Changes in analytical and volatile compositions of red wines induced by pre-fermentation heat treatment of grapes

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Abstract:

Background and Aims: Pre-fermentation heat treatment is becoming more widespread in France in the production of red wines with fruity characteristics. Between 2009 and 2011, experiments were carried out on Grenache, Carignan and Fer grapes in order to characterize the changes in nitrogen content of the musts, conventional enological parameters and aroma compounds of the wines induced by pre-fermentative (?) heating of the grapes.

Methods and Results: Pre-fermentative heat treatments followed by alcoholic fermentation in liquid phase or in solid phase were compared to a standard vinification. In 2009 and 2010, we showed that a two-hour heat treatment at 70°C induced a significant loss in several grape-derived aroma compounds (terpenols, norisoprenoids and some phenols) associated with an increase in α-terpineol, guaiacol and 2,6-dimethoxyphenol, which suggests thermal degradation. A significant increase in most of the ethyl esters, in acetates and in fatty acids were observed in wines fermented in liquid phase, together with a decrease in fusel alcohols. The substantial modification in the amino acid composition of the must seems to be a crucial element for the understanding of these changes as in 2011 we recorded gains varying from +101.2% for Carignan to 200.2 % for Fer.

Conclusions: New findings on the chemical composition of musts and wines in response to heat treatments were obtained during this study. Most consistent results (?) suggest a thermal degradation of several compounds in the two pre-fermentation heat treatments.

Significance of the study: Better knowledge of the impact of heat treatments may provide the wine industry with key elements to produce fruit driven wines.

Key words: pre-fermentative heat treatment, thermovinification, conventional enological parameters, aroma compounds, nitrogen, amino acids.

Introduction

There is an increasing interest in the world wine market in red wines with fruity characteristics. As winemakers face a lack of new references and techniques to produce wine that can fulfill these expectations, old techniques developed some decades ago in a completely different context of production, such as pre-fermentation heat treatment or thermovinification, are becoming more widespread.

The first works on heat treatment of musts were conducted more than 60 years ago in California, both in the laboratory and in an artisanal manner (Berg 1950). The development of industrial heating systems in the seventies and the large number of associated research papers published in the same decade (Marteau and Olivieri 1970, Rankine 1973; Poux 1974; Humbert and Mignonac 1975; Lowe et al. 1976) reflect the strong interest of the wine industry in this technology at that time.

The volume of wine elaborated in France with this technique was estimated some years ago at 500 million liters (Escudier et al. 2008). Pre-fermentation heat treatment is spreading quickly through French vineyards and is becoming a must-use in the production of wines either with or without Protected Geographical Indication (PGI). This pre-fermentation heat treatment consists of heating grapes between 70-75°C for a length of time varying from 30 minutes to 24 hours. When heating is limited to a short time period (<1 hour), this technique is known as "thermovinification." If the heating is extended over a longer period, up to 24 hours, it is known as pre-fermentation heat treatment (Escudier et al. 2008). Heating allows the extraction of phenolic compounds in aqueous phase, mainly anthocyanins and, to a lesser extent, tannins (Girard et al. 1997). In practice, this weak tannin/anthocyanin ratio in thermovinified wines can often lead to color instability, especially when the heating is not maintained for enough time.

Heating also assists in the extraction of grape polysaccharides, responsible for roundness in wine (Doco et al. 2007). Maceration heat treatment is often associated with straight pressing at hot temperatures, clarification and fermentation in liquid phase at low temperatures around 18°C. It was originally used on botrytised grapes in order to destroy laccase activity. Another advantage of the maceration heat treatment is that it quickly eliminates pomace and therefore decreases the need for fermentation vessels in the cellar. The recent rapid development of this technique can be explained by the availability of more advanced technologies to clarify heated must, which has a high solid content. The rotary-vacuum drum and the latest generations of cross-flow filters, centrifugation and flotation are commonly used to clarify must after heating. The treatment (?) can be associated (?) with specific technologies such as flash-détente or thermo-détente that induce a rapid vacuum or a high pressure. The must is then brought back to atmospheric pressure and the variation of pressure that occurs on weakened heated skins provokes the lysis of skin cells and allows significant tannin extraction (Escudier et al. 2008). A variant of the maceration heat treatment, used to obtain a higher extraction of polyphenolic compounds, consists of fermenting grapes after heating with pomace as a standard vinification. This alternative was found to increase phenolic compounds in wine from 25% to 45% (Cottereau and Desseigne 2007).

Most recent research on this maceration technique has been focused on phenolic extraction and antioxidant potential (Atanacković et al. 2012, Fretté et al. 2012) while just a few works on aroma chemical composition have been carried out to date. Thermovinification or pre-fermentative heat treatment is known to produce wines with a standardized sensory profile often described as “banana yogurt” by winemakers. Research works on volatile composition showed that maceration heat treatment allowed the elimination of a high amount of 3-isobutyl-2-methoxypyrazine (Roujou de Boubée 2000) and that fermentation conditions of thermovinified wines particularly enhanced ester formation (Girard et al. 1997, Fisher et al., 2000, Cottereau and Desseigne 2007), while the thermal inactivation of lipoxygenase enzyme system

contributed to reducing C6-alcohols and their subsequent esters. The purpose of the present work is to investigate the changes in the analytical and volatile composition of red wines induced by the pre-fermentation heat treatment of grapes. In 2009 and 2010, pre-fermentation heat treatments associated or not associated with fermentation with pomace ? (with and without pomace?) were compared to a standard vinification of Grenache, Carignan and Fer grapes, three cultivars which are known to produce wines with different sensory profiles. In 2011, an additional trial was carried out to study the changes in nitrogen content of the must induced by the heating of the grapes.

Material and Methods

Grape varieties and vineyard locations

Carignan and Grenache grapes used for this study were obtained from two commercial vineyards located in Cariñena (Aragon, Spain). The Grenache vineyard, non-irrigated and goblet trained, was selected as representative of an area with vines older than 50 years of age with a low production level (4-6 t/ha). The Carignan grapes were sampled from a young and productive (15-20 t/ha) espalier trained vineyard equipped with an underground irrigation system. The Fer grapes were collected from a hillside dryland vineyard located in the South West of France, in the heart of the Gaillac Protected Designation of Origin (PDO) area with moderate crop yields (10-15 t/ha).

The pre-fermentation heat treatments tested on the three varieties were replicated twice in 2009 and 2010. The harvest dates were 14 Sept 2009 and 16 Sept 2010 for the Grenache grapes; 25 Sept 2009 and 8 Oct 2010 for the Carignan grapes; and 24 Sept 2009 and 1 Oct 2010 for the Fer grapes. In both years, grapes of each variety were hand harvested in 18 cases of 20 kg each. Six homogenous lots of sixty-kg were constituted in our experimental winery by randomly

collecting bunches from each case. To ensure the homogeneity of each lot, standard analyses such as Potential Alcohol, pH, TA, tartaric and malic acids, potassium and fermentable nitrogen were performed on the grape must just after crushing. These analyses confirmed the good homogeneity between the lots. The grapes were stored overnight at 4°C before being processed the following day.

Winemaking and maceration techniques

Fermentation took place in our experimental cellar (Lisle Sur Tarn, France). In 2009 and 2010, three maceration treatments were investigated in duplicate for each variety: control vinification (CTRL), pre-fermentation heat treatment with pomace (PHTS) and pre-fermentation heat treatment without pomace (PHTL).

Vinification operations were carried out using the standard procedures validated in our experimental cellar. Due to the excellent sanitary conditions of the harvests in 2009 and 2010, sulfur dioxide addition was limited to 40 mg/L using a 10% bisulfite liquid solution. Standard vinification (CTRL) was carried out at 25°C using selected dry yeasts chosen for their ability to express and optimize the aromatic potential of each variety. Strains ICV D21® (ICV, Lattes, France), ICV GRE® (ICV, Lattes, France), and Vitilevure MT® (Martin Vialatte, Epernay, France) were applied at a rate of 200 mg/L to Grenache, Carignan, and Fer, respectively. Maceration treatments were conducted after destemming and crushing. Destemming was carried out with modern vibrating equipment (Socma, Narbonne, France).

The control treatment (CTRL) was fermented (?) with the skin for 8 days until the volumetric mass reached 994 g/l. Alcoholic fermentation was carried out at a fixed time every day and the volumetric mass was measured with a mustimeter (Dujardin-Salleron, Paris, France). A single punch down per day was performed with a stainless steel manual plunger for exactly 15 seconds

until the volumetric mass of the musts reached 1000 g/L. No extraction operations were carried out after this period.

The pre-fermentation heat treatment was performed using a water bath system. The stainless steel tank fermenter containing the crushed and destemmed grapes was submerged into heated water for 3 hours. The temperature was carefully monitored during this period and the grapes were mixed every 30 minutes using a manual plunger to homogenize their temperature. Thanks to the elongated shape of the tank (height = 75 cm ; diameter = 37 cm), the rise in temperature of the grapes from room temperature up to 70°C was fast, taking exactly 1 hour. Therefore, the effective heating time at 70°C was 2 hours; a temperature/time ratio commonly used at wineries. The first treatment (PHTL) associated with the pre-fermentation heat treatment consisted in pressing the grapes while at high temperature into a 30 L beer keg. Pectolytic enzyme addition with Rapidase CB® (DSM, Heerlen, Netherlands) was carried out at 2 mg/L once the temperature of the musts reached 40°C. The musts were then cooled down within 24 hours to 0°C, and maintained at this temperature for 72 hours to allow a good clarification (150-200 NTU). The level of turbidity expressed in Nephelometric Turbidity Units (NTU) was controlled with a 2100 AN IS Turbimeter (Hach, Loveland, USA). After clarification, heating at 15°C and yeast inoculation, the musts were fermented at 18°C. In the second heat treatment (PHTS), after heating under the same conditions as for the PHTL, the tank was kept without any pressing in a cool room (0°C) for active cooling during 6 hours. Once the temperature reached 15°C, yeasts were inoculated and fermentation was carried out in contact with pomace at 25°C as in the control vinification.

After alcoholic fermentation (less than 1 g/L of glucose plus fructose), all the wines were racked in a 30 L beer keg and inoculated with Lalvin 31® lactic bacteria (Lallemand, Montreal, Canada). At the end of the malolactic fermentation (less than 0.2 g/L of malic acid), the wines were racked and 50 mg/L of sulfur dioxide was added. The wines were stored in the cellar for 3

months at room temperature which did not exceed 8°C during winter. Free sulfur levels were measured according to the iodometric method proposed by the OIV (2009) and adjusted to 25 mg/L every month over that period. Prior to bottling, the wines were tartrate stabilized (1 month at 0°C) and filtered through a cartridge filter (Pall France, Saint Germain-en- Laye, France) equipped with 5 and 1 µm filtration cartridges (Prédel, Saint-Loubès, France). The free sulfur level was adjusted to 25 mg/L and carbon dioxide to 500 mg/L at bottling. Dissolved oxygen levels were followed as a control parameter between the treatments. The wines were then bottled into 750 mL bottles, closed with screw caps and stored at 12°C before being analyzed.

Analysis of conventional enological parameters

Conventional enological parameters were determined for the bottled wines after one month. Alcohol content was measured using an Alcoquick L200 infralyser (Unisensor, Karlsruhe, Germany) and pH with a Titromatic pHmeter (Hachlange, Düsseldorf, Germany). Titratable (?) acidity was measured according to the OIV method (2009). A Konelab Arena 20 sequential analyzer (Thermo Electron Corporation, Waltham, USA) using enzyme kits provided by several suppliers was used to determine volatile acidity (Megazyme, Wicklow, Ireland) and malic acid (Thermo Fisher Scientific, Waltham, USA). Potassium determination was done by flame photometry (Bio Arrow, France) according to the OIV method (2009) and tartaric acid determination by colorimetric titration (Hill and Caputi 2009). Anthocyanins and the Total Phenolic Index (TPI) were quantified according to the techniques described by Ribéreau-Gayon and Stonestreet (1965) and Ribéreau-Gayon (1970), respectively, using an Evolution 100 spectrophotometer (Thermo Electron Corporation, Waltham, USA). Absorbance was measured at 420, 520 and 620 nm and Color Hue and Color Intensity were calculated by the A420/A520 ratio and by summing the three color components (A420-yellow, A520-red, and A620-blue), respectively. All determinations were carried out in duplicate.

Chemical quantitative analysis of volatile compounds

Several families of volatile compounds were analyzed in the bottled wine with five 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.

Major Compounds (Liquid-Liquid Microextraction and GC-FID Analysis). The quantitative analysis of major compounds was carried out using the method proposed and validated in our laboratory (Ortega et al. 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 dichloromethane. The extract was then analyzed by GC with FID detection. The area of each analyte was normalized by that of its corresponding IS and was then interpolated in the corresponding calibration plot built by applying exactly the same analytical method (as that applied to?) to synthetic wines containing known amounts of the analytes covering the natural range of occurrence of these compounds. Details are given in the reference.

Minor Compounds (SPE and GC-Ion Trap-MS Analysis). This analysis was carried out using the method proposed and validated in our laboratory (Lopez et al. 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 isopropyl 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. Twenty-five µL of an internal standard solution (4-hydroxy-4-methyl-2-pentanone 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.

Volatile sulfur compounds (VSCs) (HS-SPME-GC-PFPD Analysis). This analysis was carried out using the method proposed and validated in our laboratory (Lopez et al. 2007). In accordance with the method, saturated NaCl brine (4.9 mL) was placed in a 20 mL standard headspace vial and sealed. The vial was then purged with a 2 bar nitrogen stream (purity 99.999%) for 1 min. Immediately after this operation, 100 µL of wine sample, 5 µL of glyoxal solution (8% w/v), and 20 µL of the IS solution (ethyl methyl sulfide, 200 µg/L in methanol) were injected through the septum with a syringe. The sample was then analyzed by HS-SPME-GC with PFPD detection under the conditions described in the reference. The compounds analyzed were hydrogen sulfide (H_2S), methanethiol (MeSH) and dimethyl sulfide (DMS).

Polyfunctional mercaptans (SPE and GC-NCI-MS Analysis). This analysis was carried out using the method first proposed and further improved in our laboratory (Mateo-Vivaracho et al. 2008 and 2010). First, 0.2 g of ethylenediaminetetracetic acid and 0.6 g of L-cysteine chlorohydrate were added to twenty five 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 internal standard (IS). The complete volume was then transferred into a 24 mL screw-capped vial together with 0.2 g of *O*-methylhydroxylamine, 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 removed 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 through 220 µL of solution (20 µL of 4-methoxy-α-toluenethiol, 150 µg/L in ethanol and 200 µL water). Mercaptans retained in the cartridge were directly derivatized by passing 1mL 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 derivatizating agent was removed by addition of 100 µL of 2000 mg L^{-1} 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 (Octafluoronaphtalene -OFN- 22.5 µL L^{-1} in hexane). The extract was finally washed with 5 x 1 mL fractions of brine (200 g L^{-1} NaCl in water). Four µL of this sample were directly injected in cold Splitless mode in the GC-negative chemical ionization MS system.

Alkylmethoxypyrazines (SPE and GC-MS Analysis). This analysis was carried out using the method proposed and validated in our laboratory (Lopez et al. 2010). In accordance with the method, 25 mL of wine was spiked with 30 ng/L of internal standard (3-isopropyl-2-ethoxypyrazine). The wine pH was adjusted to 2.0 after which it was passed through an SPE cartridge (Bond Elut Plexa PCX 60 mg). The sorbent was washed with 1 mL of milli-Q water/methanol (30%) adjusted to pH 2.0 and dried for 10 min with a nitrogen stream. A second washing step with 0.5 mL of dichloromethane was performed. Afterwards, elution with triethylamine in dichloromethane (10 g/L) was carried out: the first 600 µL of the eluate was discarded and the next 200 µL of the eluate was recovered and analyzed by GC with MS detection under the conditions described in the reference. 3-isobutyl-2-methoxypyrazine (IBMP) and 2-isopropyl-3-methoxypyrazine (IPMP) were only analyzed in Fer wines since preliminary analysis revealed that these compounds were virtually absent from wines made with Grenache and Carignan. IPMP was not detected in any of the analyzed wines.

Statistical analysis

Statistical analyses were conducted with Xlstat software (Addinsoft, Paris, France). All the 2009 and 2010 analytical data were subjected to a three-way analysis of variance (ANOVA) treatment (vintage x variety x treatment) with first-order interaction (n=36; residual degrees of freedom = 18). The results presented in this article will focus on the maceration treatment factor, while the effect of the vintage and variety factors will be very briefly discussed.

Additional trial on nitrogen content of the musts

In order to study the changes in nitrogen content of the must induced by the heating of the grapes, a complementary trial in quadruplicate was carried out in 2011. For Carignan, Fer and Grenache, 12 cases of 20 kg each were sourced from the same plot as in 2009 and 2010 in order to constitute 4 homogenous lots of 60 kg. The harvest dates were 9 Sept for Grenache, 26 Sept for Fer and 3 Oct for Carignan. The grapes were processed according to the PHTL protocol described previously. The grape musts were sampled just after crushing (before sulfur addition and heating) and just after pressing at hot temperature and homogenization in a 30 L beer keg. Before performing the analyses with a Konelab Arena 20 sequential analyzer (Thermo Electron Corporation, Waltham, USA), the grape musts were centrifuged (14 000 x *g* for 6 min). Enzymatic determinations of ammonium based on its reaction with α-ketoglutaric acid were conducted using a kit provided by Thermo Electron Corporation (Waltham, USA). The amino acid analysis was carried out using the method proposed by Dukes and Butzke (1998).

Results and discussion

Conventional enological parameters

As may be expected, the analytical results obtained from conventional enological analyses revealed that the *cultivar* and to a lesser extent the *vintage* had a strong impact on the characteristics of the wines (Table 1). 10 and 7 parameters out of 10 were significantly impacted (at least at $p<0.05$) by these factors, respectively. The Grenache wines were characterized by higher total acidity, higher alcohol content (?) and higher volatile acidity as a consequence of the high-sugar fermentation level (Table 2) whereas the Fer wines had greater potassium and anthocyanin concentrations and a weaker color hue. These features lead to a more intense red and violet perception of the color of Fer wines. The Carignan wines had a lower Total Phenolic Index (TPI) which should result in a less astringent sensation on tasting. As for the *vintage* factor, the 2010 vintage was more favorable to maturity with higher levels of alcohol, pH and polyphenolics. The few significant *vintage* x *cultivar* interactions suggest that climatic conditions over the maturation period were almost similar in Spain and France even if the two studied vineyards are almost 500 km distant and separated by the Pyrenees mountain range which has a strong climatic influence.

The *treatment* also had a large impact on the conventional enological parameters as this factor impacted significantly 8 out of the 10 measured parameters. Higher levels of ethanol observed in the PHTL wines confirm previous observations made on this technique (Girard et al. 1997) which must be mainly attributed to a weaker evaporation of ethanol during the alcoholic fermentation, carried out at 18°C rather than at 25°C (Cottrell and McLellan 1986). In most cases, potassium and tartaric concentrations in the finished wines were higher in PHTL whereas PHTS was more similar to the CRTL wines. These increases might be the consequence of a larger extraction of potassium and tartaric acid found in the pericarp tissue of the berries. The differences between the PHTL and PHTS samples may be attributed to the fact that in the former case the pressing of the grapes took place prior to fermentation at high temperatures, making the extraction more efficient. Surprisingly, volatile acidity was not significantly

impacted by the *treatment* even if, as will be discussed later, the acetic and other fatty acid concentrations were increased in PHTL wines. Higher levels of amino acids in thermovinified musts obtained under conditions similar to those of the PHTL treatment carried out in our study have been previously reported by Poux (1974). The expected high yeast assimilable nitrogen (YAN) should have (?) led to an increased formation of volatile acidity in PHTL wines as reported by Bell and Henschke (2005). The higher levels of potassium and tartaric acid found in the PHTL wines modified the acid-base balance, provoking a clear increase in total acidity without affecting the pH.

For TPI, anthocyanins, color hue and color intensity, interactions were observed which indicates that polyphenolic extractability and extraction on heated grapes especially associated with pressing and fermentation in liquid phase (PHTL) is complex and depends on several parameters such as the grape variety and vintage conditions. In our experiment, extractability by heating was particularly limited on Carignan grapes. For this cultivar, polyphenolic contents of the PHTL wines, as reflected by anthocyanins, TPI and color intensity, were decreased. For Grenache and Fer, the PHTS wines had a higher TPI which is in agreement with previous research works (Cottereau and Desseigne 2007).

Aroma-chemical composition

78 compounds from 13 chemical families were analyzed in the 36 experimental wines produced. The significance (*P*-values) of *vintage*, *cultivar*, *treatment*, *vintage* x *cultivar*, *vintage* x *treatment* and *cultivar* x *treatment* for the 77 compounds detected in the bottled wines are shown in Table 3.

Of all the factors, *cultivar* had the greatest impact on the aroma-chemical composition of the wines. As different selected dry yeasts were used on each variety, this factor reflects differences

in grape-derived volatile compounds, both in the release of these compounds by the enzymatic activities of the yeasts and in the production of fermentation aroma compounds by *Saccharomyces cerevisiae*. Some major differences were observed between the cultivars among the grape-derived compounds with the highest sensory impact in wines according to Ferreira (2002). Notably, the Carignan and Fer wines were characterized by significantly higher concentrations of β-damascenone and 4-mercapto-4-methyl-2-pentanone, respectively (Table 4). The *vintage* factor also strongly impacted the concentrations in aroma compounds including those produced by yeasts such as ethyl esters, acetates and acids. As the fermentation conditions (yeast strain, clarification level of PHTL musts, temperature) were exactly the same for the two vintages, the differences observed might be the result of distinct amino acid compositions of the musts as shown in the model solutions by Hernández-Orte et al (2002) in relation to the vintage climatic characteristics. Among other key aroma compounds detected in the experimental wines and impacted by the *vintage* factor, concentrations of terpenols and norisoprenoids were in general higher in 2010 (the vintage with a better maturity) whereas polyfunctional mercaptan concentrations were lower in the wines from the same vintage.

The heating of the grapes induced substantial changes in the aroma composition. This process, in both PHTL and PHTS, had a depreciative impact on several grape-derived flavor compounds such as β-damascenone, β-ionone, β-citronellol, o- cresol, ethyl vanillate and ethyl cinnamate. In the case of PHTL, the effect was also evident in linalool, m-cresol, γ-butirolactone and γ-nonalactone. In the cases in which a precursor has been reported for the aroma compound, the decrease can be explained by the destruction of the associated enzyme activity by denaturation. However, it might not be the only possible explanation. A research work (Loscos et al. 2007) has shown that most of the previously cited aroma compounds from the terpenol and norisoprenoid chemical families could also be released by the enzymatic activity of the yeast. The higher concentrations of α-terpineol (especially in Fer wines), guaiacol and 2,6-dimethoxyphenol observed for both PHTL and PHTS treatments would be consistent with the

hypothesis of a thermal degradation of terpenols and of phenolic compounds. α -terpineol is a known linalool and β-citronellol degradation product (Maicas and Mateo 2005) and the two phenols are end products of phenolic degradation. The odor thresholds of linalool (25 µg/L) and β-citronellol (100 µg/L) are lower than α-terpineol (250 µg/L), according to Ferreira et al (2000), Etievant (1991) and Ferreira et al. (2000), respectively. In consequence, the aromatic impact of these changes might penalize the overall perception of the terpenol family. The decrease in β-damascenone and β-ionone deserve further comment, since several studies on Merlot wines and on sweet potato *Shoch,* have shown that heat treatments, moderate or through distillation, could enhance the production of β-damascenone and β-ionone (Kotseridis et al. 1999, Yoshozaki et al. 2011). In our work, when heating was performed prior to the beginning of fermentation in the aqueous phase, the same phenomenon could not be observed. This observation supports the results from the Japanese research team (Yoshozaki et al. 2011) who found that most β-damascenone in *shochu* was formed by acid hydrolysis during fermentation and/or distillation, and not during the initial steaming of the sweet potato. This is also in agreement with the assertion that the formation of β-damascenone by carotenoid chemical degradation might need a solvent such as ethanol or benzene in order to take place (Mendes-Pinto 2009). Other varietal compounds such as 3-mercaptohexanol, 4-mercapto-4-methyl-2-pentanone and 3-mercaptohexyl acetate showed no significant differences between heat treatments and controls. However, an increase in 3-mercaptohexanol, particularly due to the expected larger amino acid content and its consequence on nitrogen catabolite repression (NCR), should have been expected in the PHTL wines (Subileau et al. 2008). These findings suggest the potential involvement of other mechanisms and a possible degradation of the precursors for 3-mercaptohexanol during pre-fermentative heating. Surprisingly, the 3-isobutyl-2-methoxypyrazine concentration was not impacted by the heat treatment even though a direct thermal degradation would have been expected (Roujou de Boubée 2000).

In addition, the PHTL treatment had a very different composition in most fermentative volatile compounds due to the fact that the fermentation was carried out in liquid phase at a relatively low temperature on a high Yeast Assimilable Nitrogen (YAN) clarified must (Moreno et al. 1988). In particular, this treatment had the highest concentrations in most ethyl esters (with the exception of ethyl butyrate, ethyl isobutyrate, ethyl 2-methylbutyrate and ethyl hexanoate), acetates, fatty acids and the lowest in fusel alcohols, in accordance with previous observations (Ferreira et al, 1996, Fisher et al. 2000). As a consequence of the higher nitrogen content of the PHTL musts, a decrease in hydrogen sulfide and methanethiol should also have been expected (?) (Rauhut 2009). It is important to note that the magnitude of the differences between the CTRL and PHTL treatments was particularly large for hexanoic, octanoid and decanoic acid concentrations. Together with the observed increases in acetates, butyric acid, diacetyl and acetoin, this should help to understand the typical "banana yogurt" sensory profile of the thermovinified wines.

In most cases, the aroma composition of the PHTS wines was more similar to those of the CTRL. However, a large increase in 2-furfurylthiol, responsible at this high level of concentration for heavy toffee notes, was observed in the PHTS treatment for Fer.

Impact of heating of the grapes on nitrogen content of the must

The impact of heating the grapes followed by pressing at high temperature on the nitrogen content of the must is presented in Figure 1. The gain in amino acids induced by a 2 hour effective heating was +101 % ± 11, +200 % ± 26 and +150 % ± 11 for Carignan, Fer and Grenache, respectively. For ammonium, the increase was lower and did not exceed +16.3% ± 3.3, +15.7% ± 14.5 and +9.3% ± 8.1 for Carignan, Fer and Grenache, respectively. The largest gain was observed for Fer, the variety showing the lowest initial nitrogen concentration in the must. These observations are consistent with previous research works (Poux 1974) but much

larger in intensity as the increase reported by the same author did not surpass 2% for ammonium and 36.5 % for amino acids. These variations can be explained by differences in the implementation conditions of the heating whose duration extended up to 2 hours in our study, which is more representative of the current industry trend. This might have induced a larger nitrogen compound extraction from the berry skin. The intensity of these changes in the nitrogen composition is essential for understanding the modifications in the aroma compounds observed in PHTL wines, especially fatty acids and acetates.

Conclusion

The present study provides a chemical characterization of wines elaborated after a pre-fermentative heat treatment. In 2009 and 2010, we showed that a two-hour heat treatment at 70°C followed by pressing at high temperature (PHTL) induced some changes in the acid-base balance of the wines by higher tartaric acid and potassium extractions from the skin. A significant increase in the alcohol concentration was also observed in PHTL wines whereas modifications to the polyphenolic contents induced by heating were complex and depended on several parameters such as variety and vintage in likely relation with the level of maturity of the grapes. The heating of the grapes provoked a loss in several grape-derived aroma compounds (terpenols, norisoprenoids and some phenols) and an increase in α-terpineol, guaiacol and 2,6-dimethoxyphenol, suggesting thermal degradation. Despite the changes in amino acids induced by the heating, the concentration of 3-mercaptohexanol was not improved in the finished wines. When the heat treatment was linked with alcoholic fermentation in liquid phase (PHTL), a significant increase in some ethyl esters, in acetates and particularly in fatty acids, and a decrease in fusel alcohols, were observed in the wines. Even if the fermentation conditions (lower temperature, clarified must) may play a role in explaining these changes, especially in fusel alcohols, the large modifications to amino acids induced by the heating of the grapes

followed by pressing at high temperature is another crucial element. In 2011, gains in amino acid concentrations varying from +101.2% for Carignan to 200.2 % for Fer were observed in PHTL musts. These results suggest that heating temperatures in relation with amino acid extraction and thermal degradation could be adjusted in order to modulate the aroma of wines elaborated after a pre-fermentative heat treatment. As current heating technologies are often implemented on non-botrytized grapes in a perfect sanitary state, new ranges of temperature, especially below 70°C, deserve to be investigated.

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Table 4: Impact of pre-fermentative heat treatments on the concentration of aroma compounds detected in bottled wines.

Aroma compounds	2009									2010								
	Carignan			Fer			Grenache			Carignan			Fer			Grenache		
	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS
Ethyl esters (mg/L)																		
Ethyl propanoate	0.07[a]	0.13	0.06	0.10	0.10	0.18	0.07	0.18	0.16	0.08	0.08	0.08	0.09	0.06	0.09	0.17	0.09	0.14
Ethyl butyrate***	10.05	7.48	12.82	14.24	6.28	9.04	12.16	7.79	10.36	9.93	3.92	7.30	6.87	2.96	4.51	3.79	3.17	3.44
Ethyl 2-methylbutyrate**	112.85	107.48	165.41	180.86	103.25	168.76	126.41	111.29	134.61	124.48	59.41	112.69	78.28	59.38	101.57	44.12	51.31	51.62
Ethyl isobutyrate	15.94	11.57	17.68	21.66	10.54	10.88	24.78	13.76	20.63	14.19	5.95	10.48	8.86	4.13	5.13	8.51	4.83	6.04
Ethyl isopentanoate***	0.07	0.40	0.07	0.18	0.33	0.12	0.06	0.33	0.07	0.14	0.70	0.11	0.33	0.67	0.26	0.12	0.82	0.12
Ethyl hexanoate***	5.36	5.81	5.86	5.75	2.78	2.16	6.20	3.96	4.51	5.95	3.75	4.48	2.62	1.22	1.00	2.88	1.07	1.48
Ethyl furoate**	73.25	72.26	53.98	105.66	72.40	123.35	36.06	36.43	38.92	56.73	52.77	44.59	68.40	43.51	72.80	36.69	19.12	38.55
Ethyl lactate	0.39	0.90	0.54	1.18	1.03	0.79	0.43	0.29	0.41	0.80	0.71	0.54	2.69	1.35	1.69	0.52	0.72	0.75
Ethyl octanoate***	0.05	0.42	0.07	0.17	0.28	0.10	0.05	0.23	0.05	0.12	0.54	0.07	0.28	0.46	0.18	0.10	0.36	0.08
Ethyl decanoate***	0.02	0.17	0.01	0.02	0.09	0.00	0.01	0.09	0.01	0.03	0.14	0.03	0.05	0.14	0.03	0.03	0.10	0.02
Diethyl succinate	2.77	4.05	1.91	2.88	2.59	3.81	1.21	1.74	1.52	0.47	0.88	0.63	2.01	2.21	4.01	0.38	0.62	1.27
Acetates (mg/L)																		
Ethyl acetate*	26.84	48.62	14.48	33.66	54.25	61.59	12.31	60.31	42.60	30.85	52.04	28.60	48.44	56.71	66.97	52.17	54.71	49.21
Hexyl acetate**	nd	0.01	nd	nd	0.02	nd	nd	nd	nd	nd	nd	nd	0.01	0.03	0.02	nd	0.02	0.01
Isoamyl acetate*	0.11	0.94	0.09	0.47	0.67	0.65	0.04	0.68	0.09	0.21	0.67	0.11	1.04	1.05	2.41	0.18	0.83	0.43
Isobutyl acetate*	41.50	156.69	65.43	116.93	115.46	135.70	31.53	74.41	36.29	115.71	156.58	90.66	194.98	179.62	411.96	37.88	116.84	99.76
Butyl acetate*	2.09	5.68	7.32	2.70	6.20	4.24	4.74	10.45	11.26	3.26	12.27	6.00	8.33	10.95	16.26	9.28	16.62	15.62
Linalol acetate	nd	nd	nd	0.29	nd	0.27	nd	nd	nd	0.41	0.31	0.57	0.70	0.50	0.56	0.43	0.48	0.24
Phenylethyl acetate	0.12	0.79	0.12	0.36	0.44	0.51	0.19	0.21	0.21	0.08	0.07	0.06	0.28	0.48	1.12	0.05	0.33	0.23
Acids (mg/L)																		
Acetic acid*	125.72	265.27	141.11	214.15	390.92	381.68	293.71	277.86	358.91	253.60	378.42	218.23	297.85	598.07	371.70	515.55	689.16	463.28
Butyric acid***	0.43	1.41	0.45	0.95	1.42	1.05	0.47	0.85	0.43	0.59	1.45	0.44	1.26	2.21	1.15	0.75	1.44	0.60
Isobutyric acid	1.66	1.71	1.46	1.90	1.00	3.29	0.65	0.80	0.68	1.90	1.37	1.44	2.20	1.45	3.25	1.17	1.34	1.11
Valeric acid	nd	nd	nd	0.72	nd	nd	nd	nd	nd	1.26	0.88	0.90	1.83	1.01	1.22	1.03	1.10	0.65
Hexanoic acid***	0.91	6.43	0.89	2.24	6.08	1.92	0.72	3.67	0.57	1.06	5.16	0.84	4.06	8.97	3.26	1.22	5.50	1.06
Octanoic acid***	0.83	7.74	0.82	2.01	6.02	1.81	0.45	4.12	0.54	0.85	5.83	0.66	2.38	7.05	2.88	0.86	4.74	0.79
Decanoic acid*	0.07	0.98	0.16	0.28	0.80	0.16	0.06	0.53	0.09	0.21	0.91	0.19	0.41	0.89	0.40	0.15	0.76	0.16
Alcohols (mg/L)																		
Isoamyl alcohol	183.04	210.67	158.56	277.69	133.01	216.69	80.82	114.17	85.78	181.76	153.40	135.91	286.94	119.53	207.23	139.51	107.66	91.19
Benzyl alcohol	0.19	0.18	0.17	0.14	0.09	0.17	0.24	0.14	0.14	0.05	0.13	0.07	0.16	0.17	0.31	0.12	0.46	0.38
Methionol*	2.17	1.45	1.95	2.91	0.57	3.43	0.55	0.44	0.35	1.60	1.23	1.43	4.93	1.13	3.97	0.76	2.00	0.74
1-hexanol***	0.74	0.41	0.50	1.23	0.41	0.61	0.70	0.39	0.47	0.82	0.54	0.56	1.31	0.41	0.40	0.91	0.34	0.36
Cis-3-hexenol	0.076	0.077	0.062	0.100	0.047	0.080	0.019	0.030	0.014	0.052	0.074	0.042	0.090	0.075	0.070	0.038	0.038	0.031
1-butanol	0.71	0.90	0.75	0.65	0.50	0.68	1.10	0.90	1.05	0.89	1.15	0.94	0.73	0.56	1.17	1.39	0.94	1.15
β-phenylethanol	33.02	43.82	38.97	34.04	13.61	22.65	10.81	10.64	10.04	38.19	22.96	34.79	46.89	15.26	33.78	18.78	26.59	18.36
Isobutanol**	54.57	49.12	44.60	86.60	27.26	81.74	13.91	12.29	13.25	54.41	29.89	37.96	75.92	33.29	64.51	21.06	15.17	15.83
Carbonyl compounds (mg/L)																		
Diacetyl*	11.87	27.01	2.02	14.61	23.98	24.67	5.78	11.61	7.17	nd	0.27	nd	nd	0.34	0.57	0.43	1.03	1.03
Phenylacetaldehyde	63.81	103.67	105.75	75.39	104.74	66.66	67.49	68.65	82.83	20.99	21.01	20.32	17.88	22.48	17.03	20.59	23.39	15.66

Acetoin*	5.18	16.64	4.67	9.59	25.91	10.44	6.91	10.66	9.98	9.08	6.86	4.42	15.56	12.16	9.53	6.22	12.24	11.02
Acetaldehyde	1.09	1.09	2.04	0.88	0.46	0.50	1.08	1.13	1.06	0.48	0.48	0.82	0.67	0.62	0.63	0.80	0.81	0.63
Benzaldehyde	30.43	17.79	17.75	21.92	nd	nd	nd	nd	nd	29.38	19.53	9.35	2.61	nd	nd	nd	nd	nd
Terpenols and norisoprenoids (µg/L)																		
Geraniol	3.07	4.93	3.94	3.64	3.86	4.29	2.48	3.59	3.76	12.64	10.60	9.99	16.10	11.79	13.87	11.54	10.34	8.95
β-citronellol ***	2.18	1.73	3.77	3.03	1.09	1.92	12.67	7.30	8.92	5.00	1.79	3.29	3.98	1.93	2.23	17.33	11.85	6.97
α-terpineol**	3.32	3.65	4.52	9.17	24.99	19.33	6.57	5.19	7.52	2.98	1.46	2.75	4.61	11.11	20.53	2.72	3.78	6.69
Linalool*	5.83	6.91	7.59	7.33	6.29	6.53	8.12	7.01	8.66	8.37	4.86	7.30	8.60	5.20	7.73	6.64	6.38	6.78
β-damascenone*	7.19	7.70	7.63	1.90	1.67	1.08	4.26	2.66	3.17	9.72	9.15	7.84	1.73	0.36	0.84	4.94	2.00	2.64
β-ionone***	0.37	0.20	0.49	0.62	0.17	0.35	0.92	0.19	0.67	0.96	0.25	0.89	1.08	0.27	0.61	0.88	0.50	0.50
Phenols (µg/L)																		
Guaiacol**	0.66	1.59	1.12	0.87	4.60	3.09	2.19	3.36	3.92	1.02	0.88	1.44	1.09	4.56	7.22	2.32	9.84	15.15
Eugenol*	5.68	2.01	4.02	0.75	0.70	0.35	0.27	nd	nd	8.49	2.83	3.55	nd	nd	nd	nd	0.67	nd
4-allyl-2,6-dimetoxyphenol	7.17	5.51	5.64	0.18	1.15	0.27	0.63	1.34	1.57	4.68	3.00	4.49	nd	nd	nd	1.81	0.95	1.07
2,6-dimetoxyphenol***	11.39	20.02	25.72	16.69	121.94	86.71	14.64	25.93	27.84	19.11	13.52	29.89	17.00	73.14	122.42	13.74	55.38	104.19
4-vinylguaiacol	111.10	91.33	153.78	12.10	17.12	12.38	1.45	1.56	2.93	76.12	47.62	79.64	7.42	9.92	10.85	1.94	1.98	1.84
4-propylguaiacol	nd	nd	0.10	nd	nd	nd	nd	nd	nd	0.21	0.17	0.27	nd	nd	nd	nd	nd	nd
4-vinylphenol	2.52	5.78	4.34	2.73	3.55	3.34	3.01	3.59	3.77	2.14	1.93	2.06	1.64	1.50	1.63	3.35	4.60	4.00
4-ethylphenol	0.35	0.44	28.35	0.56	0.71	0.15	0.48	0.20	0.34	1.31	0.09	0.59	nd	nd	nd	0.69	0.07	nd
4-ethylguaiacol	0.12	0.08	3.28	0.11	0.00	0.00	0.11	0.00	0.08	0.00	0.00	0.00	nd	nd	nd	nd	nd	nd
o-cresol***	1.82	0.84	1.42	1.43	0.90	0.62	2.73	1.09	1.43	3.75	1.21	1.95	1.73	0.71	0.86	2.34	0.86	0.73
m-cresol***	0.57	0.49	0.76	0.64	0.47	0.43	0.42	0.35	0.47	1.50	0.18	0.92	1.13	0.69	0.74	1.25	0.76	0.12
Vanillin derivates (µg/L)																		
Vanillin	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.51	11.22	9.89	nd	nd	1.41	nd	nd	6.81
Methyl vanillate*	24.35	34.23	31.65	1.71	1.78	1.02	49.49	51.58	40.51	85.03	65.76	68.90	12.76	2.49	5.25	78.82	92.82	52.97
Ethyl vanillate**	137.65	152.05	91.66	125.45	17.81	19.30	816.75	100.74	378.71	294.47	309.94	317.87	139.68	32.29	34.28	981.91	258.42	99.33
Acetovanillone	155.74	208.95	204.40	33.15	46.30	27.68	418.66	437.42	365.78	177.09	140.99	165.07	26.32	34.30	30.88	360.57	459.91	315.81
High volatile mercaptans (µg/L)																		
Hydrogen sulfide	43.95	31.55	28.57	106.21	75.24	39.74	57.29	34.33	39.16	49.51	24.14	35.04	39.91	22.00	27.65	44.21	26.96	30.56
Methanethiol	5.44	5.16	4.59	5.47	2.15	4.60	2.29	4.71	5.75	7.41	6.31	5.69	8.02	5.13	6.62	5.29	4.68	4.17
Dimethyl sulfide*	8.83	9.79	8.16	9.04	6.91	6.43	24.34	23.32	22.05	10.71	15.05	10.43	5.72	13.86	6.82	12.21	14.59	10.73
Cinnamates (µg/L)																		
Ethyl dihydrocinnamate	0.39	0.90	0.54	1.18	1.03	0.79	0.43	0.29	0.41	0.80	0.71	0.54	2.69	1.35	1.69	0.52	0.72	0.75
Ethyl cinnamate***	1.19	0.20	1.06	0.71	0.23	0.39	1.22	0.28	0.60	1.55	0.65	1.03	3.30	0.54	0.68	4.90	1.24	0.54
Polyfunctional mercaptans (ng/L)																		
2-furfurylthiol**	19.06	12.99	16.60	11.63	7.47	36.10	11.58	3.54	6.80	2.93	4.17	3.15	3.43	7.13	28.55	2.02	0.90	8.20
2-methyl-3-furanthiol	642.06	1683.8	968.77	277.58	355.98	363.31	955.91	263.01	631.40	490.54	1626.4	506.31	248.07	414.17	383.63	551.50	638.18	488.12
Benzylmercaptan	20.02	30.37	18.01	11.74	7.64	6.56	17.11	14.10	13.06	11.15	14.12	11.75	3.36	2.37	2.72	7.41	4.83	7.25
3-mercaptohexanol	399.25	381.46	587.46	899.32	745.97	879.87	737.24	833.24	1234.0	292.56	169.29	208.46	150.83	61.75	161.79	195.80	159.55	251.03
4-mercapto-4-methyl-2-pentanone	3.26	0.18	8.04	28.90	0.00	8.73	nd	8.83	4.50	14.28	18.48	25.12	27.80	52.14	39.68	4.98	3.56	6.92
3-mercaptohexyl acetate	nd	nd	nd	46.83	31.72	18.47	39.85	7.78	nd	16.25	5.46	8.67	10.54	9.67	21.09	4.13	18.35	15.79
Lactones (µg/L)																		
t-whiskylactone	0.13	3.00	1.41	3.44	4.92	1.24	1.15	3.17	2.90	4.99	4.52	3.79	1.24	0.64	2.22	1.38	4.64	2.04
γ-butirolactone**	14.04	5.47	14.60	10.01	3.08	26.42	7.03	2.77	7.68	9.05	5.52	7.54	8.75	4.60	7.90	7.69	4.00	5.08
γ-nonalactone*	22.37	11.42	25.21	8.77	5.60	4.94	31.07	15.36	28.16	26.42	9.35	24.11	5.86	3.34	6.32	26.58	22.34	22.06
γ-decalactone	2.41	2.78	3.74	1.39	2.36	2.63	3.18	1.77	4.13	2.18	1.43	2.17	0.42	0.71	1.47	1.64	3.21	1.34

δ-decalactone***	17.17	38.95	29.31	26.88	40.83	28.55	19.92	41.11	24.82	16.19	42.67	17.57	27.89	27.65	42.08	28.97	50.63	29.53
Pyrazine (ng/L)																		
3-isobutyl-2-methoxypyrazine	nd	1.55	1.00	3.80	1.50	nd	nd	nd	0.45	nd	nd	nd	0.95	0.70	3.20	nd	nd	nd

Significance of three way analysis of variance for the treatment factor *P<0.05; **P<0.01; ***P<0.001; nd. not detected; [a]Mean of two replicates

Table 1: Results of three-way analysis of variance for conventional enological parameters measured in bottled wines.

Parameter / Source of variation	*P*-values					
	Vintage (V)	**Cultivar (C)**	**Treatment (T)**	**V x C**	**V x T**	**C x T**
Alcohol	<0.001	<0.001	0.038	0.002	0.688	0.880
Total Acidity	0.298	<0.001	0.004	0.319	0.766	0.255
pH	<0.001	0.026	0.136	0.077	0.411	0.179
Tartaric acid	0.002	0.046	0.045	0.112	0.284	0.376
Potassium	<0.001	<0.001	0.005	0.121	0.387	0.494
Volatile acidity	0.097	0.001	0.121	0.861	0.670	0.614
Anthocyanins	<0.001	<0.001	0.010	0.067	0.483	0.003
Total Phenolic Index	<0.001	<0.001	<0.001	0.017	0.048	0.097
Color Hue	0.078	<0.001	0.007	0.375	0.719	0.027
Color Intensity	<0.001	0.025	0.007	0.078	0.206	0.007
Occurrence of P-*value <0.05*	*7*	*10*	*8*	*2*	*0*	*3*

Table 2: Impact of pre-fermentative heat treatments on conventional enological parameters measured in bottled wines.

Parameter	2009									2010								
	Carignan			Fer			Grenache			Carignan			Fer			Grenache		
	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS	CTRL	PHTL	PHTS
Alcohol (% vol.)*	11.2[a]	12.5	10.7	12.1	12.8	12.1	15.3	15.5	15.0	13.7	14.1	13.6	13.2	13.9	13.3	15.3	15.6	15.1
Total Acidity** ($g/l\ H_2SO_4$)	1.90	3.40	2.14	1.51	2.34	1.94	2.42	2.39	2.47	1.31	1.53	1.31	1.23	1.63	1.85	1.83	2.12	2.44
pH	3.48	3.53	3.48	3.47	3.56	3.52	3.47	3.59	3.51	3.70	3.59	3.50	3.77	3.76	3.80	3.60	3.79	3.60
Tartaric acid (g/l)*	1.90	3.40	2.14	1.51	2.34	1.94	2.42	2.39	2.47	1.31	1.53	1.31	1.23	1.63	1.85	1.83	2.12	2.44
Potassium (g/l)**	0.68	0.83	0.72	0.81	1.04	0.87	0.62	0.80	0.71	1.05	0.98	1.02	1.29	1.39	1.34	0.98	1.20	1.02
Volatile acidity (g/l acetic acid)	0.32	0.32	0.33	0.29	0.52	0.48	0.56	0.60	0.63	0.24	0.35	0.23	0.24	0.50	0.32	0.42	0.52	0.52
Anthocyanins* (mg/l)	344	252	360	419	352	422	279	237	247	607	320	626	714	702	795	548	402	371
Total Phenolic Index*** (TPI)	37	31	38	41	56	64	47	44	56	50	29	51	57	64	90	64	67	87
Color Hue** (A420/A520)	0.60	0.73	0.57	0.51	0.57	0.53	0.62	0.63	0.61	0.58	0.78	0.56	0.56	0.59	0.55	0.63	0.70	0.76
Color Intensity** (A420+A520+A620)	7.1	4.3	7.8	7.7	8.8	11.0	8.4	7.8	9.2	14.7	5.9	16.2	12.3	13.0	18.1	14.2	9.1	6.6

Significance of three way analysis of variance for the treatment factor *P<0.05; **P<0.01; ***P<0.001; [a]Mean of two replicates

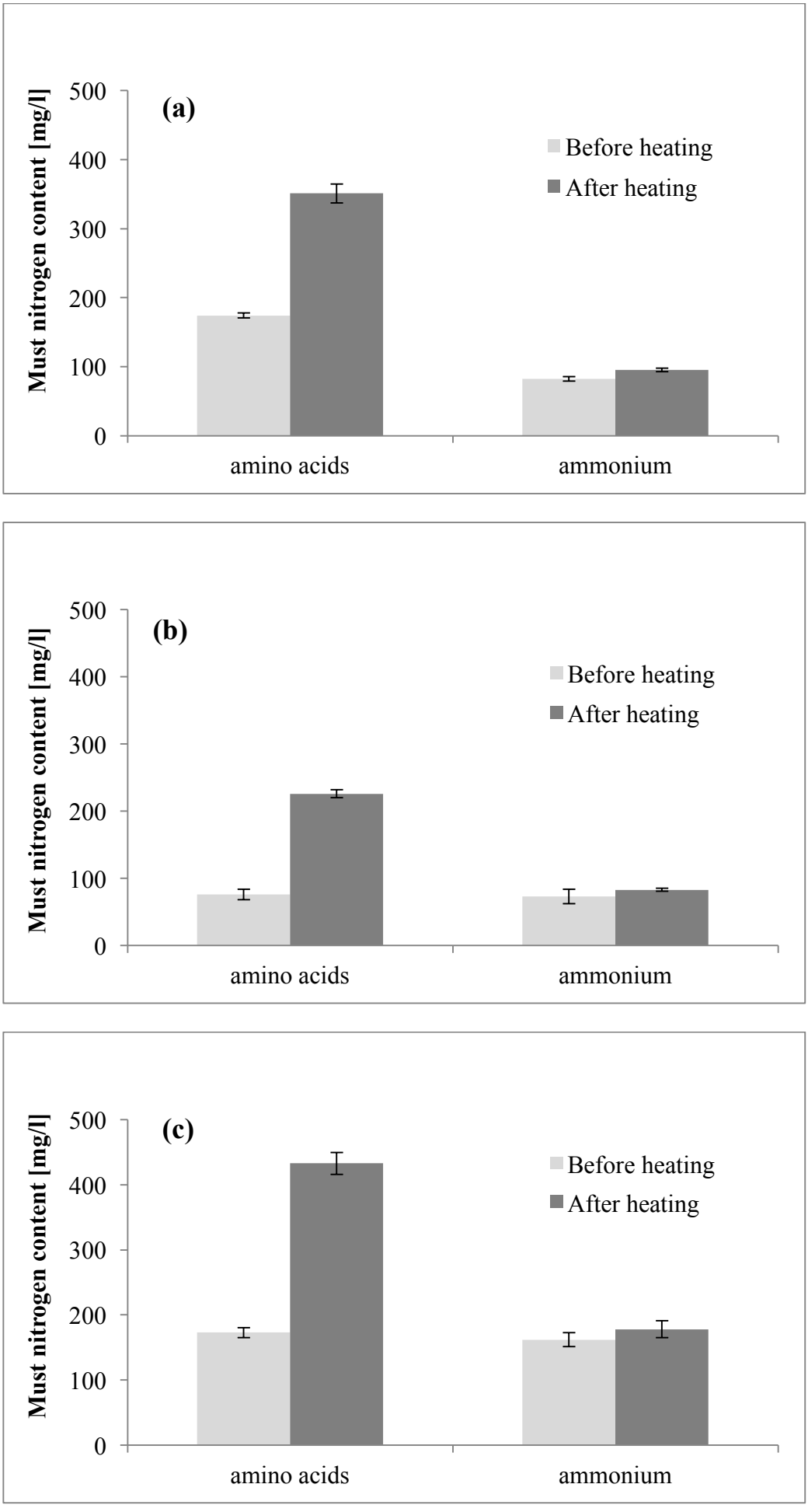

Figure 1: Impact of pre-fermentative heat treatment on nitrogen content of the must for Carignan (a), Fer (b) and Grenache (c) in 2011