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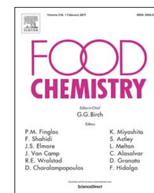

Effect of irrigation and timing and type of nitrogen application on the biochemical composition of *Vitis vinifera* L. *cv.* Chardonnay and Syrah grapeberries

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A B S T R A C T

This study reports the effect of different doses of nitrogen applied to soil and/or leaves of Syrah and Chardonnay grapevines in the Languedoc-Roussillon (France) over two years. In 2011, nitrogen treatment involved both foliar urea sprayings and soil application at two different levels, with two controls – irrigated without nitrogen and no irrigation nor nitrogen. In 2012, the same grapevines received either soil or foliar nitrogen using the same controls. Results showed that foliar application increased the amino acid content to a greater extent than soil application, but that a combination of both was the most effective. For the first time, significantly elevated proline levels in response to drought were demonstrated for the grapevine. Increased contents of aromatic compounds and glycosylated precursors closely mirrored the applied nitrogen dose. Wines produced from N-fertilized Syrah grapes in 2011 showed a statistically significant effect of irrigation and fertilization on positive sensorial perception.

1. Introduction

Vine nitrogen (N) nutrition is an important factor for wine quality and plays an essential role in the vineyard and winery. Vine N status can affect vine vigour and fruit yield (Bell & Henschke, 2005; Bell & Robson, 1999; Ewart & Kliewer, 1977; Spayd, Wample, Stevens, Evans, & Kawakami, 1993). Nitrogen is required for yeast growth and completion of alcoholic fermentation in grape juice; concentrations of 130–160 mg/L of yeast fermentable nitrogen (primary amino acids and NH_4^+) are required for complete fermentation (Agenbach, 1977; Bell & Henschke, 2005; Spayd, Nagel, & Edwards, 1995) but it has been shown that adding ammonium salts to grape juice (to increase its fermentability) can reduce by up to 30% the production of aromatic thiols such as 4-methyl-4-mercaptopentan-2-one (4MMP) from their precursors through the phenomenon of NCR – Nitrogen Catabolic Repression (Subileau, Salmon, Schneider, & Degryse, 2008). Moreover, supplementation with ammonium salts has the disadvantage that it may increase formation of ethyl carbamate, a supposed carcinogen in wine (Ough, 1991).

For this reason, new research is beginning to focus on adapting vineyard practice (for example the judicious application of nitrogen fertilization or fertigation) in order to increase the concentration of amino acids in grapes at harvest (Bindon, Dry, & Loveys, 2008; Chaves et al., 2007; Mirás-Avalos, Trigo-Córdoba, Bouzas-Cid, & Orriols-Fernández, 2016; Teles Oliveira, de Freitas, & Alves Sousa, 2012; Trigo-Córdoba, Bouzas-Cid, Orriols-Fernández, & Mirás-Avalos, 2015a)

It is known that N fertilization can increase berry N content (Choné, Lavigne-Creuge, Tominaga, Van Leewen, & Castanede, 2006; Jreij, Kelly, Deloire, & Brenon, 2009; Spayd et al., 1993, 1995) however, there is growing interest in the analysis of individual amino acids in grape juice due their pivotal role as precursors to aromas released during fermentation or ageing (Bouzas-Cid et al., 2015; Jungmin & Schreiner, 2010)

A significant relationship between must amino acids and wine aromatic composition has been described (Hernandez-Orte, Cacho, & Ferreira, 2002; Trigo-Córdoba, Bouzas-Cid, Orriols-Fernández, & Mirás-Avalos, 2015b). For example, isoamyl, isobutyl and phenylethyl alcohols are derived from respectively leucine, isoleucine and valine (Hernandez-Orte et al., 2002). The same authors also showed that threonine, phenylalanine and aspartic acid are the amino acids which most influence the fermentation process. Choné et al. (2006) showed that in the case of *Vitis vinifera* L. *cv. Sauvignon Blanc* vines, an unlimited N supply to the vine is an important factor for optimum varietal aroma expression.

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It is important to identify the optimum method of nitrogen fertilization in terms of concentration and methodology for both ecological and economic reasons (Arrobas et al., 2014; Garde-Cerdán et al., 2009; Peuke, 2009). Furthermore, grapeberry nitrogen compounds increase, but at a declining rate, as the application level of nitrogen in the vineyard increases (Bell, 1994; Bell, Ough, & Kliewer, 1979).

As consequence, the aim of this study was to study the effect of fertilization in Chardonnay and Syrah grapevines at different levels in irrigated vines and to evaluate their effects on the biochemical composition of berries and the aroma compounds in wines produced from both treated and control vines. Furthermore, in order to study the effect of severe water stress on berry composition and wine quality, one of the controls consisted of neither fertilization nor irrigation over the entire ripening period. It has been reported (Ojeda, Deloire, & Carbonneau, 2001) that water stress causes a positive and an indirect response due to the effect of berry size reduction (a concentration effect), however, it may elicit a direct response in biosynthetic pathways (particularly phenolic biosynthesis). The results of this study indicated that by regulating water supply to vines it is possible to control berry size and the relative concentration of key nitrogen and aromatic compounds.

2. Materials and methods

2.1. Plant material and experimental design

The grapevines were grown in the experimental vineyard of Pech Rouge in the Institut National de la Recherche Agronomique in Gruissan, in the south of France (43 °10′N latitude and 3 °06′E longitude). This area is characterized by a Mediterranean climate with a maritime influence. The mean rainfall is about 600 mm annually and is particularly characterized by high winds. Two varieties of grapes were used in the study – *Vitis vinifera* Syrah, planted in 1993 and *Vitis vinifera* Chardonnay, planted in 2000; both varieties were grafted on R140. The vines were trained by cordon system and were separated 1 m between plants and 2.5 m between rows. The experimental design was completely randomised and each of the experimental units were homogeneous. In 2011, irrigation and nitrogen treatments were carried out in triplicate and in 2012 they were carried out in quadruplicate.

The treatments were as follows: in 2011, I0N0: non-irrigated and non-fertilized, I1N0: irrigated and without fertilization, I1N1: irrigated and soil fertilized with 30 kg N/ha ammonium nitrate (50 and 44 days after fruit set for Chardonnay and Syrah, respectively) and 2.5 kg N/ha urea foliar application at 50% of véraison (63 and 66 days after fruit set for Chardonnay and Syrah respectively), I1N2: irrigated and soil fertilized with 60 kg N/ha ammonium nitrate and 5 kg N/ha urea foliar application at the same dates as I1N1. In 2012 I0N0 and I1N0 were as per 2011, I1NS: irrigated and soil fertilized with 60 kg N/ha ammonium nitrate 28 and 22 days after fruit set for Chardonnay and Syrah, respectively and I1NF: irrigated and fertilized with 5 kg N/ha urea foliar application at 50% véraison (71 and 69 days after fruit set for Chardonnay and Syrah, respectively). Thus in 2011 the vines received two N treatments and in 2012, one N treatment (soil or foliar).

Irrigation was effected by a drip system to maintain a moderate water stress level measured weekly by base potential pressure with a Scholander pressure chamber. Soil fertilization was applied using ammonium nitrate (33.5% purity) supplied to each plant at the front of each dropper, to obtain 30 and 60 kg of nitrogen per hectare in I1N1, I1N2 respectively in 2011 and 60 kg/ha in I1NS in 2012. Foliar fertilization was by spraying approximately 250 mL of urea (220 g of urea/L) to each plant, providing 2.5 and 5.0 kg of nitrogen per hectare in I1N1 and I1N2, respectively in 2011 and 5.0 g/ha in I1NF in 2012. Spraying was carried out early in the morning when there was no wind and it was applied mixed with a non-ionic adjutant solution based on terpene alcohols, Escapade™ to improve the quality of pulverization. One border between each treatment block was not sprayed to avoid contamination.

2.2. Sampling

In 2012 two hundred berries were sampled from each of the 16 treatment blocks once a week from 100% of véraison to harvest. In 2011 samples were taken only at harvest. In 2011 Syrah was harvested at 23.0 and Chardonnay at 23.5 °Brix, in 2012 Syrah was harvested at 21.8 and Chardonnay at 22.0 °Brix. Whole berries (including skins and seeds) were homogenised in a Waring laboratory blender for three minutes and stored at −20 °C until further processing.

2.3. Reagents and chemicals

All chemicals and reagents were of analytical or HPLC grade or equivalent. Methanol, acetonitrile, acetic acid, sodium acetate, hydrochloric acid 0.1 M and sodium hydroxide 1 M were obtained from Carlo Erba (Carlo Erba Réactifs, Val de Reuil, France). An amino acid standard mixture containing 2.5 μM of Alanine, Ammonium chloride, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine and Valine in addition to individual standards of glutamine, asparagine GABA and tryptophan were obtained from Sigma Aldrich (St Quentin-Fallavier, France). The *ortho*-phthaldialdehyde-mercaptoproprionic acid reagent and borate buffer (0.4 M, pH 10.2) were purchased from Agilent France. Doubly distilled water was used to prepare solutions and for washing all consumable materials.

2.4. General grape attributes

Homogenised samples were defrosted at room temperature. Sugar concentration was measured using a hand-held refractometer (VWR international), automatic total acidity by titration to pH 7 with 0.1 M NaOH using a Crison Basic 20 (Barcelona, Spain) pH; the total polyphenol index was measured by UV absorbance at 280 nm and the concentration of anthocyanins were measured at 520 nm, both using a Thermo Scientific (France) Evolution 300 Spectrophotometer .

Ammoniacal nitrogen was measured enzymatically and total primary amino method was measured by the NOPA method (Dukes and Butske, 1998). Fermentable nitrogen was determined by combining the total primary amino nitrogen and ammoniacal nitrogen.

2.5. Individual amino acid analysis

2.5.1. Primary amino acids – HPLC Instrumentation and operating conditions

Homogenised samples were defrosted at room temperature. Amino acids were analysed using a modified version of a previously reported method (Kelly & Larroque, 2010). A Hewlett-Packard (Agilent Technologies Massy, France) 1100 179 series HPLC instrument was used, consisting of a model G1322A 180 degasser, a G1312A binary pump, a model G1313A autosampler and a G1321A fluorescence detector set at excitation and emission wavelengths of 330 nm and 440 nm, respectively. Separations were carried out on a C_{18} 150 mm × 3mm Machery Nagel Durabond® column 5 μm dp, protected by a 1mm C_{18} SecurityGuard® cartridge supplied by Phenomenx (France). Mobile phase A consisted of 95% 0.05 M acetate buffer, pH 6.5 and 5% methanol,-acetonitrile [1:1] filtered under vacuum using a 0.22 μm nylon membrane. Mobile phase B consisted of methanol–acetonitrile [1:1]. Separations were carried out at 40 °C with a flow rate of 0.5 mL/min. The total run time (including re-equilibration of the column), was 28 min.

The in-loop derivatisation procedure (applied equally to standards and samples) was as follows: draw 1 μL from borate buffer, 3.5 μL of sample or standard, rinse needle and draw 1 μL from the OPA-MPA reagent, and mix 15 times in seat.

Berry samples (200 g from each repetition of each treatment) having being stored at −20 °C were thawed, mixed with the same weight of distilled water and crushed in a laboratory blender at speed 3 for

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