Modulating analytical characteristics of thermovinified Carignan musts and the volatile composition of the resulting wines through the heating temperature

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α-Terpineol (PubChem CID: 17100)
Geraniol (PubChem CID: 637566)
4-Mercapto-4-methyl-2-pentanone (PubChem CID: 88290)
3-Mercaptohexanol (PubChem CID: 521348)
Guaiacol (PubChem CID: 460)
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γ-Nonalactone (PubChem CID: 7710).

ABSTRACT

The impact of two temperature levels (50 °C and 75 °C) and heating times (30 min and 3 h) on the composition of thermovinified musts and wines from Carignan was investigated at the laboratory scale in 2014 and 2015. The heating temperature had a significant impact on the extraction of amino acids and a probable thermal degradation of anthocyanins was noted at 75 °C. In 2014, musts from grapes that underwent a heat treatment at 50 °C for 3 h had a similar level of phenolic compounds as those treated at 75 °C for 30 min. This indicates that the reduction of the heating temperature in some vintages can be compensated for through an extension of the heating period. Several grape-derived molecules were impacted by the rise in temperature and wines made from grapes treated at 50 °C in most cases contained larger concentrations of geraniol, β-citronellol, β-damascenone and 3-mercaptohexanol.

1. Introduction

Pre fermentation heat treatment of grapes or thermovinification is a winemaking technology first industrially developed in the seventies (Marteau & Olivieri, 1970). It consists of heating grapes between 70 and 75 °C for a length of time varying from 30 min to 24 h. The term "thermovinification" is sometimes used to describe the process in which heating is limited to a brief period (< 1 h). After pressing at a high temperature and clarification, fermentation is usually undertaken in liquid phase at a lower temperature than usual red ferments, typically between 18 °C and 25 °C. This technique is becoming increasingly popular for the production of colored, fruit driven red wines with soft tannins. The volume of wine elaborated in France in 2017 through thermovinification was estimated at 750 million liters (J.L. Favarel, personal communication, July 18, 2017).

In comparison with control macerated wines, thermovinification wines fermented at a lower temperature usually have higher levels of ethanol (Geffroy et al., 2015). Some changes in the acid base balance of the wines by higher tartaric acid and potassium extractions from the pericarp tissue of the berries were also highlighted. The typical sensory profile of thermovinified wines is due to a large extraction under the effects of heat of hydrophilic anthocyanin pigments and grape poly saccharides responsible for roundness in wine (Doco, Williams, & Cheynier, 2007; Girard, Kopp, Reynolds, & Cliff, 1997). In aqueous phase, the extraction of tannins is more moderate and the technique usually leads to wines with a lower overall phenolic content than those made using traditional maceration techniques (Auw, Blanco, O'Keefe, & Sims, 1996).

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From an olfactive point of view, thermovinification is known to produce wines with a standardized sensory profile often described as "banana yogurt" by winemakers. Past research into the volatile com position of thermovinified wines showed that maceration heat treat ment allowed the elimination through volatilization of a large amount of 3 isobutyl 2 methoxypyrazine (Roujou de Boubée, 2000) and was not favorable to the production of wines with high concentrations of hydrophobic rotundone (Geffroy, Siebert, Silvano, & Herderich, 2016). Another work reported the likely thermal degradation of several grape derived aroma compounds or their precursors (i.e. some varietal thiols, monoterpenols, norisoprenoids, phenols) when grapes were heated during 3 h at 70 °C (Geffroy et al., 2015). The fermentation conditions of thermovinified wines particularly enhanced esters, acetates and fatty acid formation (Cottereau & Desseigne, 2007; Fischer, Strasser, & Gutzler, 2000; Girard et al., 1997). Consistent with previous observa tions made in the seventies (Poux, 1974), Geffroy et al. (2015) recently showed that heating at 70 °C for 2 h followed by pressing at a high temperature induced a substantial increase in the concentration of amino acids in the must (from + 101% to 200%). The fermentation in liquid phase and at low temperature of high Yeast Assimilable Nitrogen (YAN) clarified musts enhanced the production of fermentative aroma compounds by the yeast (Moreno, Medina, & Garcia, 1988).

The modulation of the sensory profile of thermovinified wines to wards a fruiter varietal character is an issue frequently raised by winemakers. Grape derived aroma compounds imparting this character to the wine include monoterpenes, norisoprenoids, aliphatics, phenyl propanoids, methoxypyrazines, and volatile sulfur compounds (Robinson et al., 2014).

In an attempt to produce thermovinified wines with a fruiter var ietal character, two levels of must clarification (150 and 800 nephelo metric turbidity units or NTU) and fermentation temperature (18 °C and 25 °C) were previously investigated (Geffroy et al. (2014)). The results were inconclusive as both factors had an overall weak impact on the aroma composition and sensory profile of the wines. However, wines fermented at 25 °C were judged slightly less amylic and more complex. Minor differences were observed between wines made from distinct clarification levels, and these were limited to mouthfeel and taste perception.

In relation with amino acid extraction, thermal degradation and volatilization of aroma compounds, the heating temperatures could be adjusted to modulate the volatile composition of thermovinified wines. Thermovinification was originally used on botrytized grapes to destroy laccase whose activity increases with temperature up to its denatura tion point of 60 °C. This is the main reason why the heating of rotten grapes must be done very quickly at a temperature above 70 °C (Ribéreau Gayon, Dubourdieu, Donèche, & Lonvaud, 2005). Nowadays thermovinification is mainly employed on grapes of perfect sanitary status without *Botrytis cinerea* and new ranges of temperature, espe cially below 60 °C, deserve to be investigated. As a decrease in the heating temperature is likely to impact the level of phenolic compounds in wine, the heating time would need to be adapted.

The purpose of the present work is to study the impact of the temperature and the heating time on the analytical and volatile com position of thermovinified musts and wines. In 2014 and 2015, two temperature levels (50 °C and 75 °C) and heating times (30 min and 3 h) were investigated in duplicate at the laboratory scale on Carignan grapes sourced in Spain.

2. Material and methods

2.1. Grapes and vineyard location

The experiment was carried out with *Vitis vinifera* L. cv. Carignan grapes collected in the Spanish region of Catalonia in the Penedès Protected Designation of Origin (PDO) area. The vineyard (lat. 41° 25′ 4.80″′ N; long. 01° 37′ 21.79″′ E) was non irrigated and goblet trained with 2.40 m × 1.40 m vine spacing and a moderate production level (6 8 t/ha). 10 kg of grapes were hand harvested on 6 October in 2014 and on 25 September in 2015 in 1 case of 20 kg. The grapes were then destemmed manually, mixed gently and divided into eight homogenous lots of 1000 g.

2.2. Pre fermentation heat treatments

In 2014 and 2015, each pre fermentation heat treatment was re plicated twice. 1000 g of berry samples were crushed and poured into a 1 L Erlenmeyer flask (with a perforated lid to evacuate carbon dioxide during fermentation), and sulfur dioxide (40 mg/L) was added using a 10% bisulfite liquid solution. The heating was carried out at two tem perature levels (50 °C and 75 °C) using a water bath system. The rise in temperature of the grapes from room temperature up to the desired temperature was fast, taking exactly 40 min. For each target tempera ture, the heating was maintained for 30 min for 2 out of 4 samples and for 3 h for the remaining flasks. The grapes were then pressed at a high temperature under controlled conditions (200 kPa for 2 min) using a laboratory press (Paul Arauner GmbH, Kitzingen, Germany). The weight of must at pressing was measured and the extraction rate (%) expressed as the weight of must (g) obtained from the pressing of 100 g of berries was calculated. The musts were centrifuged (14,000 × *g* for 6 min) and 200 mL were sampled to perform classical enological ana lysis and determination of polyfunctional thiols precursors. To avoid any bias due to distinct levels of clarification between the studied treatments, turbidity was controlled using a 2100AN turbidimeter (Hachlange, Düsseldorf, Germany). After centrifugation, differences in turbidity between the samples were weak; the average value was 87 NTU ± 13. The musts were then inoculated with 200 mg/L of rehy drated active dried *Saccharomyces cerevisiae* yeast (Anchor NT116®, La Littorale, Servian, France). To promote the production of wine with a varietal and complex sensory profile, the musts were fermented at 25 °C for 12 days. The kinetics of fermentation was monitored daily by manual weighing of the flasks. After that period, the wines were cen trifuged (14,000 × *g* for 6 min) and received a sulfite addition of 80 mg/ L. After bottling into 200 mL bottles, the samples were stored at 4 °C until the aroma composition analysis.

2.3. Must analysis

2.3.1. Conventional enological analysis

Conventional enological parameters were determined after one day. The sugar concentration (°Brix) was determined with a digital hand held Pocket refractometer PAL (Atago, Japan) and the pH with a Titromatic pHmeter (Hachlange, Düsseldorf, Germany). The titratable acidity was measured following the OIV method (2009). A Konelab Arena 20 sequential analyzer (Thermo Electron Corporation, Waltham, USA) associated with enzyme kits provided by several suppliers was used to determine amino acids, ammonium (Megazyme, Ireland) and malic acid (Thermo Fisher Scientific, Waltham, USA). Potassium de termination was done by flame photometry (Bio Arrow, France) fol lowing the OIV method (2009) and tartaric acid determination by colorimetric titration (Hill & Caputi, 2009). Anthocyanins and the Total Phenolic Index (TPI) were quantified following the techniques de scribed by Ribéreau Gayon and Stonestreet (1965) and Ribéreau Gayon (1970), respectively, using an Evolution 100 spectrophotometer (Thermo Electron Corporation, Waltham, USA). Absorbance was mea sured at 420, 520 and 620 nm and Color Intensity was calculated by summing the three color components (A420 yellow, A520 red, and A620 blue). All determinations were carried out in duplicate.

2.3.2. Precursors of 3 mercaptohexanol and 4 mercapto 4 methyl 2 pentanone

Four precursors of polyfunctional varietal mercaptans were ana lyzed following the procedure validated by Concejero, Peña Gallego,

Fernandez Zurbano, Hernández Orte, and Ferreira (2014): cysteine 4 mercapto 4 methyl 2 pentanone (CYSMP), glutathione 4 mercapto 4 methyl 2 pentanone (GLUMP), cysteine 3 mercaptohexan 1 ol (CYSMH) and glutathione 3 mercaptohexan 1 ol (GLUMH).

2.4. Chemical quantitative analysis of volatile compounds

Several families of volatile compounds were analyzed in the wine with three different analytical methods. The analyses were performed in two different years but during the same period of each year to reduce potential variations associated with different post bottling times. 3 isobutyl 2 methoxypyrazine (IBMP) and 2 isopropyl 3 methoxypyr azine (IPMP) were not analyzed since preliminary analysis revealed that these compounds were virtually absent from wines made with Carignan.

2.4.1. Major compounds (Liquid Liquid microextraction and GC FID Analysis)

The quantitative analysis of major compounds was carried out using a validated and published method (Ortega, Lopez, Cacho, & Ferreira, 2001). In accordance with this method, 3 mL of wine containing the internal standards IS (2 butanol, 4 methyl 2 pentanol, 4 hydroxy 4 methyl 2 pentanone, and 2 octanol) and 7 mL of water were salted with 4.5 g of ammonium sulfate and extracted with 0.2 mL of di chloromethane. The extract was then analyzed by GC with FID detec tion. The area of each analyte was normalized by that of its corre sponding IS and was then interpolated in the corresponding calibration plot built by applying exactly the same analytical method as that ap plied to synthetic wines containing known amounts of the analytes covering the natural range of occurrence of these compounds. Details are given in the reference.

2.4.2. Minor compounds (SPE and GC Ion Trap MS Analysis)

This analysis was carried out using the method proposed by Lopez, Aznar, Cacho, & Ferreira (2002). In accordance with the method, 50 mL of wine, containing 25 µL of BHA solution and 75 µL of a surrogate standards solution (3 octanone, β damascone, heptanoic acid, and iso propyl propanoate), were passed through a LiChrolut EN (Merck, Darmstadt, Germany) 200 mg cartridge at a rate of about 2 mL/min. The sorbent was dried under nitrogen stream (purity 99.999%). Analytes were recovered by elution with 1.3 mL of dichloromethane. 25 µL of an internal standard solution (4 hydroxy 4 methyl 2 penta none and 2 octanol, both at 300 mg per g of dichloromethane) were added to the eluted sample. The extract was then analyzed by GC with ion trap MS detection under the conditions described in the reference.

2.4.3. Polyfunctional mercaptans (SPE and GC NCI MS Analysis)

This analysis was carried out using the method first proposed by Mateo Vivaracho, Cacho, & Ferreira (2008) and further improved by Mateo Vivaracho, Zapata, Cacho, & Ferreira (2010). First, 0.2 g of ethylenediaminetetracetic acid and 0.6 g of L cysteine chlorohydrate were added to 25 mL of wine. This sample mixture was then transferred to a 20 mL volumetric flask where it was spiked with 15 µL of an ethanolic solution containing 1400 µg/L of 2 phenylethanethiol as in ternal standard (IS). The complete volume was then transferred into a 24 mL screw capped vial together with 0.2 g of *O* methylhydrox ylamine, shaken for 15 s, purged with pure nitrogen (99.999%), sealed and incubated in a water bath at 55 °C for 45 min. Six milliliters of this incubated sample were then loaded into a 50 mg Bond Elut ENV SPE cartridge (Varian, Walnut Creek, USA). Major wine volatiles were re moved by rinsing with 4 mL of a 40% methanol water solution 0.2 M in phosphate buffer at pH 7.7. A second internal standard was also loaded into the cartridge by passing it through 220 µL of solution (20 µL of 4 methoxy α toluenethiol, 150 µg/L in ethanol and 200 µL water). Mer captans retained in the cartridge were directly derivatized by passing 1 mL of an aqueous solution of DBU (6.7%) and 50 µL of a 2000 mg/L solution of PFBBr in hexane, and letting the cartridge become imbibed with the reagent for 20 min at room temperature (25 °C). The remaining derivatizing agent was removed by addition of 100 µL of 2000 mg/L mercaptoglycerol in an aqueous solution containing 6.7% DBU, and letting the reaction take place for another 20 min at room temperature. The cartridge was further rinsed with 4 mL of 0.2 M H_3PO_4 in water containing 40% methanol (v/v) and 1 mL of water. Derivatized analytes were eluted with 600 µL of a solvent mixture (hexane 25% in diethyl ether), spiked with 10 µL of chromatographic internal standard (Octa fluoronaphthalene OFN 22.5 µL/L in hexane). The extract was finally washed with 5 × 1 mL fractions of brine (200 g/L NaCl in water). 4 µL of this sample were directly injected in cold splitless mode into the GC negative chemical ionization MS system.

2.5. Statistical analysis

Statistical analyses including regression tests were conducted with Xlstat software (Addinsoft, Paris, France). The data were subjected to a three way analysis of variance (ANOVA) treatment (vintage × heating temperature × heating time) with a first order interaction (n = 16; residual degrees of freedom = 9).

3. Results and discussion

3.1. Conventional enological parameters

Despite the pressing at high temperature that should have promoted the extraction of amino acids from the pericarp (Geffroy et al., 2015; Poux, 1974), the content in yeast assimilable nitrogen (Table 1) re flected by the sum of amino acids and ammonium was very low (less than 100 mg/L for the prefermentive heat treatment at 50 °C for 30 min in 2014). To allow this content to surpass 150 mg/L and to avoid stuck and sluggish fermentations, 300 mg/L of diammonium phosphate were added to all the flasks in 2014 and in 2015.

The three factors under study had a substantial effect on the con ventional enological parameters as 9, 9 and 10 out of 13 measured parameters were significantly impacted (at least at $P < 0.05$) by the vintage, the temperature and the heating time, respectively. Several significant interactions were noticed indicating that the extractability of berry components under the effect of temperature and heating time is complex and strongly dependent on the level of maturity of the grapes.

The 2014 vintage was characterized by a dry spring, a mild summer and rainy conditions during the ripening period. 2015 was an early vintage with warm dry weather conditions throughout the grapevine vegetative cycle, especially in July when a severe 25 day heat wave occurred. Consequently the hot dry conditions in 2015 were more fa vorable to early maturity with higher sugar concentrations and lower levels of titratable acidity and malic acid. For this vintage, the extrac tion rate was also lower which suggests that the berries were smaller and contained a lesser quantity of juice. Surprisingly, the phenolic concentrations in the experimental musts as reflected by anthocyanins and TPI were weaker in 2015 than in 2014. It has been documented that high temperature and increased water deficit up to a certain level enhanced the phenolic accumulation in berries (Jackson & Lombard, 1993). The excessive heat experienced in July 2015 after berry set with temperatures largely above 30 °C might have contributed to lower an thocyanin and proanthocyanidin synthesis (Mira de Orduña, 2010). In addition, differences in the extractability of phenolic compounds at harvest might have played a role. Distinct concentrations in must ni trogen and potassium contents might be the consequence of differences in climatic conditions impacting the mineralization of soil organic matter and assimilation, or differences in fertilization practices between the two vintages under study.

In most cases the heating temperature induced a significant increase in sugar concentration, pH, amino acids and ammonium. Differences in the localization of organic acids, sugar and potassium have been

Table 1
Results of three-way analysis of variance and impact of the heating conditions on skin to juice ratio and conventional enological parameters measured in musts.

Parameter	2014				2015				*P*-value					
	50 °C		75 °C		50 °C		75 °C		Vintage (V)	Temperature (Te)	Time (Ti)	V × Te	V × Ti	Te × Ti
	30 min	3 h	30 min	3 h	30 min	3 h	30 min	3 h						
Sugar concentration (°Brix)	21.5[a]	22.3	21.7	22.1	23.8	23.8	24.4	24.9	< 0.001	0.041	0.041	0.041	0.449	0.798
Titratable acidity (g/L tartaric acid)	6.97	7.16	6.89	7.10	6.42	6.36	6.49	6.52	< 0.001	0.597	0.062	0.059	0.036	0.574
pH	3.22	3.22	3.24	3.24	3.21	3.24	3.26	3.26	0.078	0.001	0.146	0.146	0.447	0.264
Tartaric acid (g/L)	3.91	3.01	2.84	3.54	4.64	3.64	2.94	2.81	0.073	< 0.001	0.005	< 0.001	0.027	< 0.001
Malic acid (g/L)	3.52	3.44	3.35	3.46	2.40	2.18	2.18	2.23	< 0.001	0.056	0.339	0.951	0.199	0.013
Potassium (g/L)	1.80	1.86	1.78	1.89	1.69	1.68	1.64	1.75	< 0.001	0.714	0.013	0.922	0.513	0.082
Amino acids (mg/L)	91	120	123	146	91	133	159	178	< 0.001	< 0.001	< 0.001	< 0.001	0.226	0.004
Ammonium (mg/L)	3.6	3.1	8.3	18.2	14.7	18.9	17.0	18.9	< 0.001	0.002	0.011	0.001	0.523	0.132
Anthocyanins (mg/L)	952	1448	1483	1329	495	1004	1232	1193	< 0.001	< 0.001	< 0.001	< 0.001	0.095	< 0.001
Total Phenolic Index (TPI)	51.7	68.0	70.4	80.8	33.2	55.2	67.5	80.8	< 0.001	< 0.001	< 0.001	< 0.001	0.059	0.005
Color Intensity (A420 + A520 + A620)	31.5	47.6	48.6	44.3	19.6	42.4	52.2	51.7	0.070	< 0.001	< 0.001	< 0.001	0.007	< 0.001
Extraction rate (%)	64.1	68.1	68.8	70.3	51.1	60.6	56.0	56.1	< 0.001	0.203	0.019	0.263	0.457	0.052

[a] Mean of two replicates.

previously reported (Possner & Kliewer, 1985). Larger concentrations of glucose, fructose and potassium and lower levels of tartaric acid have been reported 120 days after anthesis in the skin and/or in the outer part of the pulp in comparison with the inner part of the pulp and the area around the seeds. When crushing and pressing grapes, juices from the center of the berries were first released. Then, through the fragili zation and destruction of cell membranes, the heating must have pro moted the extraction of barely extractable juice from the periphery and the release of components from the skin into the musts. This led to an enrichment of the must in sugar and potassium and to a dilution in tartaric acid. The fact that no significant increase was observed for potassium can be explained by a higher precipitation of potassium tartrate which also contributed to intensify the decrease in tartaric acid and induced a clear increase in pH. In accordance with previous find ings (Geffroy et al., 2015; Poux, 1974), the heating temperature en hanced the extraction of amino acids and to a lesser extent of ammo nium contained in the pericarp. For anthocyanins, TPI and color intensity, the same increase was observed in most cases. Surprisingly, the musts obtained in 2014 from grapes heated at 50 °C for 3 h had a higher level of anthocyanins and color intensity than those treated at 75 °C for the same period, which suggests thermal degradation. Patras, Brunton, O'Donnell, and Tiwari (2010) demonstrated that anthocyanins were significantly affected in blackberry and strawberry puree by thermal process treatments of 70 °C during holding times of 2 min. The reasons why this phenomenon was not observed in the 2015 must re main unclear. However, we can assume that the anthocyanin con centration in thermovinified musts is the result of extraction from the skin, thermal degradation and enzymatic degradations through poly phenol oxidase. In our experimental conditions, we can assume that enzymatic oxidation usually inhibited from 50 °C to 60 °C was limited in both temperature treatments. To suffer from degradation, anthocyanins need first to be solubilized. The lesser extractability of anthocyanins in 2015 as reflected by the levels in musts from grapes heated at 50 °C for 30 min might have contributed to reducing the time spent in solubilized form and therefore have limited their thermal degradation.

As expected, the longer heating time improved the extraction rate and generally enhanced the extraction of components located in the pericarp or in the outer part of the pulp such as sugars, potassium, amino acids, ammonium, TPI, anthocyanins and color intensity (except in 2014 at 75 °C for the reasons previously discussed). This could not be always confirmed for tartaric acid, an acid involved together with po tassium in salt precipitation mechanisms and for which several first order interactions were noticed. When the extension of the heating time led to a decrease in tartaric acid, we can suppose that the excess of tartaric acid has been precipitated by the excess of potassium. The fact that potassium was not lowered suggests that its extractability was higher than those of tartaric under an increase of the heating time.

It is important to emphasize that in 2014, the vintage with the higher extractability of phenolic compounds, the musts obtained from grapes heated at 50 °C for 3 h had a similar level of anthocyanins, TPI and color intensity as those treated at 75° for 30 min. This finding de monstrates that in some vintages the reduction of the heating tem perature can be compensated for through an extension of the heating time.

3.2. Aroma chemical composition

Sixty four compounds from 11 chemical families were analyzed and detected in the 16 experimental wines produced. The results of the three way analysis of variance and the effect of the heating conditions on the aroma composition of the wines are shown in Table 2.

Of all the factors studied, the vintage had the greatest effect on the aroma chemical composition of the wines as 49 out of 64 parameters were impacted. It is commonly accepted that the vintage, owing to varying climatic conditions, has a major influence on fruit quality and aroma composition. Without being exhaustive, several studies have confirmed this observation analytically for white grape varieties (Schneider, Razungles, Augier, & Baumes, 2001) and for red wines made with traditional winemaking techniques (Kotseridis, Beloqui, Bertrand, & Doazan, 1998) and after a pre fermentative heat treatment of the grapes (Geffroy et al., 2015). Under our experimental conditions, the vintage factor significantly impacted most of the grape derived aroma compounds belonging to the terpenol and norisoprenoid, phenol, vanillin derivate, cinnamate, polyfunctional mercaptan and lactone chemical families. The reasons why changes in the fermentative aroma compounds (i.e. ethyl esters, acetates, acids and alcohols) were also noticed deserve further comment. Previous works highlighted that the amino acid composition in must had an impact on the production of fermentative volatile compounds in a model wine (Hernández Orte, Cacho, & Ferreira, 2002). It has been documented that the vintage and environmental conditions modified the amino acid profile of grapes and that a high Yeast Assimilable Nitrogen (YAN) must promoted the pro duction of ethyl esters, acetates, acids, carbonyl compounds and, in contrast, limited the synthesis of fusel alcohol (Bell & Henschke, 2005). In accordance with these findings, the same conclusions could be drawn on our data in most cases in 2015, the vintage with the larger YAN level.

30 and 14 variables were significantly impacted for temperature

Table 2
Results of three-way analysis of variance and impact of the heating conditions on the aroma composition of wines.

Parameter	2014				2015				*P*-value					
	50 °C		75 °C		50 °C		75 °C		Vintage (V)	Temperature (Te)	Time (Ti)	V × Te	V × Ti	Te × Ti
	30 min	3 h	30 min	3 h	30 min	3 h	30 min	3 h						
Ethyl esters (mg/L)														
Ethyl butyrate	0.05[a]	0.05	0.07	0.08	0.15	0.15	0.100	0.100	0.001	0.283	0.911	0.019	0.920	0.723
Ethyl 2-methylbutyrate	7.27	6.01	5.39	6.42	10.30	10.18	8.84	9.74	< 0.001	0.216	0.830	0.868	0.702	0.221
Ethyl isobutyrate	20.9	24.9	24.3	16.3	161.3	112.3	181.0	256.8	< 0.001	0.018	0.701	0.013	0.599	0.072
Ethyl hexanoate	0.19	0.23	0.20	0.20	0.25	0.21	0.59	0.62	0.001	0.002	0.834	0.002	0.773	0.865
Ethyl lactate	36.14	3.56	2.94	3.25	3.81	3.90	3.57	3.78	0.232	0.193	0.216	0.201	0.208	0.203
Ethyl octanoate	0.08	0.07	nd	nd	0.24	0.16	0.22	0.26	< 0.001	0.627	0.675	0.114	0.834	0.324
Diethyl succinate	0.23	0.34	0.20	0.15	0.22	0.24	0.29	0.24	0.440	0.138	0.632	0.011	0.360	0.033
Ethyl isovalerate	6.3	75.1	84.0	47.0	4.54	21.7	87.7	69.4	0.367	< 0.001	0.343	0.027	0.310	0.001
Acetates (mg/L)														
Ethyl acetate	15.4	19.0	17.4	18.8	37.8	40.7	33.6	34.8	< 0.001	0.412	0.373	0.239	0.925	0.692
Hexyl acetate	nd	nd	nd	nd	0.022	0.021	0.007	0.007	< 0.001	0.013	0.916	0.013	0.916	0.898
Isoamyl acetate	0.37	0.22	0.37	0.54	1.01	0.91	0.41	0.51	0.025	0.238	0.920	0.026	0.915	0.362
Isobutyl acetate	9.7	7.1	10.9	15.4	12.9	11.1	6.1	7.4	0.429	0.904	0.833	0.016	0.728	0.166
Butyl acetate	2.0	2.7	2.0	2.8	14.5	14.9	13.7	13.9	< 0.001	0.542	0.443	0.511	0.733	0.978
Linalol acetate	0.64	0.63	0.69	0.88	1.74	1.81	1.68	1.68	< 0.001	0.742	0.456	0.152	0.729	0.680
Phenylethyl acetate	42.3	20.6	30.3	57.7	135.6	146.8	52.1	102.6	0.001	0.092	0.247	0.020	0.329	0.138
Acids (mg/L)														
Acetic acid	241	483	207	288	118	154	276	362	0.275	0.621	0.129	0.053	0.474	0.690
Butyric acid	0.27	0.24	0.26	0.32	0.43	0.44	0.32	0.36	0.001	0.215	0.372	0.018	0.913	0.170
Isobutyric acid	1.47	0.80	1.02	1.19	0.97	0.73	0.64	0.76	0.008	0.395	0.175	0.578	0.371	0.018
Isovaleric acid	1.67	1.43	1.12	1.61	1.12	0.86	0.73	0.87	< 0.001	0.077	0.751	0.968	0.364	0.014
Hexanoic acid	1.04	0.99	0.89	1.13	1.81	1.87	1.31	1.45	0.001	0.101	0.480	0.112	0.996	0.490
Octanoic acid	1.54	1.18	1.25	1.36	4.27	4.75	2.86	3.24	< 0.001	0.047	0.653	0.061	0.419	0.784
Decanoic acid	0.61	0.58	0.40	0.49	0.49	0.46	0.74	0.58	0.673	0.893	0.760	0.174	0.584	0.987
Alcohols (mg/L)														
Isoamyl alcohol	143	144	118	138	146	123	92	96	0.007	0.002	0.937	0.089	0.142	0.095
Benzyl alcohol	0.035	0.043	0.022	0.027	0.020	0.023	0.028	0.027	< 0.001	0.002	0.006	< 0.001	0.022	0.136
Methionol	1.89	0.92	0.59	0.91	1.99	1.57	0.65	1.00	0.035	< 0.001	0.073	0.132	0.138	< 0.001
1-hexanol	0.55	0.69	0.66	0.49	0.34	0.30	0.33	0.28	< 0.001	0.403	0.319	0.684	0.675	0.040
Cis-3-hexenol	0.045	0.054	0.047	0.041	0.065	0.052	0.058	0.050	0.001	0.038	0.047	0.743	0.018	0.280
1-butanol	0.48	0.65	0.53	0.58	0.73	0.80	0.76	0.76	< 0.001	0.753	< 0.001	0.752	0.032	0.008
β-phenylethanol	26.4	25.6	19.1	26.2	31.1	26.5	21.4	23.9	0.447	0.023	0.560	0.441	0.256	0.057
Isobutanol	30.1	27.2	25.6	30.8	12.5	10.4	8.8	9.9	< 0.001	0.263	0.740	0.456	0.467	0.026
Carbonyl compounds (mg/L)														
Diacetyl	2.3	3.5	2.5	1.7	nd	3.1	11.2	10.7	< 0.001	< 0.001	0.231	< 0.001	0.384	0.048
Syringaldehyde	0.99	0.97	0.73	0.62	0.39	nd	0.40	0.38	< 0.001	0.502	0.125	0.013	0.381	0.391
Acetoin	7.9	12.7	19.0	6.3	3.3	6.2	17.7	10.1	0.318	0.020	0.158	0.135	0.711	0.080
Acetaldehyde	9.9	12.9	9.7	6.9	4.3	6.8	22.6	16.6	0.002	< 0.001	0.233	< 0.001	0.198	< 0.001
Benzaldehyde	2.5	2.4	2.2	2.0	10.0	8.3	11.6	10.6	< 0.001	0.077	0.092	0.021	0.168	0.724
Terpenols and norisoprenoids (μg/L)														
Geraniol	56.3	51.7	44.7	50.2	5.6	8.4	5.8	5.3	< 0.001	0.022	0.619	0.115	0.820	0.270
β-citronellol	8.65	8.67	6.94	7.79	5.35	4.28	4.62	3.57	< 0.001	0.024	0.425	0.459	0.077	0.579
α-terpineol	6.89	6.01	6.05	8.42	2.84	3.02	3.18	3.95	< 0.001	0.025	0.047	0.783	0.617	0.006
Linalool	20.5	17.9	18.8	19.9	15.4	14.5	14.3	14.9	< 0.001	0.890	0.547	0.750	0.689	0.117
β-damascenone	5.42	3.21	2.43	1.11	6.53	5.21	3.97	2.34	< 0.001	< 0.001	< 0.001	0.594	0.379	0.380
α-ionone	nd	nd	nd	nd	0.027	0.060	0.049	0.078	0.004	0.493	0.290	0.493	0.290	0.945
β-ionone	0.32	0.29	0.32	0.39	0.33	0.34	0.35	0.31	0.990	0.364	0.878	0.226	0.494	0.574
Phenols (μg/L)														
Guaiacol	4.01	3.71	7.68	9.86	2.92	3.42	3.33	4.17	0.001	0.001	0.218	0.006	0.827	0.276
Eugenol	1.40	1.86	1.49	1.80	0.87	1.07	0.94	1.04	< 0.001	0.782	0.001	0.944	0.070	0.307
4-allyl-2,6-dimetoxyphenol	3.21	5.32	3.98	6.43	1.80	2.40	2.38	2.75	< 0.001	0.010	< 0.001	0.303	0.002	0.910
2,6-dimetoxyphenol	14.7	19.8	31.1	74.4	14.2	20.7	22.4	45.7	0.017	< 0.001	< 0.001	0.016	0.179	0.002
4-vinylguaiacol	17.6	25.4	25.0	41.5	17.1	23.3	27.6	37.1	0.470	< 0.001	< 0.001	0.909	0.163	0.069
4-vinylphenol	38.3	37.7	47.8	72.0	30.0	40.1	29.4	32.4	0.056	0.252	0.239	0.108	0.727	0.561
4-ethylphenol	0.055	0.079	0.180	0.190	0.149	0.194	0.193	0.211	0.044	0.020	0.370	0.128	0.787	0.699
o-cresol	0.32	0.41	0.17	0.37	0.30	0.26	0.31	0.28	0.507	0.418	0.253	0.271	0.091	0.509
m-cresol	nd	1.46	1.44	1.53	nd	nd	nd	nd	< 0.001	0.012	0.011	0.012	0.011	0.020
Vanillin derivates (μg/L)														
Vanillin	5.06	2.64	2.79	2.61	6.20	3.85	4.33	4.87	0.001	0.026	0.005	0.250	0.519	0.002
Methyl vanillate	32.3	38.3	39.8	40.6	10.6	11.7	12.1	11.5	< 0.001	0.043	0.153	0.104	0.217	0.173
Ethyl vanillate	17.4	12.6	6.9	7.3	13.2	10.7	9.8	8.6	0.684	0.001	0.096	0.043	0.890	0.165
Acetovanillone	42.6	50.2	54.0	61.6	34.0	41.6	44.9	47.4	0.001	0.001	0.011	0.456	0.533	0.548
Cinnamates (μg/L)														
Ethyl dihydrocinnamate	0.28	0.29	0.39	0.52	0.80	0.69	0.54	0.57	0.001	0.865	0.756	0.008	0.361	0.265
Polyfunctional mercaptans (ng/L)														

(*continued on next page*)

Table 2 (*continued*)

Parameter	2014				2015				*P*-value					
	50 °C		75 °C		50 °C		75 °C		Vintage (V)	Temperature (Te)	Time (Ti)	V × Te	V × Ti	Te × Ti
	30 min	3 h	30 min	3 h	30 min	3 h	30 min	3 h						
2-furfurylthiol	2.52	2.08	1.96	1.16	3.76	4.35	4.84	2.86	0.004	0.382	0.235	0.618	0.946	0.189
2-methyl-3-furanthiol	235	126	119	61	718	790	672	384	< 0.001	0.021	0.125	0.263	0.832	0.205
Benzylmercaptan	5.41	5.48	4.80	0.81	0.63	4.32	3.22	2.62	0.020	0.059	0.690	0.014	0.007	0.003
3-mercaptohexanol	2395	2188	1131	613	870	2220	2803	2045	0.106	0.259	0.886	0.001	0.176	0.025
Log(3-mercaptohexanol)	3.38	3.34	3.05	2.79	2.94	3.35	3.45	3.31	0.023	0.048	0.858	< 0.001	0.011	0.002
4-mercapto-4-methyl-2-pentanone	7.3	8.0	14.0	20.5	nd	nd	nd	11.2	< 0.001	0.001	0.016	0.230	0.530	0.023
3-mercaptohexyl acetate	11.0	nd	nd	nd	11.0	35.0	19.7	15.3	0.006	0.291	0.674	1.000	0.154	0.396
Lactones (μg/L)														
γ-butirolactone	3.99	3.33	2.80	4.03	3.30	3.49	3.47	4.06	0.811	0.714	0.076	0.102	0.759	0.008
γ-nonalactone	2.15	4.75	5.62	6.05	1.02	1.37	2.23	3.12	< 0.001	0.001	0.020	0.265	0.274	0.314

nd. not detected.

[a] Mean of two replicates.

and heating time, respectively. This indicates that temperature has a greater effect than heating time on the aroma composition of the wines. In most cases, and especially for free aroma compounds extracted from the berry skin or volatiles originating from precursors mainly located in the pericarp, the extension of the heating period led to wines with a higher concentration by promoting their extraction.

Surprisingly, the heating temperature did not induce any changes in ethyl esters, acetates or acids with the exception of ethyl isobutyrate, ethyl hexanoate, ethyl isovalerate, hexyl acetate and octanoic acid. For the reasons previously discussed, the increase in YAN induced by this factor should have promoted the production of most of these com pounds. This might be explained by the low initial nitrogen level of the musts and the addition of diammonium phosphate (300 mg/L) to all the flasks, which contributed to reducing the percentage difference in YAN between the treatments under study. The impact of the temperature on carbonyls (diacetyl, syringaldehyde, acetaldehyde) remains difficult to discern as significant interactions involving the vintage and tempera ture factors were observed. For each of these compounds, an increase or decrease recorded in the first year of study was followed by the opposite behavior in the second.

Differences in monoterpenols were noted between the temperature treatments. These aroma compounds originate from free and glycosi dically bound forms mainly located in the berry skin (Gomez, Martinez, & Laencina, 1994). While the increase in temperature should have led to an overall increase in monoterpenols, the wines obtained from grapes heated at 75 °C had a lower level of geraniol, β citronellol and a higher concentration of α terpineol. Precursors (bound forms) are released through grape derived and yeast β glycosidase enzymes (Robinson et al., 2014). If the decrease in geraniol and β citronellol can be ex plained by the destruction of the endogenous enzyme activity by de naturation, the increase in α terpineol deserves further comments. Higher levels of this monoterpenol could be the consequence of the thermal degradation of geraniol and β citronellol, a phenomenon pre viously observed when grapes undergo a prefermentation heat treat ment at 70 °C (Geffroy et al., 2015). As the odor thresholds of β ci tronellol (100 μg/L) and geraniol (30 μg/L) are lower than that of α terpineol (250 μg/L), this degradation is likely to have an overall de preciative impact on the sensory characteristics of wine (Ferreira, Lopez, and Aznar (2002)).

The β damascenone content in red wine is generally around 1 1.5 μg/L (Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2007). The concentrations found in our experimental wines, especially those made from grapes heated at 50 °C, are to the best of our knowledge among the highest ever reported in the literature. In accordance with previous research into thermovinification (Geffroy et al., 2015), the temperature had a depreciative effect on β damascenone, which has been described as an enhancer of fruity aromas in red wine (Pineau et al., 2007). This aroma compound is produced from the neoxanthin carotenoid mostly found in the skin of mature grapes by both enzymatic and non enzy matic reactions (Mendes Pinto, 2009). After an initial dioxygenase cleavage, the products undergo an enzymatic transformation to give the non aroma intermediate metabolites which are then converted through acid catalyze into β damascenone. Despite a likely initial larger ex traction of carotenoids from the pericarp under the heat effect, the denaturation of the carotenoid cleavage dioxygenase at 75 °C might have contributed to limit the production of β damascenone from its precursor. The fact that this variable was also impacted by the heating time independently of the temperature tends to strengthen this suppo sition, and the hypothesis of thermal degradation can be discarded. It also suggests that the enzyme is sensitive to a temperature of 50 °C when the heating is maintained for an extended period.

The lower concentrations of 4 ethylphenol and *m* cresol combined with higher levels of guaiacol, 4 allyl 2,6 dimetoxyphenol, and 2,6 di metoxyphenol in wines from grapes heated at 75 °C reflects a thermal degradation of phenols. Indeed, the three latter compounds are known end products of phenolic degradation (Michałowicz, & Duda, 2007).

Vanillin, methyl vanillate, ethyl vanillate and acetovanillone are components originating from the ligneous material in the berries (Pearl, 1942). As the grapes were destemmed, these compounds were mainly extracted from the seeds. The increase in temperature should have promoted their extraction but this was not obvious in our experimental conditions. A significant decrease in vanillin and ethyl vanillate com bined with an increase in methyl vanillate and acetovanillone was ob served with an increase in temperature.

Despite major differences in 3 mercaptohexanol (3MH) concentra tion between the treatments under study, none of the main factors had a significant impact on this key molecule involved in the varietal char acter of wine. A significant interaction involving the temperature and vintage factors was observed and the residuals did not have a normal distribution, which suggests the existence of multiplicative factors. As proposed by Berry (1987), a logarithmic transformation of the variable was performed to convert the multiplicative session factor into an ad ditive component. Data treatment using a 3 way ANOVA highlighted a significant effect of vintage and temperature with significant first order interactions. In some experimental wines, 3MH concentrations ex ceeded 2000 ng/L and were in the same range as those found in Sau vignon blanc wines (Lund et al., 2009). Considering the perception threshold of 60 ng/L for this aroma compound (Tominaga, Baltenweck Guyot, Gachons, & Dubourdieu, 2000), 3MH should make an important contribution to wine aroma. In our experiment, the temperature had a clear depreciative impact on 3MH in 2014 while this could not be confirmed in 2015. For a given grape material, the concentration of

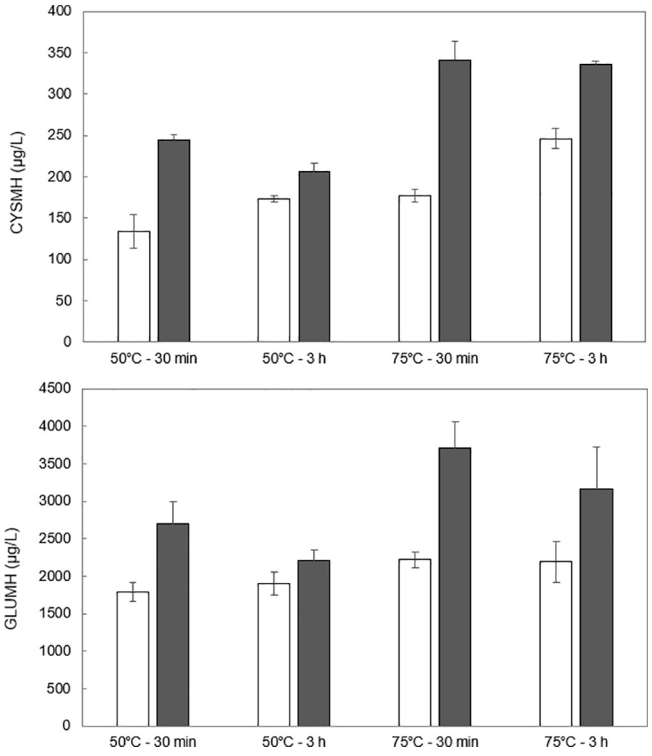

Fig. 1. Impact of the heating conditions on the precursors of 3-mercaptohexanol in 2014 (□) and in 2015 (■). Error bars represent the standard error of the mean.

3MH in wine is the result of the extraction of its precursors mostly from the berry skin, their release by the yeast β lyase (Roland, Schneider, Razungles, & Cavelier, 2011) which depends on the fermentation conditions (yeast strain, temperature, level of turbidity) and especially the nitrogen composition of the must (Subileau, Schneider, Salmon, & Degryse, 2008). It was also recently proposed that 3MH precursors might be thermally degraded by a pre fermentation heat treatment of grapes (Geffroy et al., 2015). Levels of 3MH precursors in musts (Fig. 1) were much higher than those previously reported for Sauvignon blanc, a cultivar for which 3MH is an impact odorant (Capone, Sefton, Hayasaka, & Jeffery, 2010). This is likely to explain the substantial levels of 3MH found in our experimental wines. As Cys3MH is mostly localized in the skin (Roland et al., 2011), we can suppose that the maceration at a temperature above 50 °C particularly enhanced the extraction of 3MH precursors in comparison with a typical white winemaking process which can include a short period of skin contact at a temperature below 20 °C. The analysis of 3MH precursors indicates that their extraction is significantly enhanced by the temperature at $P < 0.001$ for both compounds which allows us to reject the hypothesis of thermal degradation. In our experiments carried out on the 2014 vintage, the extension of the heating period led to musts with a higher level of precursors while in 2015 the opposite change was observed. Both precursors were impacted by the vintage at $P < 0.001$ with higher concentrations found in 2015, the vintage with the higher nitrogen status, in accordance with previous findings (Choné et al. (2006)). No relationship could be identified between 3MH in wines and CYSMH ($R^2 = 0.01$), GLUMH ($R^2 = 0.04$), and the sum of CYSMH and GLUMH ($R^2 = 0.03$) in musts. These discrepancies between the precursors and the released thiols have been found previously by other authors (Peña Gallego, Hernández Orte, Cacho, & Ferreira, 2012). This indicates the eventual presence in must of other precursors and/or suggests that differences in the aroma composition are likely to be explained by distinct fermentation conditions such as the nitrogen or aminoacidic composition (Alegre, Culleré, Ferreira, & Hernández Orte, 2017). Furthermore, *trans* caftaric acid is oxidized to *o* quinones by polyphenol oxidase (PPO) in the presence of oxygen which can favor the disappearance of varietal thiols (Roland et al., 2011). This non flavonoid phenolic compound is likely to be found at elevated levels in thermovinified wines and the inhibition of the tyrosinase (PPO) occurring at 75 °C might also have impacted the 3MH concentrations. However, this phenomenon must have a small contribution in red wines as it was highlighted that their very high concentration of tannin should make these compounds very effective at scavenging quinone electrophiles, and preventing the loss of thiols (Waterhouse, & Nikolantonaki, 2015).

4 Mercapto 4 methyl 2 pentanone (4MMP), another important contributor to varietal aroma whose perception threshold has been established at 0.8 ng/L (Tominaga et al., 2000), was systematically enhanced by a higher temperature and a longer heating time. As suggested by Roland et al. (2011), this observation tends to indicate that enzymatic mechanisms leading to the production of 4MMP and 3MH differ. The difference in localization of 3MH and 4MMP precursors within the skin or pulp of the berry (Peyrot des Gachons, Tominaga, & Dubourdieu, 2002) might also play a role.

Surprisingly, the increase in temperature led to a decrease in 2 methyl 3 furanthiol, an aroma compound imparting meaty notes formed through the thermal degradation of thiamin and through Maillard reactions (Bouchilloux, Darriet, & Dubourdieu, 1998).

γ nonalactone, a scarcely studied aroma compound with an odor reminiscent of coconut (Nakamura, Crowell, Ough, & Totsuka, 1988), was also enhanced by a higher temperature and a longer heating time. As this odorant is produced from the yeast metabolism of amino and keto acids (Etievant, 1991), this increase can be explained by the greater extraction of amino acids from the pericarp. Concentrations analyzed in experimental wines are well under the sensory threshold of the molecule (30 µg/L) and the sensory contribution of this volatile should remain weak.

Before concluding, it is important to mention that our data are valid for Carignan and might not be transposable to other grape cultivars. Indeed, it has been shown that the extractability of skin compounds (i.e. phenolic compounds, aroma precursors) from grapes that underwent a similar prefermentative heat treatment can differ between grape varieties (Geffroy et al., 2015). However, our novel findings might stimulate further studies on cultivars grown in wine regions where thermovinification is popular. Another advantage for wineries of implementing such heating would be to reduce the environmental impact and the cost of the thermovinification process by saving energy both to heat the grapes and to cool down the musts after pressing.

4. Conclusion

The present study provides an in depth characterization of the effect of temperature and heating time on the composition of two vintages of must and wine from Carignan. Among other findings, we have shown that, as expected, the heating temperature had a substantial impact on the extraction of amino acids and that probable thermal degradation of anthocyanins occurred when the grapes were heated at 75 °C. In the case of the 2014 vintage, which had the higher extractability of phenolic compounds, the reduction of the heating temperature was compensated for thanks to an extension of the heating time. The musts obtained from grapes heated at 50 °C for 3 h had a similar level of phenolic compounds as those treated at 75° for 30 min. The heating temperature had a greater effect than the heating time on the aroma composition of the wines. Surprisingly, the increase in temperature did not lead to wines with a higher level of volatile fermentative compounds. Several aroma compounds, especially grape derived molecules, were impacted by the rise in temperature, probably through degradation and enzyme denaturation mechanisms or as a consequence of the modification of fermentation conditions. Notably, wines made from grapes treated at 50 °C had in most cases higher concentrations of geraniol, β citronellol, β damascenone and 3 mercaptohexanol. This

indicates that the heating temperature can be modulated to produce wines with a substantial concentration of grape derived volatiles.

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