LWT - Food Science and Technology 66 (2016) 193–200

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Modelling phenolic and volatile composition to characterize the effects of pre-fermentative cold soaking in Tempranillo wines

José L. Aleixandre-Tudó [a, c, *], Inmaculada Álvarez [c], Victoria Lizama [c], Hélène Nieuwoudt [a, b], María J. García [c], José L. Aleixandre [c], Wessel J. du Toit [a]

[a] Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] Institute for Wine Biotechnology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[c] Departamento de Tecnología de Alimentos, Universitat Politecnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form
17 September 2015
Accepted 12 October 2015
Available online 22 October 2015

Keywords:
Cold soak
Dry ice
Phenolics
Volatiles and OPLS-DA

ABSTRACT

The impact of pre-fermentative cold soak, alone or in combination with dry ice addition, on colour, phenolic and volatile composition of Tempranillo wines at 12 months after bottling was studied. A control wine without cold soak was also evaluated. A sample set consisting of 66 wines was investigated. The results from ANOVA and PCA analysis showed significant treatment-related differences for a number of chemical measurements, as well as overlapping effects. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) of the data showed that the dry ice addition treatment had a major effect on the anthocyanin fraction and on the levels of ethyl decanoate, 2-phenylethyl acetate and decanoic acid. In comparison, the cold soak treatment only had a slight effect on the bisulphite bleaching anthocyanins and volatile composition.

1. Introduction

Polyphenols and volatiles are essential for wine colour, mouth-feel and flavour. The relative proportions of anthocyanins and tannins can be adjusted during skin maceration to achieve a wine capable of undergoing good evolution during aging (Glories & Galvin, 1990). Therefore, when fruity and full bodied red wines are required, pre-fermentative cold maceration appears as an alternative for winemakers (Cai et al., 2014). However, the effects of pre-fermentative techniques are highly dependent on grape sanitary status and phenolic ripeness (Alvarez, Aleixandre, García, & Lizama, 2006), as well as on the aromatic nature of the variety (Moreno-Pérez, Vila-López, & Fernández-Fernández, 2013).

Skin polyphenols and volatile compounds are extracted throughout the pre-fermentative cold soak in the absence of ethanol (Gómez-Míguez, González-Miret, & Heredia, 2007). Dry ice (solid carbon dioxide) addition appears as a common method to obtain the cold temperatures required for this technique (Heredia et al., 2010). After crushing and dry ice addition, grape skin cells are broken and disorganized through freezing, which facilitates aroma and phenolic extraction (Álvarez, Aleixandre, García, Lizama, & Aleixandre-Tudó, 2009).

Contradictory results have been observed in the literature regarding the effect of pre-fermentative cold soak techniques on chemical wine composition. Several studies have shown that cold soak has either no effect, while a decrease in phenolic levels was also observed (De Beer, Joubert, Marais, & Manley, 2006; Heatherbell, Dicey, Goldsworthy, & Vanhanen, 1997; Marais, 2003; Okubo, Goto-Yamamoto, & Okazaki, 2003). Other authors noticed an increased phenolic content and higher sensory scores when wines were cold soaked before fermentation (Gil-Muñoz et al., 2009; Gordillo, López-Infante, Ramírez-Pérez, González-Miret, & Heredia, 2010; Heredia et al., 2010; Koyama, Goto-Yamamoto, & Hashizume, 2007). During the cold soak step numerous reactions between grape phenolics also occur, which may influence wine sensory properties (Parenti, Spugnoli, Calamai, Ferrari, & Gori, 2004). Moreover, other compounds (proteins and polysaccharides) are also extracted, which may participate in condensation reactions (Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández, 2001).

Pre-fermentative techniques have been used extensively in the

* Corresponding author. Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.
E-mail address: joaltu@sun.ac.za (J.L. Aleixandre-Tudó).

http://dx.doi.org/10.1016/j.lwt.2015.10.033
0023-6438/

production of white and rosé wines and have been recommended as a means for enhancing wine aroma (Sánchez Palomo, González-Viñas, Díaz-Maroto, Soriano-Pérez, & Pérez-Coello, 2007). Cold soak has also been tested in red winemaking (Sacchi, Bisson, & Adams, 2005). However, little is known about its effect on the volatile composition. Moreno-Pérez et al. (2013) cited differences between cold soak treated red wines and conventional winemaking after six months of bottling, although no differences between pre-treatments (cold soak, freezing grapes and dry ice addition) were observed. Moreover, in Monastrell wines increased volatile compound levels were reported for Monastrell wines produced by dry ice addition after 6 months of bottle storage (Alvarez, Aleixandre, García, & Lizama, 2006).

Based on these contradictory reports, the aim of this work was thus to evaluate the impact of cold soak techniques on color, phenolic and volatile composition of red Tempranillo wines at 12 months of bottle storage. Tempranillo is one of the most widely planted grape cultivars in Spain and wines made from this cultivar are increasingly being accepted in new world wine producing countries such as Australia and the United States (Cynkar, Dambergs, Smith, & Cozzolino, 2010; USDA, 2014).

2. Materials and methods

2.1. Wine samples

Tempranillo grapes from a commercial vineyard (Utiel-Requena, Valencia, Spain) were harvested in 2008. At harvest, the grapes had 221.33 ± 9.29 g/L of total sugar content, total acidity of 6.1 ± 0.2 g/L as tartaric acid, pH of 3.34 ± 0.12 and potential alcohol of 13.07 ± 0.64% vol. (N = 3). Wines were produced at an experimental wine production centre (Universitat Politècnica de València (UPV)). 40 kg of grapes were destemmed, crushed, mixed and divided into closed 50 L stainless steel tanks. Potassium bisulphite was added at 100 mg/kg before fermentation. Treatments consisted of traditional vinification wines (T–V) without cold soak (control); cold soaked wines at 6 to 8 °C for four days (C–S); and dry ice addition (0 to 2 °C) followed by cold soak at 6 to 8 °C for four days (D–I). Twenty-two small scale vinifications were made for each treatment. After the cold soak period wines were left to warm up in the fermentation room and commercial yeasts were inoculated at 20 g/hL (*Saccharomyces cerevisiae* strain EP 841, Agrovin, Spain). The highest temperature during fermentation was 25 °C. Manual punching down was carried out twice a day. T–V wines were left on the skins for 15 days to ensure that sugar levels were lower than 2 g/L. Following the same workflow, C–S and D–I wines were also left on the skins for 15 days after the four days cold soak treatment. After fermentation wines were pressed and the first 5 L were mixed with 20 L free-run wine. *Oenococcus oeni* strain OE 104 (Agrovin, Spain) lactic acid bacteria was inoculated and malolactic fermentation (MLF) was conducted at room temperature (~20 °C). Potassium bisulphite was added at 50 mg/L before bottling. Wines were stored at room temperature (15 ± 2 °C) and cork closures were used.

2.2. Analytical methods

A UV–Visible JASCO V-530 spectrophotometer, and a JASCO MD-2010 Plus high-performance liquid chromatography instrument coupled with a diode array detector (DAD) (JASCO LC-Net II/ADC, Tokyo, Japan) were used for phenolic measurements. All the spectrophotometric measurements were performed in triplicate. Using the analytical methods described by Glories (1984) colour intensity, hue, gelatin (astringency) and EtOH (tannin-polysaccharide molecules) indexes were estimated. The Ribéreau-Gayon and Stronestreet (1965) method was used for the determination of bisulphite bleached anthocyanins. Catechins were quantified using the method reported by Sun, Ricardo Da Silva, and Spranger (1998). The modified version of the MCP tannin assay reported by Mercurio, Dambergs, Herderich, and Smith (2007) was used for tannin quantification. The method reported by Boulton (1996) was used to analyze the contribution of the copigmented, free and polymeric anthocyanins to the total wine colour. PVPP (anthocyanin-tannin complexes) and DMACH (tannin degree of polymerization) indexes were calculated according to Vivas and Glories (1995). The Folin-Ciocalteu index was determined using the method developed by Singleton and Rossi (1965).

HPLC was used to quantify individual phenolic compounds using the method reported by Jensen, Blachez, Egebo, and Meyer (2007). Gallic acid, (+)-catechin and (−)-epicatechin were quantified at 280 nm. Flavan-3-ols were defined as the sum of (+)-catechin and (−)-epicatechin. Hydroxycinnamic acids were quantified at 316 nm. Phenolic acids were calculated as the sum of gallic and caffeic, coutaric, *p*-coumaric and caftaric acid. Flavonols (quercetin rutinoside, quercetin glucoside, myricetin, quercetin and kaempherol) were quantified at 365 nm. Delphinidin, cyanidin, peonidin, petunidin and malvidin acetyl and coumaryl glucosides resulted in the derivated anthocyanins. Total anthocyanins were calculated as the sum of anthocyanidin-3-glucosides and derivated anthocyanins. Within each phenolic group, compounds were identified based on their intrinsic spectral features and retention times. Commercially available standards were used to build the calibration curves for phenolics quantifications: gallic acid (Fluka, Milwaukee, WI, USA), (+)-catechin (Fluka, Milwaukee, WI, USA) for flavan-3-ols, caffeic acid (Fluka, Milwaukee, WI, USA) for hydroxycinnamic acids, rutin (Sigma–Aldrich, St Louis, MO) for flavonols and malvidine-3-glucoside (Sigma–Aldrich, St Louis, MO) for anthocyanins. 20 μL of the wine sample were injected twice after centrifugation (5000 rpm) and filtration (0.45 μm membrane Millipore filter). Separation was carried out on a Gemini NX (Phenomenex, Torrance, CA) 5 μm, 250 mm × 4.6 mm i.d. column at 40 °C. Acetonitrile and *o*-phosphoric acid were used as solvents. Solvents composition and the elution gradient were reported elsewhere (Jensen et al., 2007).

An Agilent gas chromatograph (GC) (Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless capillary injection port and flame ionization detector (FID) was used for the analysis of the wine aroma composition. Separations were performed on a ZB- WAX Plus column (50 m × 0.25 mm i.d., 0.25 μm film thickness) from Phenomenex (Aschaffenburg, Germany). Duplicate injections were performed using the following conditions: injector temperature, 250 °C; detector temperature, 300 °C; carrier gas flow (N2), 1 mL/min. Injections were made in split mode (split ratio, 1/60; sample size, 1 μl). The oven temperature was maintained at 40 °C for 7 min, from 40 to 110 °C at 4 °C/min, from 110 to 170 °C at 10 °C/min, and then held for 10 min. The comparison of retention times with those of standard compounds was used to identify volatile compounds. Preparation of the samples was carried out following the method proposed by Hernanz, Heredia, Beltran, and Recamales (1999). Twenty volatile compounds were quantified with 2-octanol as internal standard.

2.3. Statistical analysis

Statgraphics Plus 5.1 software was used for the ANOVA treatment of the data. Principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) (Trygg & Wold, 2002) was performed using SIMCA version 13.0.3 software (www.umetrics.com). Chemical measurements

were Pareto ($1/\sqrt{\text{Standard Deviation}}$) scaled. The advantages of Pareto scaling have been defined as a means of reducing the impact of noise and artefacts in the models (Wiklund et al., 2008).

3. Results and discusion

3.1. Effect of pre-fermentative maceration techniques on wine phenolic composition

ANOVA results showed no differences between the control and pre-fermentative treated wines in colour density and hue after 12 months of bottling (Table 1). Bisulphite bleaching anthocyanins showed higher values in cold soaked (C–S) and dry ice added (D–I) wines. Higher levels of malvidine-3-monoglucoside, sum of anthocyanidins and total anthocyanins were found in D–I wines. These results are in accordance with those obtained by Alvarez et al. (2006) and Gil-Muñoz et al. (2009) which indicate an increase in the anthocyanin concentrations due to cold soak with dry ice addition. The higher anthocyanin derivates values observed might support the idea that dry ice addition is capable of increasing not only the free anthocyanin fraction. The extraction of other low molecular weight phenolics that could react with free anthocyanins might give rise to more complex anthocyanin moieties (Aleixandre-Tudó et al., 2013). Gil-Muñoz et al. (2009) also reported increased phenolic extraction and an improvement on the chromatic characteristics in cold soaked wines.

Pre-fermentative cold soak techniques have been reported to influence the presence of phenolic compounds with high copigmentation potential (Álvarez, Aleixandre, García, Lizama, & Aleixandre-Tudó, 2009; Heredia et al., 2010). Despite the reported results no differences in the copigmentation fraction were observed. Copigmented anthocyanins account for almost half of the observed colour in young red wines and it is thought that anthocyanins may take part in future condensation reactions. Moreover, it is also believed that at early stages of fermentation the anthocyanin-copigment molecules can prevent anthocyanin oxidation, favouring further covalent tannin-anthocyanin interaction (Boulton, 2001).

The colour resulting from the free non-copigmented anthocyanins and the flavan-3-ol concentration was also higher in D–I wines. Parenti et al. (2004) reported increased flavan-3-ol, tannin and anthocyanin concentrations in cold soaked wines after bottling, which led to more anthocyanin-tannin interactions. Contrarily to what was reported, anthocyanin-tannin interactions (PVPP index and % of colour due to polymeric anthocyanins) were not increased in this study at 12 months of bottling.

3.2. Effect of pre-fermentative cold soak techniques on wine volatile composition

The ANOVA results showed that pre-fermentative cold soak techniques caused significant differences (Table 2) in fatty acids (octanoic and decanoic acid), ethyl esters (ethyl hexanoate and ethyl decanoate), an ester (2-Phenylethyl acetate), a volatile phenol (4-vinylphenol) and a lactone (γ-butyrolactone). The concentration of octanoic acid was lower in C–S and D–I wines, while the opposite was found for decanoic acid, although only significant for D–I wines. Moreover, no trends were observed in the esters and ethyl esters levels found for the different treatments. The levels of 4-vinylphenol were lower for D–I wines when compared to T–V and C–S treatments. Finally γ-butyrolactone levels seem to be diminished by cold soak treatments, with T–V wines having the highest levels of this compound. Moreno-Pérez et al. (2013) were not able to discriminate between the volatile composition of cold soaked Monastrell, Cabernet Sauvignon and Syrah cold soaked wines (freezing grapes, dry ice and cold soak) and concluded that these effects were variety dependent.

The large within treatment variations (as indicated by high standard deviations), probably caused by the large number of small scale fermentations, could possibly explain why non-significant differences were observed for some compounds (Table 2). Contrarily to univariate techniques such as ANOVA that

Table 1
Colour and phenolic profile of Tempranillo wines after 12 month of bottle aging. Mean ± standard deviation values of the phenolic measurement.

		T–V (n = 22)	C–S (n = 22)	D–I (n = 22)	p-value
Color density (1)	CDe	12.71 ± 1.55	12.52 ± 0.88	12.21 ± 1.06	0.39
Hue (2)	Hue	71.20 ± 2.63	69.93 ± 2.28	70.84 ± 2.28	0.20
Bisulfite bleaching ant. (mg/L) (3)	BBA	271.85 ± 27.83 a	297.59 ± 38.96 b	287.27 ± 30.64 b	0.04*
Delphinidin-3-glucoside (mg/L) (4)	Del	33.10 ± 2.84	32.21 ± 1.85	33.58 ± 1.73	0.19
Cyanidin-3-glucoside (mg/L) (5)	Cya	2.17 ± 2.15	2.15 ± 0.25	2.33 ± 0.03	0.65
Petunidin-3-glucoside (mg/L) (6)	Pet	30.67 ± 31.93	31.93 ± 1.90	32.61 ± 2.42	0.08
Peonidin-3-glucoside (mg/L) (7)	Peo	5.13 ± 1.01	5.20 ± 0.87	5.49 ± 0.90	0.49
Malvidin-3-glucoside (mg/L) (8)	Mal	255.42 ± 18.40 a	257.34 ± 26.61 a	280.43 ± 25.88 b	0.00*
Anthocyanidins (mg/L) (9)	Ant	326.49 ± 24.64 a	328.83 ± 30.30 a	354.45 ± 29.36 b	0.00*
Anthocyanin derivates (mg/L) (10)	ADe	46.84 ± 3.69 a	48.33 ± 7.28 a	53.34 ± 8.63 b	0.00*
Total anthocyanis (mg/L) (11)	ToA	373.33 ± 25.24 a	377.16 ± 34.48 a	407.78 ± 32.23 b	0.00*
% Copigmented anthocyanins (12)	%CA	10.48 ± 2.65	10.61 ± 2.39	10.48 ± 2.25	0.91
% Free anthocyanins (13)	%FA	39.49 ± 1.21 a	40.72 ± 2.91 ab	41.98 ± 2.76 b	0.04*
% Polimerized anthocyanins (14)	%PA	50.03 ± 7.62	48.67 ± 3.54	47.54 ± 4.70	0.77
PVPP index (15)	PVI	32.02 ± 3.07	32.79 ± 2.90	33.92 ± 2.98	0.07
Folin index (16)	FoI	46.22 ± 2.23	45.65 ± 1.95	45.56 ± 1.95	0.50
Phenolic acids (mg/L) (17)	PhA	146.74 ± 29.40	148.56 ± 26.35	137.98 ± 26.56	0.34
Flavonols (mg/L) (18)	Flo	61.27 ± 20.59	57.19 ± 20.87	63.38 ± 19.34	0.59
Catechin assay (mg/L) (19)	Cat	85.49 ± 17.59	84.93 ± 15.05	85.49 ± 13.88	0.99
Flavan-3-ols (mg/L) (20)	Fla	65.45 ± 19.01 a	70.16 ± 20.00 ab	80.89 ± 18.34 b	0.03*
Tannins (g/L) (21)	Tan	2.15 ± 0.25 b	2.01 ± 0.20 a	2.03 ± 0.23 a	0.03*
DMACH index (22)	DMI	43.41 ± 4.23	43.76 ± 3.21	44.82 ± 3.99	0.29
Ethanol index (23)	EtI	21.75 ± 5.89	23.96 ± 4.39	22.78 ± 4.83	0.25
Gelatin index (24)	GeI	64.97 ± 5.96	65.45 ± 10.12	65.53 ± 8.65	0.96

n: number of samples involved in the analysis from each treatment. T–V: traditional vinification; C–S: cold soak; D–I: dry ice.*Statistical analysis ANOVA at 95% confidence level with same letters indicating no significant difference for Tukey's test.

Table 2
Volatile composition of Tempranillo wines after 12 month of bottle aging. Mean ± standard deviation values (μg/L) of the quantified volatile compounds.

		T–V (n = 22)	C–S (n = 22)	D–I (n = 22)	p-value	PT[a,b]
Isoamyl acetate (25)	IsA	449.55 ± 221.45	704.91 ± 349.04	675.18 ± 550.59	0.07	30(I)[#]
Ethyl hexanoate (26)	EHe	173.27 ± 125.21 b	105.48 ± 34.87 a	85.90 ± 19.71 a	0.00*	50(I)[#]
Ethyl lactate (27)	ELa	12797.10 ± 10021.19	23283.53 ± 19508.42	21629.45 ± 20015.09	0.10	14000(II)[¥]
Cis-3-hexen-1-ol (28)	C3H	26.30 ± 19.55	18.22 ± 9.84	21.14 ± 17.22	0.25	400(III)[α]
Ethyl 3-hydroxybutyrate (29)	EHb	21.21 ± 11.84	21.79 ± 9.90	22.33 ± 15.42	0.96	20000(III)[ρ]
Isobutyric acid (30)	IbA	2731.74 ± 1398.11	2998.24 ± 1646.48	3032.73 ± 2053.87	0.82	2300(IV)[g]
4-Vinylphenol (31)	4Vp	27.29 ± 22.48 b	26.58 ± 18.69 b	8.43 ± 9.04 a	0.00*	180(III)[§]
Butyric acid (32)	BtA	6.60 ± 4.54	7.45 ± 4.78	7.27 ± 5.65	0.84	173(III)[α]
Ethyl decanoate (33)	EDe	425.89 ± 228.45 b	190.63 ± 68.13 a	573.95 ± 119.04 c	0.00*	200(I)[¥]
γ-Butyrolactone (34)	Btl	40.58 ± 23.75 b	26.77 ± 10.83 a	23.77 ± 9.69 a	0.00*	35(III)[ρ]
Isopentanoic acid (35)	IpA	3.69 ± 1.63	3.86 ± 2.44	4.03 ± 2.68	0.89	30(III)[α]
Diethyl succinate (36)	DeS	535.60 ± 317.26	614.19 ± 299.84	713.82 ± 170.98	0.10	200000(III)[¥]
2-Phenylethyl acetate (37)	2 PA	163.38 ± 74.92 a	213.14 ± 50.23 a	306.31 ± 140.36 b	0.00*	250(IV)[#]
Hexanoic acid (38)	HeA	3121.63 ± 1459.38	3161.95 ± 1632.91	2717.16 ± 1619.91	0.59	420(V)[g]
2-Methoxyphenol (39)	2 MP	197.77 ± 68.82	249.24 ± 77.78	247.83 ± 111.20	0.09	9.5(III)[α]
2-Phenylethanol (40)	2Pe	40792.84 ± 30714.08	47685.85 ± 29690.71	50851.00 ± 30873,27	0.54	14000(IV)[¥]
4-Ethylguaiacol (41)	4 Eg	283.54 ± 97.22	238.30 ± 64.57	209.23 ± 150.89	0.09	33(IV)[#]
Octanoic acid (42)	OcA	576.25 ± 259.34 b	422.5 ± 233.28 a	350.92 ± 185.84 a	0.01*	500(III)[α]
4-Ethylphenol (43)	4Ep	51.40 ± 19.30	39.36 ± 15.64	43.57 ± 21.57	0.11	440(IV)[§]
Decanoic acid (44)	DeA	308.73 ± 104.77 a	340.20 ± 66.34 ab	408.89 ± 170.14 b	0.03*	1000(III)[α]

n: number of samples involved in the analysis from each treatment. T–V: traditional vinification; C–S: cold soak; D–I: dry ice. PT: perception threshold. *Statistical analysis ANOVA at 95% confidence level with same letters indicating no significant difference for Tukey's test.
[a]The reference from which the value was obtained is given in parentheses: I (Swiegers, Bartowsky, Henschke, & Pretorius, 2005); II (Li, Tao, Wang, & Zhang, 2008); III (Culleré, Escudero, Cacho, & Ferreira, 2004); IV (Francis & Newton, 2005); V (Ferreira, López, & Cacho, 2000).
[b]Symbols correspond to different matrices from which the PT was obtained: [#]10% ethanol/water solution; [¥]12% ethanol/water solution; [§]12% ethanol/water solution with 8 g/L glycerol and different salts; [ρ]10% ethanol/water solution with 5 g/L of tartaric acid at pH 3.2; [α]11% ethanol/water solution with 7 g/L glycerol and 5 g/L tartaric acid; [g]10% ethanol/water solution with 7 g/L glycerol at pH 3.0.

compare only one variable at the time, multivariate techniques include more than one statistical variable and include the effect of all variables on the responses of interest. PCA and OPLS-DA were thus performed to explore the parameters that could contribute to the variation between treatments.

3.3. Multivariate data analysis

In order to investigate the relationship between chemical composition and treatments in a more holistic approach, PCA was performed on the 66 samples and 44 variables (24 phenolic measurements and 20 volatile compounds). The bi-plot (Fig. 1 (a)) showed that the first two principal components captured 93.7% of the explained variance (PC1 = 60.6% and PC2 = 33.1%) in the dataset. Although some differences were shown in the ANOVA, no clear trend based on treatments was observed in the PCA plot (i.e. no grouping of samples), thereby making it difficult to understand which chemical compounds were correlated to the applied pre-fermentative techniques.

As has been reported by several authors, cold pre-fermentative techniques might initially increase anthocyanin and phenolic extraction (Gil-Muñoz et al., 2009), although the reactions that occur during storage could reduce or even negate this effect. In a study reported by Ortega-Heras, Pérez-Magariño, and González-Sanjosé (2012) the higher extraction of some phenolic compounds induced by cold soak was no longer evident at the end of bottle aging (12 months). Other authors reported that the effect might depend on the vintage, which could be related to the phenolic maturity of the grapes (Álvarez, Aleixandre, García, & Lizama, 2006, De Beer et al., 2006). The discrepancies in the above mentioned studies indicated that the effect could also depend on the variety used (Bussé-Valverde et al., 2010; Gil-Muñoz et al., 2009;).

With the aim of further investigating the differences between treatments, the OPLS-DA technique was applied to the data. OPLS-DA removes variability in X (chemical data) that is orthogonal to Y (treatments), by separating systematic variation into predictive components and orthogonal components (Lee, Hong, & Lee, 2009).

Fig. 1 (B) shows OPLS-DA bi-plot of pre-fermentative cold soaked (C–S and D–I) and control wines (T–V) using the complete set of 66 wines and 44 variables (chemical data). The model resulted in two predictive components and five orthogonal components, with cross-validated predictive ability $Q^2(Y) = 52.3\%$, total explained variance $R^2(X) = 98.7\%$, and variance related to treatment effect $R^2p(X) = 7\%$. Compared to the results obtained with PCA, OPLS-DA exhibited an improved separation of cold soaked and control wines (Fig. 1 (b)). C–S and D–I wines were mostly located towards the negative end of PC1, and T–V wines towards the positive end. Moreover, a differentiation between C–S and D–I wines was observed on PC2, with C–S wines located towards the positive end, and D–I towards the negative end.

In order to further investigate the effect of cold soak treatments on the chemical composition of the wines, only the statistically significant compounds identified in the ANOVA were considered. In addition, pairwise comparisons of the chemical composition of T–V, C–S wines and D–I wines were performed. Fig. 2 shows the cross-validated OPLS-DA scores plots (a.1, b.1 and c.1) and the S-plots (a.2, b.2 and c.2) that were constructed on the pair-wise comparisons. S-plots are scatter plots of the predictive values obtained by cross validation vs. the correlation vectors. The S-plot often takes an "S" shape if the X variables are scaled to Pareto variance. X variables located far out of the "S" wings combine high covariance and correlation and are therefore important contributors to the differences between treatments.

The comparison of significant variables in T–V and C–S wines is shown in Fig. 2(a.1 and a.2), and a clear separation between samples of the different treatments was not observed (Fig. 2(a.1)). The volatile compounds ethyl decaoate (EDe), ethyl hexanoate (EHe) and octanoic acid (OcA) appeared located on the lower edge of the "S" wing and are therefore highly correlated with T–V wines. On the other hand, looking at the opposite side of the S-plot, the volatile compounds decanoic acid (DeA) and 2-phenylethyl acetate (2 PA) together with the bisulphite bleaching anthocyanins (BBA) were important contributors to C–S wines (Fig. 2(A.2)).

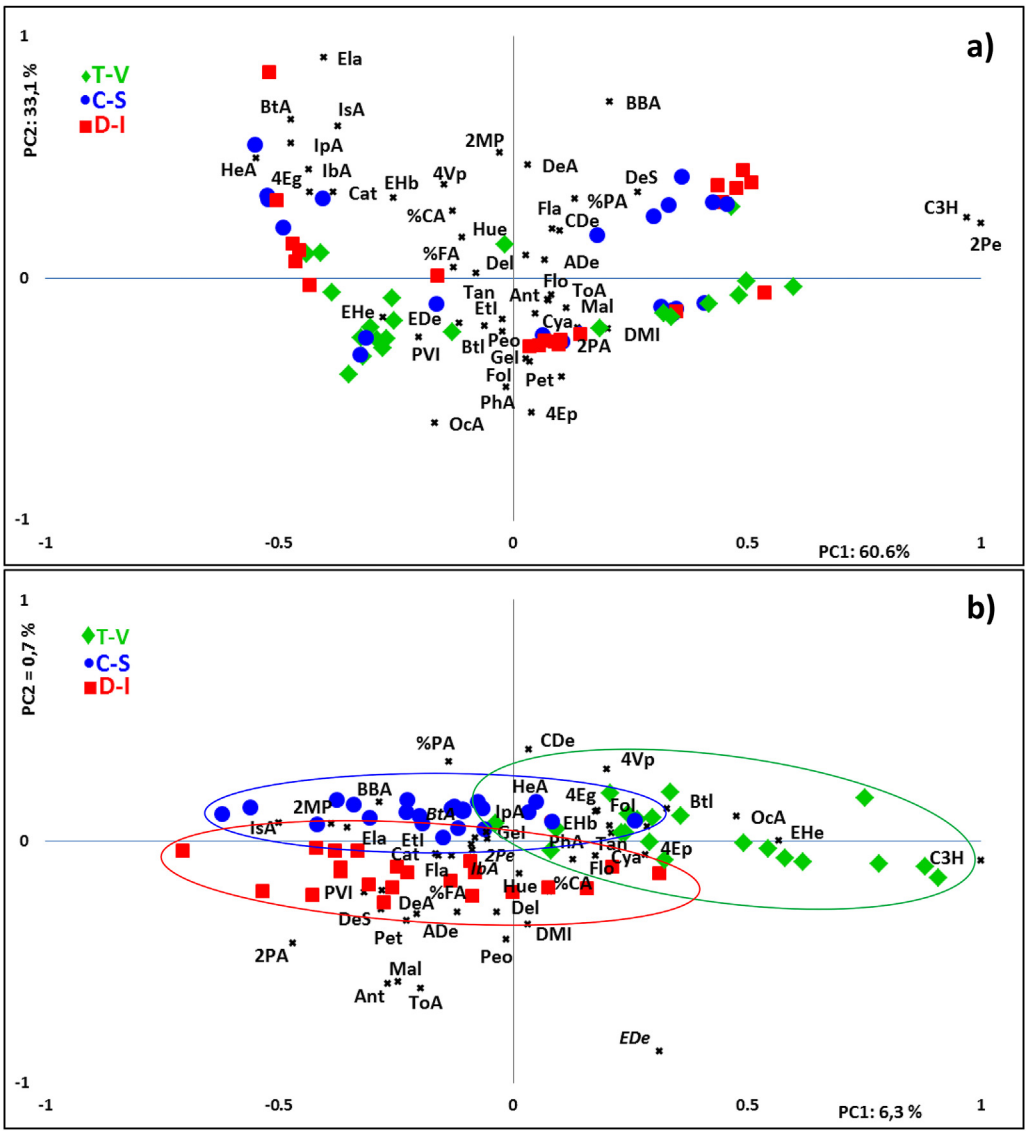

Fig. 1. Principal component analysis bi-plot (PCA) (A) and orthogonal projections on latent structures discriminant analysis bi-plot (OPLS-DA) (B) of phenolic determinations and volatile compounds of wines elaborated with pre-fermentative cold maceration techniques. ◆T–V wines; ●C–S wines; ■D–I wines. Phenolic and volatile abbreviations correspond to Tables 1 and 2.

Fig. 2(b.1 and b.2) also show the cross-validated OPLS-DA scores plot and the S-plot for the pair T–V vs. D–I. A better separation between samples was clearly observed, which could be interpreted as bigger differences in chemical composition between wines, than those observed between C–S and T–V treatments (Fig. 2(b.1)). For the D–I and T–V pair, the volatile compounds octanoic acid (OcA), ethyl hexanoate (EHe), γ-butirolactone (Btl) and 4-vinilphenol (4VP) appeared to be correlated with T–V wines, while ethyl decanoate (Ede), 2-phenylethyl acetate (2 PA) and decanoic acid (DeA), together with the parameters related with the anthocyanin fraction (malvidine (Mal), anthocyanidins (Ant), anthocyanin derivates (ADe), total anthocyanins (TAn), bisulfite bleaching anthocyanins (BBA) and % free anthocyanins (%FA)) and the parameter flavan-3-ols (Fla) appeared highly related with dry D–I wines (Fig. 2(b.2)).

Finally, the direct comparison between pre-fermentative techniques (C–S vs. D–I) is shown in Fig. 2(c.1 and c.2). A clear separation between treatments was also observed (Fig. 2(c.1)). These results suggested that the dry ice addition treatment (D–I wines) had the biggest influence on the volatile and phenolic composition of Tempranillo wines after 12 months of bottle aging. C–S wines also differed from T–V wines, although the variation in phenolic and volatile composition was less pronounced. The volatile compounds ethyl decanoate (EDe), 2-phenylethyl acetate (2 PA) and decanoic acid (DeA), together with some of the mentioned anthocyanin related parameters, were strongly correlated with the D–I wines. On the other hand, volatile compounds such as octanoic acid (OcA), ethyl hexanoate (EHe), γ-butirolactone (Btl) and 4-vinilphenol (4VP) were correlated with C–S wines (Fig. 2(C.2)).

3.4. OPLS-DA classification

Classification models were established using half of the samples (N = 33) for the calibration set, while the other 33 samples were used to validate the models. The classification results are shown in Table 3. In order to investigate which group of chemical compounds had the biggest influence on the differences between cold soak treatments the parameters were grouped based on the corresponding phenolic family and also on their aromatic chemical

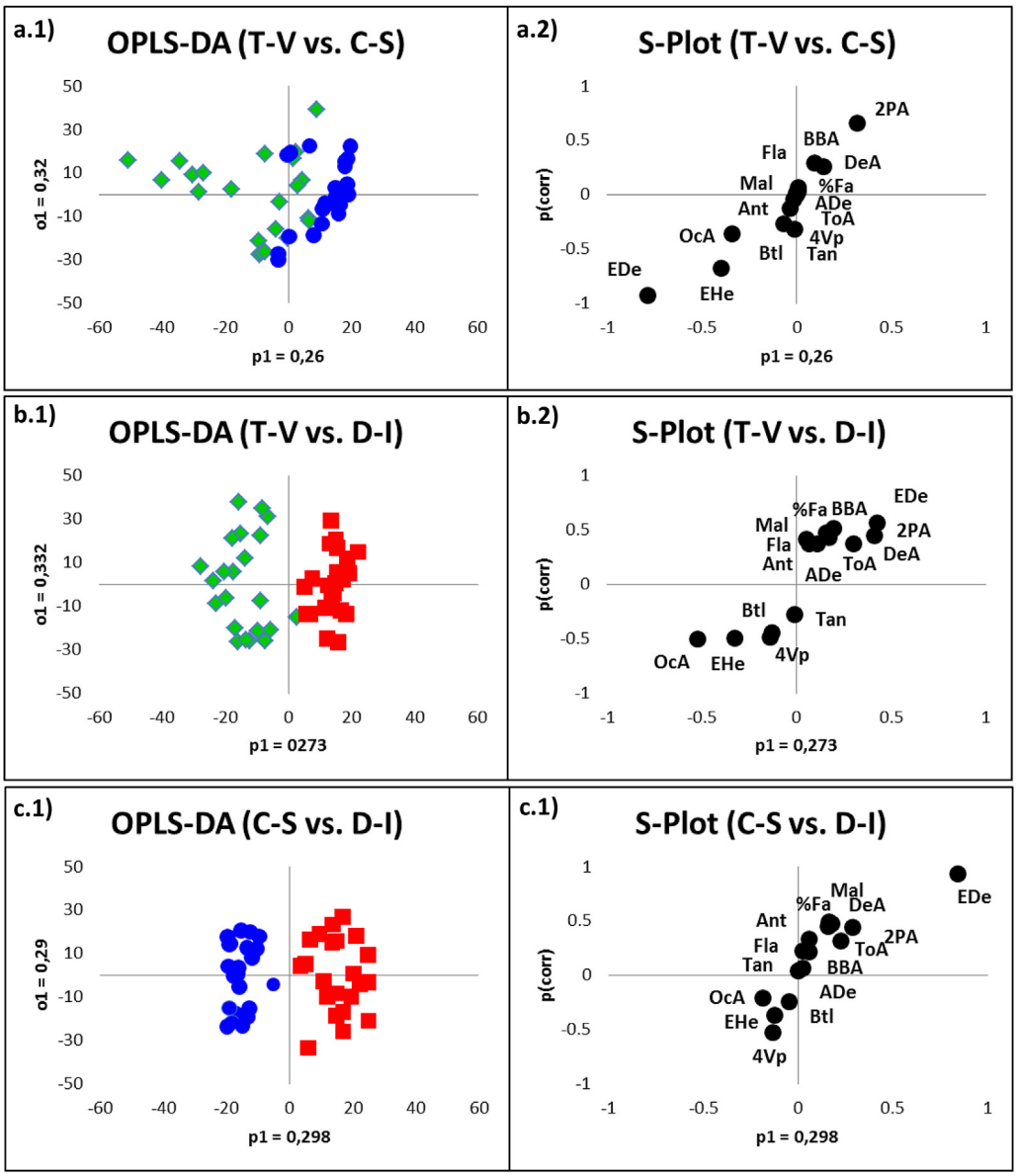

Fig. 2. OPLS-DA scores plots and S-Plots considering the significant phenolic and volatile parameters identified in the ANOVA of wines elaborated with pre-fermentative cold maceration techniques. Comparison among T–V and C–S wine (A.1 and A.2), T–V and D–I wines (B.1 and B.2) and C–S and D–I wines (C.1 and C.2). ◆T–V wines, ●C–S wines, ■D–I wines. Phenolic and volatile abbreviations correspond to Tables 1 and 2.

families (esters, higher alcohols, volatile phenols, fatty acids and lactones). Therefore groups containing anthocyanin (BBA, Del, Cya, Pet, Peo, Mal, Ant, Ade, ToA, %CA, %FA, %PA, PVI), tannin (PVI, Cat, Fla, Tan, DMI, EtI, GeI), phenolic acids (PhA) and flavonol (Flo) related parameters were tested. Two additional groups, one including the total phenolics parameter (FoI) and another considering the two wine colour related parameters (CDe and Hue) were also performed. Two groups including all the phenolic measurement and all the volatile compounds were also considered. Finally the classification was again performed taking into account all the variables included in the study (phenolic + volatiles). This last model showed a validation accuracy of 87.88% in classifying the cold soak techniques, thus highlighting the impact that pre-fermentative cooling techniques have on wine chemical composition.

Within phenolic compounds, anthocyanin related parameters were 60.61% accurately classified in validation showing the effect of these techniques on the anthocyanin fraction. Finally when all the phenolic measurements were included in the model 54.55% of the samples were accurately classified.

Regarding volatile compounds, the ester group appeared as the group of compounds with the better ability to classify samples, with 63.64% of validation accuracy. Otherwise other groups of volatile compounds showed lower accuracy in classifying Tempranillo wines (Table 3). An important increase in ester levels has also been observed in cold soaked and dry ice added pre-fermentative macerated wines (Table 2). Compounds such as ethyl decanoate (soap, floral, grape, fruity, fatty, and pleasant) and 2-phenylethyl acetate (rose, honey, tobacco, fruity, flowery, pleasant) were identified as important contributors of D–I wines aroma. In addition, when the volatile compounds were all included in the prediction model, 81.82% of the samples were correctly classified.

Table 3
Summary of orthogonal projection to latent structures discriminant analysis (OPLS-DA) classification results.

Model	Parameters included[a]	Cold soak	
		Calibration (%) N = 33	Validation (%) N = 33
Color	1,2	39,39	27,27
Total phenolics	16	39,39	39,39
Anthocyanins	3–15	63,64	60,61
Tannins	15,19–24	45,45	39,39
Phenolic acids	17	36,36	33,33
Flavonols	18	48,48	27,27
Phenolics	1–24	69,70	54,55
Esters	25-27,29,33,36,37	84,85	63,64
Higher alcohols	28.40	42,42	42,42
Fatty acids	30,32,35,38,42,44	45,45	42,42
Volatile phenols	31,39,41,43	66,67	48,48
Lactones	34	45,45	42,42
Volatile compounds	25–44	100	81,82
Phenolics + Volatiles	1–44	100	87,88

[a] Parameters included in each model, numbers correspond to Tables 1 and 2 33 random selected samples were included in the calibration set while the remaining 33 were used for model validation.

4. Conclusions

Dry ice addition led to significant effects on anthocyanins and flaval-3-ols levels. However an increased formation of tannins and polymeric pigments was not observed. Dry ice also influenced volatile composition with increased levels of ethyl decanoate, 2-phenylethyl acetate and decanoic acid. Pre-fermentative cold soak caused minor changes in the anthocyanins and volatile fraction. An extended cold soak period might cause larger effects, but in this case economic reasons need to be considered. Moreover from the classification analysis cold soak treatments seem to strongly influence the volatile composition, especially esters, together with the anthocyanin fraction. Finally it is important to mention that these results should be confirmed with further investigations which include at least three seasons due to our study only being conducted on a single vintage.

Acknowledgements

The research reported here is a project financially supported by the Spanish Government (AGL 2006-10723-C02-02) which the authors gratefully acknowledge.

References

Aleixandre-Tudó, J. L., Álvarez, I., Lizama, V., García, M. J., Aleixandre, J. L., & du Toit, W. J. (2013). Impact of caffeic acid addition on phenolic composition of Tempranillo wines from different winemaking techniques. *Journal of Agricultural and Food Chemistry, 61*, 11900–11912.

Álvarez, I., Aleixandre, J. L., García, M. J., Lizama, V., & Aleixandre-Tudó, J. L. (2009). Effect of the prefermentative addition of compigments on the polyphenolic composition of Tempranillo wines after malolactic fermentation. *European Food Research and Technology, 228*, 201–210.

Alvarez, I., Aleixandre, J. L., García, M. J., & Lizama, V. (2006). Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. *Analytica Chimica Acta, 563*, 109–115.

Boulton, R. B. (1996). Methods for the assement of copigmentation in red wines. In *Presented at the 47th Annual Meeting of the American Society for Enology and Viticulture, Reno, NV*.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *American Journal of Enology and Viticulture, 52*, 67–87.

Bussé-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I., & Bautista-Ortín, A. (2010). Effect of different enological practices on skin and sees proanthocyanidins in three varietal wines. *Journal of Agricultural and Food Chemistry, 58*, 11333–11339.

Cai, J., Zhu, B. Q., Wang, Y. H., Lu, L., Lan, Y. B., Reeves, M. J., & Duan, C. Q. (2014). Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chemistry, 154*, 217–229.

Culleré, L., Escudero, A., Cacho, J., & Ferreira, V. (2004). Gas chromatography–olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry, 52*(6), 1653–1660.

Cynkar, W., Dambergs, R., Smith, P., & Cozzolino, D. (2010). Classification of Tempranillo wines according to geographic origin: combination of mass spectrometry based electronic nose and chemometrics. *Analytica Chimica Acta, 660*(1–2), 227–231.

De Beer, D., Joubert, E., Marais, J., & Manley, M. (2006). Maceration before and during fermentation: effect on Pinotage wine phenolic composition, total antioxidant capacity and objective colour parameters. *South African Journal of Enology and Viticulture, 27*, 137–150.

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*, 1659–1667.

Francis, I. L., & Newton, J. L. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research, 11*, 114–126.

Gil-Muñoz, R., Moreno-Pérez, A., Vila-López, R., Martínez-Cutillas, A., Fernández-Fernández, J. I., & Gómez-Plaza, E. (2009). Influence of low temperature prefermentative techniques on chromatic and phenolic characteristics of Syrah and Cabernet sauvignon wines. *European Food Research and Technology, 228*, 777–788.

Glories, Y. (1984). La coleur des vins rouges. Mesure, origine et interpretation. *Connaissance de la Vigne et du Vin, 18*(4), 253–271.

Glories, Y., & Galvin, C. (1990). Les complexes tanin-anthocyanes en présence d'éthanal. Conditions de leur formation. *Acttualités œnologiques, 89*, 408–413.

Gómez-Míguez, M., González-Miret, M., & Heredia, F. (2007). Evolution of color and anthocyanin composition of Syrah wines elaborated with pre-fermentative cold maceration. *Journal of Food Engineering, 79*, 271–278.

Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J. M., Martínez-Cutillas, A., & Fernández-Fernández, J. I. (2001). Phenolic compounds and color stability of red wines: effect of skin maceration time. *American Journal of Enology and Viticulture, 52*(3), 266–270.

Gordillo, B., López-Infante, M. I., Ramírez-Pérez, P., González-Miret, M., & Heredia, F. (2010). Influence of prefermentative cold maceration on the color and anthocyanin copigmentation of organic Tempranillo wines elaborated in warm climate. *Journal of Agricultural and Food Chemistry, 58*, 6797–6803.

Heatherbell, D., Dicey, M., Goldsworthy, S., & Vanhanen, L. (1997). Effect of prefermentation cold maceration on the composition, color and flavor of Pinot noir wine. In G. F. Steans (Ed.), *Proceedings of the New Zealand Grape & Wine Symposium* (pp. 30–42). New Zealand Society for Viticulture and Oenology, P.O. Box 90-276, Auckland Mail Centre, Auckland 1, New Zealand.

Heredia, F. J., Escudero-Gilete, M. L., Hernanz, D., Gordillo, B., Meléndez-Martínez, A. J., Vicario, I. M., & González-Miret, M. L. (2010). Influence of the refrigeration technique on the color and phenolic composition of Syrah wines obtained with pre-fermentative cold maceration. *Food Chemistry, 118*, 377–383.

Hernanz, D., Heredia, F. J., Beltran, R., & Recamales, M. A. F. (1999). Optimization of an extraction method of aroma compounds in white wine using ultrasound. *Talanta, 50*, 413–421.

Jensen, J. S., Blachez, B., Egebo, M., & Meyer, A. S. (2007). Rapid extraction of polyphenols from red grapes. *American Journal of Enology and Viticulture, 58*(4), 451–461.

Koyama, K., Goto-Yamamoto, N., & Hashizume, K. (2007). Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins

and seed of grape (Vitis Vinifera). *Bioscience Biotechnology Biochemistry, 71*(4), 958–965.
Lee, J. E., Hong, Y. S., & Lee, C. H. (2009). Characterization of fermentative behaviors of lactic acid bacteria in grape wines through 1H NMR- and GC-based metabolic profiling. *Journal of Agricultural and Food Chemistry, 57*, 4810–4817.
Li, H., Tao, Y. S., Wang, H., & Zhang, L. (2008). Impact odorants of Chardonnay dry white wine from Changli County (China). *European Food Research and Technology, 227*(1), 287–292.
Marais, J. (2003). Effect of different wine-making techniques on the composition and quality of Pinotage wine I. Low-temperature skin contact prior to fermentation. *South African Journal of Enology and Viticulture, 24*, 70–75.
Mercurio, M. D., Dambergs, R. G., Herderich, M. J., & Smith, P. A. (2007). High throughput analysis of red wine and grape phenolics: adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. *Journal of Agricultural and Food Chemistry, 55*(12), 4651–4657.
Moreno-Pérez, A., Vila-López, R., & Fernández-Fernández, J. I. (2013). Influence of cold pre-fermentation treatments on the major volatile compounds of three wine varieties. *Food Chemistry, 139*(1–4), 770–776.
Okubo, K., Goto-Yamamoto, N., & Okazaki, N. (2003). Effect of prefermentative cold soak on extraction of anthocyanin during red wine making. *Journal of the Brewing Society of Japan, 98*, 193–200.
Ortega-Heras, M., Pérez-Magariño, S., & González-Sanjosé, M. (2012). Comparative study of the use of maceration enzymes and cold prefermentative maceration on phenolic and anthocyanic composition and color of a Mencía red wine. *LWT-Food Science and Technology, 48*, 1–8.
Parenti, A., Spugnoli, P., Calamai, L., Ferrari, S., & Gori, C. (2004). Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *European Food Research and Technology, 218*, 360–366.
Ribéreau-Gayon, P., & Stronestreet, E. (1965). Le dosage des anthocyanes dans le vin rouge. *Bulletin de la Société Chimique de France, 9*, 119–142.
Sacchi, K., Bisson, L., & Adams, D. (2005). A review of the effect of winemaking techniques on phenolic interaction in red wines. *American Journal of Enology and Viticulture, 53*(3), 197–206.
Sánchez Palomo, E., González-Viñas, M. A., Díaz-Maroto, M. C., Soriano-Pérez, A., & Pérez-Coello, M. S. (2007). Aroma potential of Albillo wines and effect of skin-contact treatment. *Food Chemistry, 103*, 631–640.
Singleton, V. L., & Rossi, J. (1965). A Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.
Sun, B., Ricardo Da Silva, J. M., & Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry, 46*, 4267–4274.
Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research, 11*, 139–173.
Trygg, J., & Wold, S. (2002). Orthogonal projections to latent structures (O-PLS). *Journal of Chemometrics, 16*, 119–128.
USDA. (2014). *National Agricultural Statistics Service. California grape Crush report. Washington, DC*.
Vivas, N., & Glories, Y. (1995). Ranking of red wines matured in barrels. A tentative classification of racking techniques. *Australian and New Zealand Wine Industry Journal, 10*, 241–243.
Wiklund, S., Johansson, E., Sjostrom, L., Mellerowicz, E. J., Edlund, U., Shockcor, J. P., Gottfries, J., Moritz, T., & Trygg, J. (2008). Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Analytical Chemistry, 80*, 115–122.