4-EG. Nevertheless, Marín et al. (2005) using a PDMS coated stir bar without dilution and obtained the lower reported LOD for 4-EG, in the ng/L range, but could not decrease the LOD for 4-EP below the values observed in the method of Díez et al. (2004). Zhou et al. (2015) used an ethylene glycol PDMS coated stir-bar and after dilution of the wine sample by a factor of 1:5 obtained low LOD for both compounds, especially for 4-EP that is more polar than 4-EG. For all SBSE methods used in wine for VPs analysis, the recovery values were within the acceptable range and with good repeatability.

The risk of cross-contamination in SBSE sample preparation and its high cost lead to the evaluation of disposable silicon polymers as sorptive agents (Carpinteiro et al. 2010). Also, for simultaneous determination of all VPs including 4-EC by gas-chromatography an *in sample* derivatisation with acetic anhydride was developed. After extraction of wine, desorption was performed with a small volume (0.2 mL) of ethylacetate and directly analysed by GC-MS. The recoveries obtained by using disposable silicon polymers were identical to those obtained by SBSE. The linear range was higher, the precision was similar and quantification limits were higher than the sorptive techniques based on the traditional SBSE technique, except for the method developed by Diez et al. (2004), although well below the olfactory detection threshold.

3.2. Liquid chromatography

The number of methods developed for quantification of 4-EP and 4-EG in wines employing liquid chromatography are considerably lower when compared to gas-chromatography, nevertheless due to the lower volatility and higher polarity of 4-EC, liquid chromatography seems to be the preferable method. Reverse phase columns (C-18) have been successfully used for separation of VPs from the other phenolic present in wine, either by isocratic elution (Larcher et al. 2007; Nicolini et al. 2007; Nikfardjam et al. 2009), or gradient elution (Caboni et al. 2007; Valente et al. 2013; Garcia et al. 2015; Fontana & Bottini 2017) (Table 6). Various detection methods have been used: UV diode-array detector (Caboni et al. 2007; Garcia et al. 2015), fluorescence (Caboni et al. 2007; Nikfardjam et al. 2009; Valente et al. 2013; Garcia et al. 2015), tandem mass spectrometry (Caboni et al. 2007), and coulometric array detectors (Larcher et al. 2007). All detection methods allowed obtaining similar LOD and LOQ, below the olfactory detection threshold, although a 10-fold increase in injection volume was used in the direct injection when DAD was used for detection by Caboni et al. (2007).

3.2.1. Direct injection

The major advantage of HPLC analysis is the fact that samples can be directly analysed without any sample pre-treatment and pre-concentration, besides filtration, which reduces considerably the analysis time and cost of these analysis without reported matrix effects (Caboni et al. 2007; Larcher et al. 2007; Nicolini et al. 2007). Only one method was applied for the simultaneous determination of the three VPs (Larcher et al. 2008). Nevertheless, a long term degradation of the analytical performance of the column has been observed (Fontana & Bottini 2013; Valente et al. 2013).

3.2.2. Quick Easy Cheap Effective Rugged and Safe (QuEChERS)

A QuEChERS approach has been applied for red wine pretreatment for preventing the long-term column degradation in the analysis of VPs by HPLC (Fontana & Bottini 2013; Valente et al. 2013). QuEChERS is a fast and simple procedure that has been used for the determination of several analytes of different nature in foods (Lesueur et al. 2008; Stubbings & Bigwood 2009). This technique involves a single-phase solvent extraction with polar organic solvents, most usually acetonitrile, and a phase separation after salting out with the addition of a salt mixture ($NaCl + Na_2SO_4$) and centrifuging the sample. For extract clean-up, dispersive solid-phase extraction (dSPE) is used, by addition of sorbent material (commonly primary secondary amine sorbent, PSA) into the extract followed by centrifugation (Valente et al. 2013). The dSPE approach for extract clean up avoids the use of SPE columns, therefore low amounts of sorbent and solvent are used. Extraction solvent volume employed in QuEChERS is lower in comparison to traditional LLE methods employed for VPs analysis (Chatonnet & Boidron 1988; Monje et al. 2002; Hesford & Schneider 2004), with exception of three (Pollnitz et al. 2000; Rayne & Eggers 2007; Milheiro et al. 2017). These last methods used smaller volumes of organic solvents, with one extraction step, and no need of a sample concentration step. Fontana and Bottini (2017) developed a modified QuEChERS method based on a combination of in-vial filtration with d-SPE as a convenient sample clean-up step for QuEChERS. The main disadvantage of the QuEChERS approach for sample clean-up and concentration is the difficulty in automation (Payá et al. 2007).

3.2.3. Solid phase extraction (SPE) - molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers (MIPs) are synthetic polymers that are polymerised in the presence of a target molecule (template), allowing the formation of recognition sites in their structure with ability to specifically interact with the analyte. When MIPs are added to a sample containing the target analyte, the MIP specifically binds the analyte, and then the MIP/ target molecule complex can be removed from the sample (Garde-Cerdán et al. 2008) and afterwards the analyte can be desorbed from the polymer. This MIP-molecule interaction can be via

hydrogen bonding, covalent or ionic, however the two last forms of interaction are normally less employed (Belbruno & Kelm 2016). This approach has been recently applied with success as an SPE sorbent for extraction of 4-EP and related precursors (4-vinylphenol, coumaric acid and coumaric acid ethyl ester) in red wine and as column stationary phase for their separation before quantitation showing good selectivity with minimal matrix effects (Garcia et al. 2015).

4. Conclusions

This review discusses the formation and impact of volatile phenols on wine quality and consumer acceptance. Also the preventive and remedial approaches to mitigate this sensory defect are reviewed as well as the available analytical methods for the quantification of volatile phenols in wines. The authorised methods by the OIV for the prevention of *Dekkera/Brettanomyces* wine spoilage with the formation of 4-EP, 4-EG and 4-EC, namely the use of sulphur dioxide, chitosan and DMDC, does not seem to completely resolve this problem as there are still wines on the market with this sensory defect. Therefore, some preventive alternatives have been studied, including non-thermal technologies like high pressure processing, pulsed electric field, low electric current and ultraviolet C light, that have the advantage of not changing the wine sensory profiles, a problem associated with the thermal technologies like flash pasteurisation. Meanwhile some remedial treatments have also been tested to remove these volatile phenols from wines in order to remove this sensory defect. Of the additives allowed to use in oenology activated carbons have shown the highest efficiency in the removal of VPs, although the careful selection of the physical chemical characteristics of activated carbons are required to not impact negatively on the wine sensory quality. Also the use of chitosans with high deacetylation degree, although not removing VPs from wine, decrease their headspace abundance and the wine negative phenolic attribute. Further studies are needed to develop more efficient preventive processes for avoiding the spoilage of wines by *Dekkera/Brettanomyces* and while this is not efficiently accomplished remedial treatments for removing these VPs should be explored in order to decrease the impact of these treatments on the wine sensory quality and promote their application in the wine industry. While the risk of wine contamination with *Dekkera/Brettanomyces* and the formation of volatile phenols is not completely eliminated, the control of VPs in wines is of upmost importance in order to avoid the commercialisation of wines with this defect. At present, there are a variety of methods available for volatile phenols analysis nevertheless not all have been subjected to detailed validation studies. However, when available, they show adequate detection limits, recoveries and precision (repeatability). Although most of the techniques reviewed are well established and can be successfully applied for the analysis of 4-EP, 4-EG and 4-EC at concentrations well below their olfactory detection threshold, the time spent with the analysis, the cost and the simplicity of the method are important issues that must be considered when choosing the methodology. For the analysis of 4-EC the HPLC methods are more appropriate as there is no need for sample preparation for all volatile phenols and no derivatisation procedures for 4-EC. Thus, the challenge for food analysts is to focus on developing reliable methods achieving VPs quantification through new green sample preparation approaches and, at best, using an automated technique.

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