**ORIGINAL RESEARCH ARTICLE**

# Influence of non-irrigation and seasonality on wine colour, phenolic composition and sensory quality of a grapevine (*Vitis vinifera* cv. Callet) in a Mediterranean climate

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## ABSTRACT

Most vineyards in Mediterranean areas are cultivated using a training system and drip irrigation. However, the increasing risk of water deficit stress due to global warming will mean that viticulture need to adapt to a tougher water-saving policy. Thus, we investigated the effects of total suppression of irrigation on a grapevine (*Vitis vinifera* cv. Callet) and the phenolic composition and sensory quality of this native red variety wine from the Balearic Islands over three seasons. Significant yield reductions of up to 15.6 %, 17.2 % and 22.2 % were observed in non-irrigated (NI) plants in 2016, 2017 and 2018 respectively, compared to irrigated plants (I); however, wine quality parameters improved. In the years with the highest rainfall (715 mm in 2016 and 799 mm in 2017), NI favoured the enrichment of sugars, anthocyanins and phenolic compounds in the wine and enhanced the development of aromatic components. However, with lower rainfall (524 mm in 2018), the NI treatment appeared to diminish the quality of the wine, particularly affecting the global sensory quality of the wines. Thus, development of specific water strategies tailored to the vineyard, year, vintage and grape variety may regulate the phenolic composition of red wines to meet production goals and reduce total water consumption.

# INTRODUCTION

Viticulture worldwide is being affected by climate change, which is threatening the sustainability of grape and wine production (Gambetta *et al.*, 2020). Moreover, reference evapotranspiration (ETo; the sum of evaporation from the soil and transpiration from a reference crop) is expected to increase in many parts of the world by 2055, thus increasing total irrigation water needs (Gondim *et al.*, 2012). Accordingly, under future climate projections, an increase in vine irrigation of between 3.5 % and 7.5 % has been predicted for arid and semi-arid regions, depending on the properties of the soil in the months of maximum demand (Phogat *et al.*, 2020). Concretely, under Mediterranean climatic conditions in Spain, irrigation water requirements are expected to increase by 40 to 250 %, depending on crop type, by 2100 (Savé *et al.*, 2012).

Water deficit affects the vegetative and generative growth of vines in multiple ways, depending on the severity of drought and the season (Baeza *et al.*, 2019; Scholasch and Rienth, 2019). Decreased early plant growth is one of the first symptoms of water scarcity. Moreover, plant water status has a major influence on the physiological behaviour of vines, as well as on the quantity and quality of the grapes and wines (Baeza *et al.*, 2019). Water deficit that occurs early in the season is assumed to lead to a smaller number of berries per bunch; however, severe deficit between the fruit set and veraison period causes a reduction in berry weight and a higher skin to pulp ratio (Ojeda *et al.*, 2001), which results in higher concentrations of phenolic compounds, such as anthocyanins, proanthocyanidins, and flavonols (Chacón-Vozmediano *et al.*, 2021; Ojeda *et al.*, 2001; Pérez-álvarez *et al.*, 2021; Santesteban *et al.*, 2011). Other studies have shown that water deficit consistently promotes higher anthocyanin concentrations in red grapes and their wines, but has limited effects on proanthocyanidin content and flavonols, thus decoupling the ripening of grapes (Savoi *et al.*, 2020; Yu *et al.*, 2016).

Phenolic compounds, flavonoids and non-flavonoids are key factors determining the quality of wines, especially red wines (Gutiérrez-Escobar *et al.*, 2021). Phenolic composition is related to important wine sensory attributes, such as colour, taste, mouthfeel, flavour, astringency and bitterness, as well as ageing ability (Cejudo-Bastante *et al.*, 2017; Hornedo-Ortega *et al.*, 2020). Flavonoids are located in grape skins, seeds and stems, and they comprise anthocyanins, flavan-3-ol monomers, oligomeric and polymeric proanthocyanidins, flavonols, flavanonols, and flavones. Non-flavonoids are mainly derived from the pulp and skins of grape berries and comprise hydroxycinnamic and hydroxybenzoic acids and stilbenes. Thus, given the importance of wine phenolic compounds, a better understanding of their content and profile in monovarietal wines and of the effects of different water deficit conditions on these compounds is essential. In fact, such knowledge is fundamental for winemaking management in order to be able to predict wine sensory properties, oxidative stability and ageing (Gris *et al.*, 2013).

Thus, it is important to determine the sensitivity of grapevines to water deficit, as this depends on a number of factors intrinsic to the vine site and to the genetic background of the grapevine. Niculcea *et al.* (2015) have reported that the accumulation of phenolic compounds and the compositional responses to sustained deficit irrigation during berry growth and ripening are variety-dependent.

In this study, we characterised the yield and quality traits of Callet *Vitis vinifera* L. cv. - a well appreciated autochthonous red grape variety from the Balearic Islands (Spain) - to obtain a deeper understanding of its performance when grown in well-irrigated or water deficit conditions. This variety originated from a drought-prone environment and is one of the main autochthonous grapevine cultivars from the Balearic Islands within the Denomination of Origin (D.O.) Pla i Llevant, Mallorca (Cretazzo *et al.*, 2010). Callet grapes have a low alcohol content and a medium colour depth due to low phenolic concentration (Mulet *et al.*, 1992), and they are sometimes associated with other more polyphenolic wines, such as those from Cabernet Sauvignon and Merlot (Hidalgo-Togores, 2018). Callet wines have a high content of higher alcohols, which are responsible for the fusel character of the aroma (Escalona *et al.*, 2006), and are characterised by spicy and citric attributes (García-Muñoz *et al.*, 2014). The overall aim of this work was to study the influence of total water suppression on the colour, phenolic composition and sensory quality of varietal wines made from Callet over three consecutive seasons in a typical Mediterranean climate, with water stress typically occuring in summer when light and temperature levels are high.

# MATERIALS AND METHODS

## 1. Plant material and irrigation treatments

The trial was carried out over three growing seasons (2016–2018) on *V. vinifera* L. 'Callet' vines grafted on SO4 rootstock in a 23-year-old estate vineyard known as Can Axartell (UTM: 31S 501616.434, 4409438.756, Mallorca Island, Spain). The plantation was settled with vertical shoot positioning trellis and has a density of 3200 plants per ha. The soil had been previously classified by López-García *et al.* (2020) as a Calcaric Regosol as defined by the Reference Soil Groups (FAO, 2015); this corresponds to a chalky, alkaline (pH 8,30) soil with high-water retention capacity, a field capacity of 38 %, high clay content (USDA, 17 % sand, 31 % silt, 52 % clay), total organic carbon of 20.7 g/Kg and total nitrogen of 1.6 g/Kg. Average rootable depth is 55 cm. In the horizon of root exploration there is no skeleton content or salinity that can affect root growth development (C.E. 0.43 mmhos/cm). Regarding the management of the soil in the vineyard, a spontaneous green cover was maintained in alternate rows (i.e., one not tilled, the other tilled) in the alleys between vine rows (inter-rows). The cover was maintained in the central part of the inter-rows, while the vegetation between vine plants in the same row was removed several times a year by shallow cultivation in a strip about 1 m wide. The climate here is classified as typical sub-tropical-Mediterranean,

with mild winters and hot, dry summers (BSk by the Köppen-Geiger system). The dry drought period usually occurs between May and September, but its length is highly variable between years. Meteorological data were provided by an automatic meteorological station belonging to the Agroclimatic Information System for Irrigation (SiAR) located 10 km from the experimental site in Sa Pobla.

A drip irrigation system was available with one drip per plant (2.3 L/m$^2$). The irrigation system could be adjusted according to the water demand. In this experiment, six selected plants were exposed to water-deficit conditions (non-irrigated, NI) by total suppression of irrigation, while six other plants were well-watered (irrigated, I) on a weekly basis, receiving 100 % of their total water needs based on potential evapotranspiration (ETP). The ETP values were taken from the aforementioned automatic meteorological station. The water balance was thus simply based on the sum of the ETP values, as well as precipitation. Additionally, two rows of separation were established between the NI plants and the I ones. The TDR technique was employed to monitor soil water content (SWC) using a Moisture Meter type HH2 (Delta-T Devices Ltd.) and expressed as m$^3$ of water per m$^3$ of soil. The measurements were taken once a week between watering periods at two different depths (25 and 50 cm), installing one tube attached to each selected plant.

## 2. Yield components

The total yield per vine, number of bunches, bunch weight and single berry weight were recorded on the six plants per treatment in each experimental year at harvest. Each year, the evolution of grape maturity was evaluated by random berry sampling.

## 3. Harvest and winemaking

The harvest date was determined after monitoring for maturation three times during the month prior to harvest. Harvest was performed manually when the soluble solids content had almost reached 12 degrees of probable alcohol. Three 3 L micro-fermentations were carried out for each treatment using randomly selected bunches from the six vines in each treatment, as described in Sampaio *et al.* (2007). Grapes were crushed by hand inside the jar and sulphited (60 mg/100 L). A commercial *Saccharomyces cerevisiae* yeast strain was then added (0.20 g/100 L, Zymaflore RJA 64, Laffort, Bordeaux, France), and fermentation was carried out at a constant temperature of 29 °C. After approximately two weeks, when alcoholic fermentation had finished, the wine was manually pressed and SO$_2$ was adjusted to 30 mg/L. The wines were kept in cold storage at 2 °C for two weeks before being racked; the sample for analysis were taken at this time point, before racking.

## 4. Oenological parameters

Total soluble solids (TSS) (Brix) in the musts was analysed by refractometry. The titratable acidity (TA), pH, alcoholic content (degrees) and volatile acidity of the wines were determined according to standard methods (OIV, 2019).

## 5. Analysis of colour parameters and total polyphenol index

The red colour of the wine (WC), monomeric anthocyanin colour (MAC), copigmentation colour (CC) and bisulphite-stable colour (BSC) were determined according to the methodology described by Levengood & Boulton (2004) using a Cary 300 Scan UV–vis spectrophotometer (Varian Inc., Madrid, Spain). The stable colour of the wine (SC) was calculated as the sum of CC and BSC. CIELab parameters (L*, a* and b*, illuminant D65 and 10° observer conditions) were calculated according to the methodology reported by Ayala *et al.* (1997). Colour intensity (CI) was calculated as the sum of the absorbance values at 420, 520 and 620 nm, and the hue was recorded as A420/A520 at wine pH. The total polyphenol index (TPI) was determined by absorbance at 280. All measurements were performed in triplicate using 10-mm path length quartz cells.

## 6. Analysis of monomeric phenolics

The analysis of the phenolic compounds was based on the methodology reported by Gómez-Alonso *et al.* (2007). Anthocyanins, hydroxycinnamic acids, hydroxybenzoic acids, flavonols and flavan-3-ols were analysed by HPLC-DAD by direct injection of 25-µL wine previously filtered through 0.45-µm membranes. Separation was achieved with an ACE HPLC (Teknokroma, Barcelona, Spain) 5 C18-HL (particle size 5 µm; 250 mm x 4.6 mm) column protected with a guard column. The identification of the phenolic compounds was performed using the retention times of available pure compounds and the UV–Vis characteristics of authentic standards. Quantification was achieved using DAD chromatograms recorded at 520 nm for anthocyanins, 360 nm for flavonols, 280 nm for hydroxybenzoic acids and flavanols and 320 nm for hydroxycinnamic acids.

## 7. Analysis of proanthocyanidins

The wine samples were directly fractionated by gel permeation chromatography on a Toyopearl gel HP-50F column (particle size distribution, 30–60 µm; exclusion limit, 1.8 × 104 Da; resolution, 1.3 min) as previously reported (Guadalupe *et al.*, 2006). The first fraction (F1) was eluted with ethanol/water/trifluoroacetic acid (55:45:0.05, *v/v/v*); the second fraction (F2) containing proanthocyanidins was recovered by elution with acetone/water (60:40, *v/v*). Phloroglucinol adducts were analysed in the F2 fractions using reverse-phase HPLC (Kennedy and Jones, 2001). The column was an ACE HPLC [5 C18-HL, particle size 5 µm; 250 × 4.6 mm (Teknokroma)] protected by a guard column. Proanthocyanidin cleavage products were estimated using the response factors relative to (+)-catechin, which was used as the quantitative standard. Total proanthocyanidin content was calculated as the sum of extension subunits (phloroglucinol adducts) and terminal subunits (catechin, epicatechin and epicatechin-gallate). The apparent mean degree of polymerisation (mDP) was calculated as the sum of all subunits divided by the sum of the terminal subunits.

## 8. Sensory analysis

Sensory analysis was performed one month after bottling in a sensory room in accordance with ISO 8589 Standards (2010) by eleven expert tasters (seven males and four females, 29–68 years old). In the first session, the tasters established similar qualitative and quantitative criteria and selected a consensus group of descriptors. In the second session, the wine samples were evaluated using a structured numerical scale, whereby 0 represented no intensity and 10 the highest intensity. The wines were presented in standard wine-tasting glasses in random order. The tasters rated the visual, gustatory, olfactory and overall quality of the wines.

## 9. Statistical analysis

Statistical analyses of the yield components, oenological parameters, colour parameters, phenolic compounds and the sensory analysis of the wines were performed using Students *t*-tests at the 95 % probability level. Multivariate factorial analysis (MFA) of the yield components, oenological parameters, colour parameters and phenolic compounds was performed using SPSS v. 15.0 for Windows (SPSS Statistics, Inc., Chicago, IL). The percentages of variance attributable to each factor (treatment and season) were calculated from the ratio of the sum of squares of each factor and the total multiplied by 100.

## RESULTS

### 1. Climate and Irrigation Treatments

The experiment was conducted over three growing seasons (2016–2018). Total annual rainfall was 715, 799 and 524 mm in 2016, 2017 and 2018 respectively (Supplementary Table 1). The monthly temperatures in 2016 to 2018 measured by the meteorological station are shown in Figure 1. Spring precipitation (from April to June) varied between years: precipitation was lower in the spring of 2018 (85 mm) than in the spring of 2016 (169 mm) and 2017 (123 mm; Figure 1). Accordingly, more water was applied to the irrigated plants in 2018 (110 L/m$^2$) than in 2016 and 2017 (93 and 71 L/m$^2$ respectively), and a larger difference in total water availability was observed between the well-watered (I) plants than the non-irrigated (NI) plants in 2018 compared to 2016 and 2017 (Supplementary Table 1). Indeed, the dynamics of soil water content (SWC) - indicated here by m$^3$ of water per m$^3$ of soil and measured using the TDR technique - varied depending on the annual rainfall conditions. Thus, the 2018 vintage experienced the highest difference in the SWC between I and NI plants at pea-size and veraison (Figure 2).

### 2. Yield Components

At harvest, grape yield was higher for the I plants than the NI plants (Table 1). Reductions in yield of up to 15.6 %, 17.2 % and 22.2 % were observed for the NI plants in 2016, 2017 and 2018 respectively (Table 1). In 2016 and 2017, this difference was mainly due to a higher bunch weight and rather than to a difference in the number of bunches per vine (Table 1). However, the high yield of I vines in 2018 was due to a higher number of bunches compared to the NI vines.
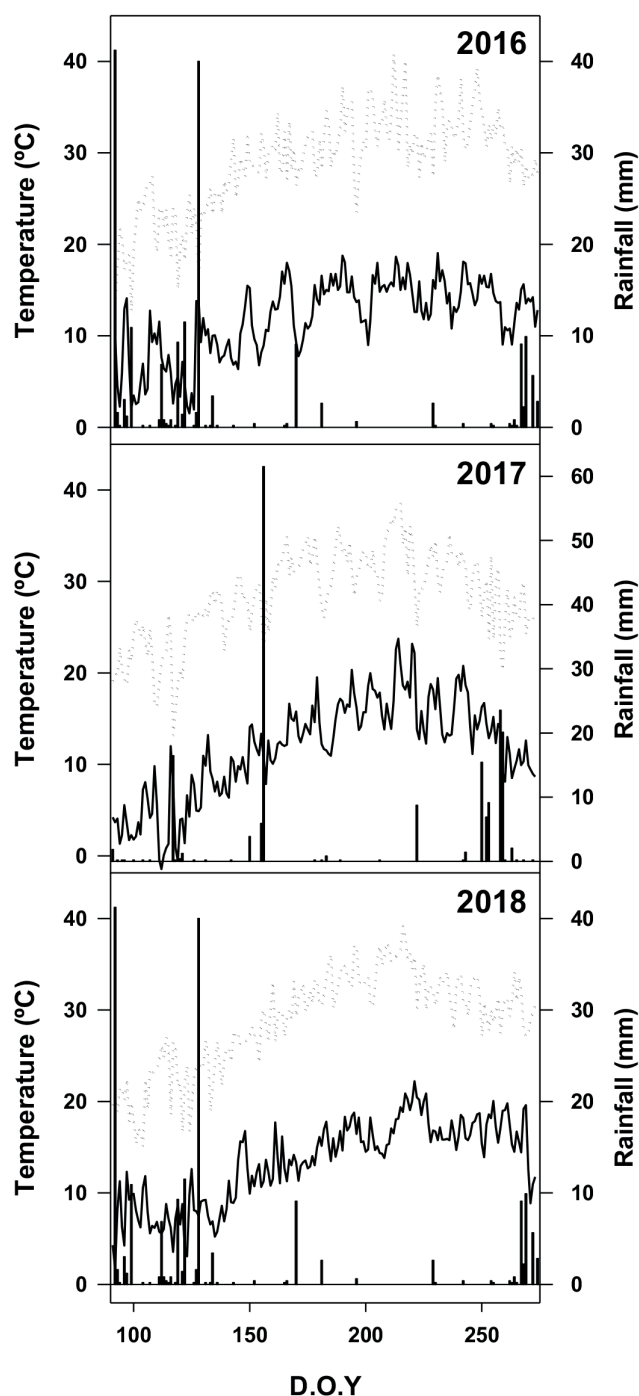


**FIGURE 1.** Climatic variables during the experimental period (2016, 2017 and 2018).

Measuring days are expressed as DOY. The displayed values are (solid line) daily minimum air temperature (°C), (dotted line) daily maximum air temperature (°C), and rainfall (mm).
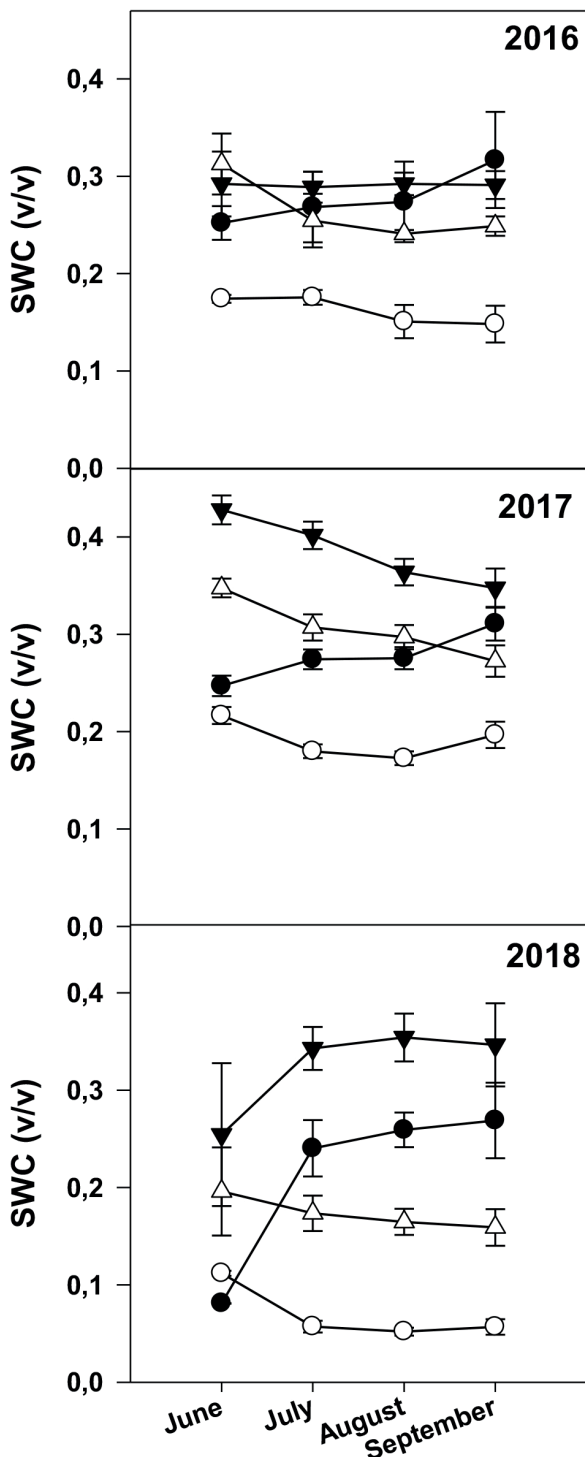
**FIGURE 2.** Soil water content from TDR probes in 2016, 2017 and 2018 seasons. Data are means ± SE of 6-8 probes. (●) 25 cm Irrigated, (○) 25 cm Non-irrigated, (▼) 60 cm Irrigated, (△) 60 cm Non-irrigated.

The grape yield, number of bunches per vine, mean bunch weight and single berry weight were higher in 2016 and 2017 than in 2018, which may be directly related to the lower precipitation recorded during the late spring of 2018 (Figure 1). These changes occurred despite a higher irrigation dosage being applied at the beginning of June (before flowering) in 2018 in an attempt to compensate for increased water demand.

## 3. Effects of water deficit on sugar and oenological parameters

The different water regimes resulted in variations in yield and to significant differences in the oenological parameters of the must and wines obtained from the I and NI grapevines (Table 2).

The grapes from NI plants had a higher sugar content than I plants in 2016 and 2017, but no differences were observed between the irrigation treatments in 2018. Similarly, the wines from NI plants had higher alcohol contents than wines from I plants in 2016 and 2017. However, the wines from I vines had lower total and volatile acidity values than those from NI plants in 2018, but no differences were observed for either of these parameters between the different water regimes in 2016 and 2017. Significant differences in pH between treatments were only observed in 2016.

When comparing the seasons, the grapes from NI plants from 2016 and 2017 were found to have higher sugar content than those from NI plants in 2018. Consequently, in the NI treatment, the wines from 2016 and 2017 had a higher alcohol content than those from 2018. Moreover, the wines obtained from the 2017 vintage (both I and NI) had the highest total acidity values and lowest volatile acidity values and the wines from 2018 had the highest pH values and the lowest total acidity values.

The seasonal effect was the dominant factor that explained total acidity and volatile acidity, whereas the treatment was the dominant factor that explained the variation in total soluble solids (ªBrix) and the alcohol content. The treatment x season interaction only accounted for a small fraction of the observed variation in all parameters, except for the pH values and the alcohol content.

## 4. Effects of water deficit on colour parameters and total polyphenol index

The colour characteristics and total polyphenol indices (TPI) of the wines obtained from the I and NI grapevines are shown in Table 3.

Except for the L* value, the treatment affected all the colour parameters and the total polyphenol index (TPI). Compared to the wines from I vines, the wines from NI vines had higher values for colour intensity (CI), wine colour (WC), monomeric anthocyanin colour (MAC), bisulfite-stable colour (BSC), copigmentation colour (CC) and TPI, and lower values for hue (A420/A520). The MAC value was considerably higher than the CC and BSC values for all wines, with MAC contributing an average of 56 % to the wine colour. Withholding water also tended to affect

**TABLE 1.** Mean values of yield components at harvest for each treatment, year, and their interaction.

| | 2016 | | 2017 | | 2018 | | Multifactorial analysis[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Irrigated | Non-irrigated | Irrigated | Non-irrigated | Irrigated | Non-irrigated | T | S | TxS |
| Yield (Kg/vine) | 5.71 ± 0.31 b | 4.82 ± 0.24 a | 6.10 ± 0.29 b | 5.01 ± 0.25 a | 3.03 ± 0.17 b | 2.36 ± 0.15 a | 13.42* | 58.61*** | 0.36 NS |
| Bunches (N°/vine) | 12.83 ± 1.54 | 12.00 ± 1.84 | 12.67 ± 1.99 | 11.67 ± 1.54 | 9.50 ± 1.33 b | 6.00 ± 1.13 a | 10.62* | 67.75*** | 4.99 NS |
| Bunches weight (g) | 426.62 ± 62.18 b | 383.07 ± 55.55 a | 475.41 ± 53.16 b | 429.49 ± 66.49 a | 416.38 ± 58.56 | 389.17 ± 62.51 | 11.41 NS | 15.88 NS | 0.52 NS |
| 100-Berry weight (g) | 299.70 ± 14.25 | 298.17 ± 31.35 | 326.33 ± 25.57 | 312.33 ± 22.43 | 266.20 ± 9.59 | 264.20 ± 29.85 | 0.96 NS | 56.32** | 0.94 NS |

All the parameters are given with their standard deviation (n = 6). For each parameter and season, different lower-case letters indicate significant differences between well-irrigated and non-irrigated plants by Student's t test (p <0.05). a Percentage of variance attributable to T: Treatment, S: Season and TxS: Interaction between treatment and season factors. Statistically significant at * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, respectively. NS: Not significant.

**TABLE 2.** Enological parameters in must and wines obtained from irrigated and non-irrigated Callet grapevines during 2016, 2017 and 2018 seasons.

| | 2016 | | 2017 | | 2018 | | Multifactorial analysis[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Irrigated | Non-irrigated | Irrigated | Non-irrigated | Irrigated | Non-irrigated | T (%) | S (%) | TxS (%) |
| TSS (°Brix) (Must) | 19.10 ± 0.20 a | 20.35 ± 0.15 b | 19.50 ± 0.19 a | 20.25 ± 0.14 b | 19.14 ± 0.12 | 19.24 ± 0.54 | 39.23*** | 27.81** | 17.70** |
| TA (g/L) (Wine) | 4.59 ± 0.00 | 4.90 ± 0.00 | 6.04 ±0.33 | 6.35 ±0.11 | 3.83 ± 0.16 a | 4.13 ± 0.00 b | 2.68*** | 95.53*** | 0.00 NS |
| pH (Wine) | 3.73 ± 0.04 b | 3.61 ± 0.05 a | 3.60 ± 0.12 | 3.69 ±0.06 | 3.80 ± 0.05 | 3.72 ± 0.03 | 4.35 NS | 31.11* | 29.17* |
| VA (Wine) | 0.43 ± 0.04 | 0.44 ± 0.05 | 0.22 ± 0.05 | 0.28 ± 0.00 | 0.39 ± 0.02 a | 0.46 ± 0.02 b | 5.65* | 82.44*** | 1.99 NS |
| Alcohol (Wine) | 11.20 ± 0.28 a | 12.30 ± 0.20 b | 11.48 ± 0.04 a | 12.13 ± 0.06 b | 11.31 ± 0.21 | 11.41 ± 0.32 | 46.02*** | 18.82** | 20.72** |

Nomenclature abbreviation: TSS, Total soluble solids (°Brix); TA, Titratable acidity as g tartaric acid equivalents/L; VA, volatile acidity as g acetic acid/L; % ethanol by volume at 20°C. All the parameters are given with their standard deviation (n = 3). For each parameter and season, different lower-case letters indicate significant differences between well-irrigated and non-irrigated plants by Student's t test (p <0.05). a Percentage of variance attributable to T: Treatment, S: Season and TxS: Interaction between treatment and season factors. Statistically significant at * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, respectively. NS: Not significant.

**TABLE 3.** Color parameters and total polyphenol index (absorbance units) in wines obtained from irrigated and non-irrigated Callet grapevines during 2016, 2017 and 2018 seasons.

| | 2016 | | 2017 | | 2018 | | Multifactorial analysis[a] | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Irrigated | Non-irrigated | Irrigated | Non-irrigated | Irrigated | Non-irrigated | T (%) | S (%) | TxS (%) |
| CI | 2.43 ± 0.25 a | 3.69 ± 0.47 b | 6.01 ± 0.34 a | 6.61 ± 0.34 b | 2.25 ± 0.20 a | 2.80 ± 0.13 b | 5.25*** | 91.29*** | 0.86** |
| Hue | 1.28 ± 0.00 b | 1.15 ± 0.05 a | 0.90 ± 0.01 b | 0.87 ± 0.01 a | 1.29 ± 0.00 b | 1.12 ± 0.02 a | 10.25*** | 85.10*** | 3.04*** |
| a* | 2.44 ± 0.23 a | 4.63 ± 1.99 b | 12.51 ± 0.68 a | 14.40 ± 0.65 b | 3.12 ± 0.16 a | 5.43 ± 0.32 b | 5.10*** | 91.72*** | 0.04 NS |
| b* | 3.37 ± 0.41 | 3.26 ± 0.53 | 2.37 ± 0.11 | 1.88 ± 0.77 | 4.13 ± 0.22 a | 3.55 ± 0.16 a | 5.44** | 71.27*** | 1.38 NS |
| L* | 93.31 ± 4.20 | 91.53 ± 4.67 | 82.83 ± 3.74 | 80.67 ± 3.70 | 96.69 ± 4.33 | 94.83 ± 4.25 | 1.83 NS | 69.87*** | 0.01 NS |
| WC | 1.16 ± 0.01 a | 1.61 ± 0.29 b | 2.98 ± 0.06 a | 3.49 ± 0.02 b | 1.00 ± 0.02 a | 1.45 ± 0.03 b | 6.08*** | 92.51*** | 0.02 NS |
| MAC | 0.67 ± 0.02 a | 1.02 ± 0.17 b | 1.79 ± 0.03 a | 2.02 ± 0.06 b | 0.48 ± 0.02 a | 0.74 ± 0.06 b | 5.65*** | 92.63*** | 0.16 NS |
| BSC | 0.38 ± 0.02 a | 0.49 ± 0.09 b | 0.81 ± 0.03 a | 0.96 ± 0.03 b | 0.37 ± 0.03 a | 0.44 ± 0.01 b | 5.59*** | 91.20*** | 0.52 NS |
| CC | 0.10 ± 0.01 | 0.10 ± 0.03 | 0.38 ± 0.04 a | 0.51 ± 0.11 b | 0.15 ± 0.01 a | 0.27 ± 0.05 b | 6.53*** | 81.21*** | 3.01** |
| TPI | 23.18 ± 0.88 a | 28.03 ± 2.09 b | 41.08 ± 1.54 a | 46.09 ± 1.33 b | 20.56 ± 0.60 a | 22.47 ± 0.24 b | 3.94*** | 94.16*** | 0.52** |

Nomenclature abbreviation: CI, color intensity as sum of absorbances at 420, 520 and 620 nm; Hue, A420/A520; a*: from green to red; b*: from blue to yellow; L*: lightness; WC: red wine color; MAC: monomeric anthocyanin color; BSC: bisulfite stable color; CC: copigmentation color; TPI: total polyphenol index. All the parameters are given with their standard deviation (n = 3). For each parameter and season, different lowercase letters indicate significant differences between well-irrigated and non-irrigated plants by Student's *t* test (p <0.05). a Percentage of variance attributable to T: Treatment, S: Season and TxS: Interaction between treatment and season factors. Statistically significant at * p ≤ 0.05 , ** p ≤ 0.01 , *** p ≤ 0.001 , respectively. NS: Not significant.

**TABLE 4.** Monomeric phenolics (mg/L) in wines obtained from irrigated and non-irrigated Callet grapevines during 2016, 2017 and 2018 seasons. (part 1/2)

| Flavonoids | 2016 | | 2017 | | 2018 | | Multifactorial analysis[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Irrigated | Non-irrigated | Irrigated | Non-irrigated | Irrigated | Non-irrigated | T (%) | S (%) | TxS (%) |
| Anthocyanins | | | | | | | | | |
| Delphinidin-3-glc | 0.11 ± 0.01 a | 0.31 ± 0.02 b | 1.37 ± 0.07 a | 1.64 ± 0.29 b | 0.30 ± 0.03 a | 0.75 ± 0.03 b | 6.94*** | 88.66*** | 0.79 NS |
| Cyanidin-3-glc | 0.09 ± 0.01 a | 0.16 ± 0.07 b | 0.34 ± 0.02 a | 0.44 ± 0.09 b | 0.20 ± 0.02 a | 0.29 ± 0.06 b | 11.63*** | 73.61*** | 0.18 NS |
| Petunidin-3-glc | 0.30 ± 0.03 a | 0.71 ± 0.07 b | 2.63 ± 0.12 a | 3.17 ± 0.41 b | 1.06 ± 0.15 a | 2.25 ± 0.12 b | 11.26*** | 83.52*** | 2.52*** |
| Peonidin-3-glc | 0.37 ± 0.02 a | 1.37 ± 0.09 b | 2.26 ± 0.10 a | 3.45 ± 0.63 b | 1.34 ± 0.08 | 1.42 ± 0.08 | 14.85*** | 72.95*** | 6.01*** |
| Malvidin-3-glc | 2.40 ± 0.16 a | 4.97 ± 0.25 b | 31.82 ± 1.15 a | 35.99 ± 1.78 b | 18.27 ± 1.34 a | 33.70 ± 1.71 b | 7.31*** | 87.62*** | 4.38*** |
| Total non-acylated | 3.26 ± 0.19 a | 7.52 ± 0.36 b | 38.41 ± 1.41 a | 44.69 ± 2.62 b | 21.17 ± 1.61 a | 38.41 ± 1.93 b | 8.30*** | 87.74*** | 3.15*** |
| Delphinidin-3-acglc | 0.09 ± 0.00 a | 0.11 ± 0.01 b | 0.40 ± 0.07 b | 0.28 ± 0.02 a | 0.18 ± 0.01 a | 0.25 ± 0.05 b | 0.11 NS | 77.52*** | 12.89*** |
| Cyanidin-3-acglc | 0.12 ± 0.01 | 0.11 ± 0.02 | 0.36 ± 0.02 | 0.37 ± 0.04 | 0.12 ± 0.01 | 0.14 ± 0.03 | 0.09 NS | 96.32*** | 0.22 NS |
| Petunidin-3-acglc | 0.12 ± 0.02 | 0.11 ± 0.03 | 0.34 ± 0.07 | 0.39 ± 0.05 | 0.25 ± 0.02 a | 0.37 ± 0.05 b | 5.16*** | 79.30*** | 4.88** |
| Peonidin-3-acglc | 0.10 ± 0.00 | 0.09 ± 0.01 | 0.73 ± 0.04 a | 1.33 ± 0.27 b | 0.18 ± 0.03 a | 0.24 ± 0.01 b | 5.42*** | 80.88*** | 8.65*** |
| Malvidin-3-acglc | 0.13 ± 0.01 b | 0.11 ± 0.01 a | 2.91 ± 0.14 b | 2.31 ± 0.33 a | 1.43 ± 0.12 a | 2.97 ± 0.18 b | 1.62*** | 82.47*** | 14.19*** |
| Total acetylated | 0.55 ± 0.04 | 0.52 ± 0.05 | 4.74 ± 0.23 | 4.68 ± 0.58 | 2.16 ± 0.17 a | 3.97 ± 0.31 b | 2.52*** | 89.45*** | 5.82*** |
| Delphinidin-3-cmglc | 0.42 ± 0.04 a | 0.53 ± 0.02 b | 0.75 ± 0.05 | 0.68 ± 0.03 | 0.33 ± 0.03 a | 0.84 ± 0.05 b | 24.17*** | 28.07*** | 44.18*** |
| Cyanidin-3-cmglc | 0.11 ± 0.02 | 0.13 ± 0.05 | 0.64 ± 0.11 b | 0.52 ± 0.05 a | 0.16 ± 0.04 a | 0.25 ± 0.01 b | 0.00 NS | 89.35*** | 4.62*** |
| Petunidin-3-cmglc | 0.21 ± 0.01 | 0.24 ± 0.04 | 1.78 ± 0.08 | 1.80 ± 0.10 | 1.00 ± 0.15 a | 1.74 ± 0.09 b | 3.55*** | 89.42*** | 5.63*** |
| Peonidin-3-cmglc | 0.17 ± 0.01 | 0.25 ± 0.11 | 0.73 ± 0.07 a | 1.17 ± 0.16 b | 0.79 ± 0.11 a | 1.19 ± 0.08 b | 14.14*** | 77.10*** | 3.74*** |
| Malvidin-3-cmglc | 0.75 ± 0.04 a | 0.92 ± 0.17 b | 7.67 ± 0.28 a | 9.61 ± 0.74 b | 4.90 ± 0.62 a | 9.33 ± 0.50 b | 8.77*** | 84.27*** | 5.64*** |
| Total coumaroylated | 1.66 ± 0.09 a | 2.07 ± 0.38 b | 11.56 ± 0.39 a | 13.78 ± 0.86 b | 7.18 ± 0.95 a | 13.35 ± 0.73 b | 8.45*** | 84.49*** | 5.69*** |
| Total anthocyanins | 5.48 ± 0.31 a | 10.12 ± 0.76 b | 54.71 ± 1.67 a | 63.14 ± 3.90 b | 30.51 ± 2.71 a | 55.72 ± 2.94 b | 7.80*** | 87.45*** | 3.83*** |

Nomenclature abbreviation: glc, glucoside; acglc, acetylglucoside; cmglc, coumaroylglucoside; gal, galactoside; glcU, glucuronide. All the parameters are given with their standard deviation (n = 3). For each parameter and season, different lowercase letters indicate significant differences between well-irrigated and non-irrigated plants by Student's t test (p <0.05). a Percentage of variance attributable to T: Treatment, S: Season and TxS: Interaction between treatment and season factors. Statistically significant at *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, respectively. NS: Not significant.

**TABLE 4.** Monomeric phenolics (mg/l) in wines obtained from irrigated and non-irrigated Callet grapevines during 2016, 2017 and 2018 seasons. (part 2/2)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Flavonols** | | | | | | | | | |
| Myricetin-3-gal | 0.08 ± 0.02 a | 3.16 ± 0.14 | 0.23 ± 0.13 b | 3.11 ± 0.15 | 0.43 ± 0.04 a | 0.87 ± 0.12 b | 0.46*** | 98.34*** | 0.58*** |
| Myricetin-3-glc+Myricetin-3glcU | 1.79 ± 0.18 | 6.26 ± 0.29 b | 1.64 ± 0.35 | 5.87 ± 0.29 a | 2.62 ± 0.13 a | 6.87 ± 0.36 b | 7.74*** | 67.68*** | 23.27*** |
| Quercetin-3-gal | 1.54 ± 0.34 | 3.29 ± 0.60 | 1.28 ± 0.37 | 2.56 ± 2.30 | 1.00 ± 0.05 a | 1.35 ± 0.07 b | 0.79 NS | 40.60*** | 3.29 NS |
| Quercetin-3-glc+Quercetin-3-glcU | 6.28 ± 1.31 | 3.81 ± 0.24 | 5.45 ± 1.01 | 5.43 ± 2.27 | 3.13 ± 0.35 a | 9.41 ± 0.42 b | 26.69*** | 9.46** | 41.84*** |
| Isorhamnetin-3-glc+Kaempferol-3-glc | 4.29 ± 0.47 | 1.89 ± 0.23 a | 4.68 ± 0.63 | 2.74 ± 0.20 b | 2.82 ± 0.14 a | 3.26 ± 0.21 b | 7.77*** | 80.61*** | 1.07 NS |
| Free myricetin | 1.62 ± 0.11 a | 2.60 ± 0.13 b | 2.82 ± 1.19 b | 2.34 ± 0.21 a | 1.44 ± 0.07 a | 2.63 ± 0.16 b | 25.98*** | 6.65 NS | 24.19*** |
| Free quercetin | 15.22 ± 2.06 | 12.00 ± 0.64 | 19.27 ± 5.09 | 12.71 ± 0.85 | 10.97 ± 0.57 a | 13.89 ± 0.63 b | 13.89** | 44.36*** | 4.08 NS |
| Free isorhamnetin | 1.19 ± 0.20 | 0.62 ± 0.03 | 1.49 ± 0.39 | 0.61 ± 0.04 | 0.72 ± 0.06 | 0.77 ± 0.04 | 2.36 NS | 73.79*** | 3.32 NS |
| Free kaempferol | 1.94 ± 0.10 | 0.97 ± 0.05 a | 2.47 ± 0.76 | 1.11 ± 0.08 b | 1.39 ± 0.13 a | 1.64 ± 0.09 b | 6.97** | 65.88*** | 1.99 NS |
| Total flavonols | 33.96 ± 1.52 | 34.61 ± 1.64 | 39.33 ± 9.90 | 36.48 ± 1.64 | 24.52 ± 1.24 a | 40.68 ± 1.95 b | 35.58*** | 6.81 NS | 21.62*** |
| **Flavanol** | | | | | | | | | |
| Catechin | 10.51 ± 0.49 | 112.59 ± 8.19 | 10.93 ± 0.57 | 109.21 ± 5.2 9 | 13.45 ± 6.73 | 17.02 ± 7.43 | 0.00 NS | 98.65*** | 0.09 NS |
| **Non-flavonoids** | | | | | | | | | |
| **Hydroxycinnamic acids (HCAs)** | | | | | | | | | |
| cis-Caftaric acid | 1.84 ± 0.12 b | 3.39 ± 0.16 | 1.53 ± 0.11 a | 3.14 ± 0.31 | 1.33 ± 0.10 a | 2.15 ± 0.15 b | 0.31 NS | 85.10*** | 10.68*** |
| trans-Caftaric acid | 24.51 ± 1.50 a | 45.07 ± 2.09 a | 28.61 ± 2.46 b | 57.14 ± 2.70 b | 28.53 ± 1.46 a | 35.48 ± 1.62 b | 11.26*** | 84.07*** | 2.07*** |
| cis-Coutaric acid | 3.21 ± 0.27 b | 4.14 ± 0.26 b | 2.65 ± 0.35 a | 3.65 ± 0.18 a | 2.72 ± 0.13 a | 3.88 ± 0.23 b | 0.09 NS | 43.20*** | 43.00*** |
| trans-Coutaric acid | 7.67 ± 0.41 | 20.40 ± 0.92 a | 7.40 ± 0.53 | 22.77 ± 1.24 b | 8.08 ± 2.03 | 9.72 ± 0.64 | 0.93** | 95.88*** | 0.74** |
| Caffeic acid | 1.20 ± 0.07 a | 3.85 ± 0.17 a | 2.33 ± 0.55 b | 4.42 ± 0.21 b | 1.24 ± 0.06 | 1.24 ± 0.06 | 4.50*** | 89.46*** | 2.99*** |
| trans-Fertaric acid | 2.91 ± 0.15 a | 4.09 ± 0.21 | 3.30 ± 0.15 b | 3.92 ± 0.18 | 2.10 ± 0.10 a | 2.78 ± 0.17 b | 4.62*** | 84.57*** | 6.26*** |
| p-Coumaric acid | 0.48 ± 0.02 a | 5.95 ± 0.30 a | 0.65 ± 0.05 b | 8.30 ± 0.78 b | 0.35 ± 0.15 | 0.41 ± 0.09 | 1.78*** | 94.60*** | 2.66*** |
| Total HCAs | 41.80 ± 2.36 a | 86.91 ± 3.93 a | 46.46 ± 2.28 b | 103.35 ± 5.38 b | 44.34 ± 3.16 a | 55.65 ± 2.52 b | 5.17*** | 92.04*** | 1.03*** |
| **Hydroxybenzoic acids (HBAs)** | | | | | | | | | |
| Gallic acid | 18.47 ± 2.33 | 62.87 ± 10.42 | 21.07 ± 3.77 | 64.23 ± 3.59 | 34.73 ± 2.06 | 40.03 ± 6.28 | 0.68 NS | 91.85*** | 0.19 NS |
| Syringic acid | 0.80 ± 0.09 a | 1.92 ± 0.39 b | 0.94 ± 0.12 b | 1.54 ± 0.07 a | 2.40 ± 0.98 | 1.77 ± 0.16 | 4.49 NS | 55.39*** | 5.47 NS |
| Total HBAs | 19.27 ± 2.42 | 64.80 ± 10.82 | 22.01 ± 3.88 | 65.78 ± 3.64 | 37.13 ± 2.86 | 41.80 ± 6.17 | 0.54 NS | 91.80*** | 0.16 NS |

Nomenclature abbreviation: glc, glucoside; acglc, acetylglucoside; cmglc, coumaroylglucoside; gal, galactoside; glcU, glucuronide. All the parameters are given with their standard deviation (n = 3). For each parameter and season, different lowercase letters indicate significant differences between well-irrigated and non-irrigated plants by Student's t test (p <0.05). a Percentage of variance attributable to T: Treatment, S: Season and TxS: Interaction between treatment and season factors. Statistically significant at *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, respectively. NS: Not significant.

CIELab colour by increasing the a* component (measure of redness) and decreasing the b* (measure of yellowness) and L* (measure of lightness) components in comparison with the wines from I vines, although the differences in these parameters were not significant (Table 3).

In terms of the colour components, and in agreement with CI values, wines from 2017 showed the highest wine colour (WC) values for MAC, BSC, and CC. In addition, wines from 2017 showed the highest TPI. The CI values of wines from 2016 and 2018 were abnormally low for red wines. Regarding the CIELab colour coordinates, the wines from 2017 exhibited the highest value for the a* component (measure of redness) and the lowest values for the b* (measure of yellowness) and L* (measure of lightness) components.

The treatment x season interaction affected the CI, Hue, CC and TPI values. The seasonal effect was the dominant factor that explained the variation in all colour parameters determined and TPI, whereas the treatment accounted for a small fraction of the observed variation.

## 5. Effects of water deficit on monomeric phenolics

Table 4 presents the monomeric phenolic content of the wines obtained from the I and NI wines.

### 5.1. Anthocyanins.

Total anthocyanins were higher in the wines from NI plants than those from I plants, as result of higher contents of total non-acylated and coumaroylated anthocyanins in the wines from NI grapes. In fact, the increases in total anthocyanins in the wines from NI grapes ranged from 15 % in 2017 to 83 % and 85 % in 2016 and 2018 respectively. In general, the wines from NI plants exhibited higher contents of delphinidin-3-glc, cyanidin-3-glc, petunidin-3-glc and malvidin-3-glc. The wines from NI vines in 2016 had higher delphinidin-3-cmglc and malvidin-3-cmglc content and lower malvidin-3-acglc content than the wines from I grapes. Wines from NI grapes in 2017 had higher peonidin-3-acglc, peonidin-3-cmglc

and malvidin-3-cmglc content and lower malvidin-3-acglc content than the wines from I grapes. In general, the wines from NI vines in 2018 had a higher content of all acetylated and coumaroylated anthocyanins.

Moreover, the season affected the content of all anthocyanins in the wines. The content of several anthocyanins and the total anthocyanin content were lower in the wines produced in 2016 than 2017 and 2018. The treatment x season interaction affected the content of all the evaluated anthocyanins, except for delphinidin-3-glc, cyanidin-3-glc and cyanidin-3-acglc.

### 5.2. Flavonols and flavanols.

The wines from NI plants tended to have higher total flavonol content than those from I plants, although the differences between treatments were not significant in 2016 or 2017. Specifically, and with some differences between seasons, water deficit affected the content of myricetin-3-gal, myricetin-3-glc+myricetin-3glcU, quercetin-3-glc+quercetin-3-glcU, isorhamnetin-3-glc+kaempferol-3-glc, free myricetin, free quercetin, free kaempferol and total flavonols. Free quercetin was the main flavonol found in the wines.

The season affected the content of individual flavonols and flavanols, except for free myricetin and total flavonols. In general, the wines from the 2016 season had a lower flavonol 3-*O*-glycoside content and higher free aglycones content than the wines from 2017 and 2018. The wines from 2017 had a higher catechin content than those from 2016 and 2018.

The treatment x season interaction affected the content of myricetin-3-gal, myricetin-3-glc+myricetin-3glcU, quercetin-3-glc+quercetin-3-glcU, free myricetin and total flavonols.

### 5.3. Non-flavonoids.

The water deficit affected the content of *trans*-caftaric acid, *trans*-coutaric acid, caffeic acid, *trans*-fertaric acid and *p*-coumaric acid, resulting in higher total hydroxycinnamic

**TABLE 5.** Proanthocyanidin concentration (mg/L), % catechin, % epicatechin, % epicatechin-gallate terminal subunits, and mean degree of polymerization (mDP) in wines obtained from irrigated and non-irrigated Callet grapevines during 2016, 2017 and 2018 seasons.

| | 2016 | | 2017 | | 2018 | | Multifactorial analysis[a] | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Irrigated | Non-irrigated | Irrigated | Non-irrigated | Irrigated | Non-irrigated | T (%) | S (%) | TxS (%) |
| PA | 32.99 ± 2.75 | 29.89 ± 5.32 | 192.34 ± 36.34 a | 353.87 ± 17.36 b | 90.58 ± 2.56 | 87.45 ± 14.51 | 5.13*** | 81.34*** | 11.53*** |
| Cat | 10.50 ± 1.00 b | 8.77 ± 1.04 a | 7.80 ± 0.86 | 8.43 ± 1.26 | 15.20 ± 1.66 | 15.85 ± 1.40 | 0.05 NS | 86.54*** | 2.64* |
| Epi | 7.22 ± 1.09 b | 4.90 ± 0.66 a | 6.97 ± 0.27 b | 5.61 ± 0.21 a | 2.61 ± 0.30 | 2.98 ± 0.64 | 8.76*** | 73.36*** | 8.91*** |
| Epi-gal | 4.13 ± 0.31 a | 5.78 ± 0.45 b | 5.89± 0.16 b | 5.25 ± 0.55 a | 2.41 ± 0.26 b | 2.08 ± 0.22 a | 0.51 NS | 84.81*** | 10.48*** |
| mDP | 6.31 ± 0.68 | 6.77 ± 0.24 | 6.33 ± 0.38 a | 6.88 ± 0.36 b | 6.99 ± 0.77 | 6.80 ± 0.59 | 5.87 NS | 7.75 NS | 8.89 NS |

Nomenclature abbreviation: PA, total proanthocyanidins content (mg/L); Cat, % catechin terminal subunits; Epi, % epicatechin terminal subunits; Epi-gal, % epicatechin-gallate terminal subunits. All the parameters are given with their standard deviation (n = 3). For each parameter and season, different lower-case letters indicate significant differences between well-irrigated and non-irrigated plants by Student's t test (p <0.05). a Percentage of variance attributable to T: Treatment, S: Season and TxS: Interaction between treatment and season factors. Statistically significant at *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, respectively. NS: Not significant.
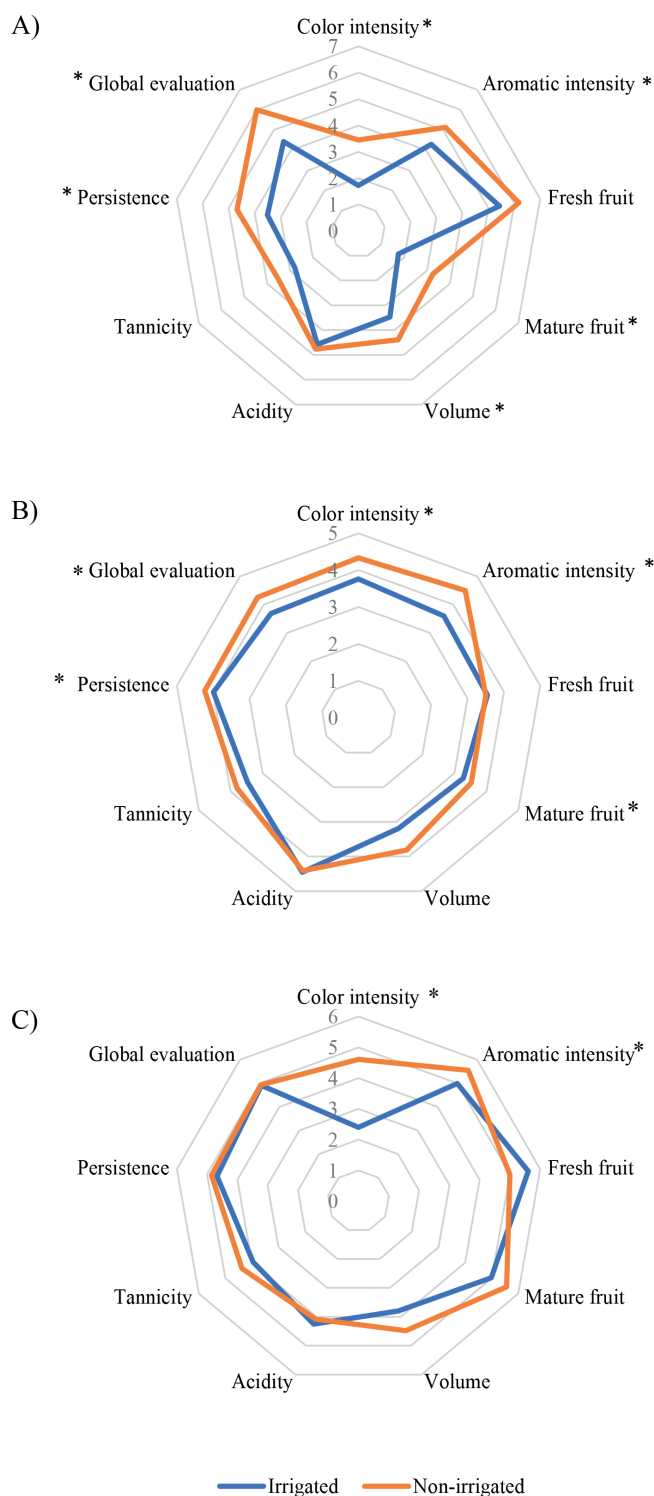
**FIGURE 3.** Sensory evaluation in wines obtained from irrigated and non-irrigated Callet grapevines during 2016 (A), 2017 (B) and 2018 (C) seasons.

Descriptors marked with an asterisk differed significantly between treatments (p < 0.05).

acid content in the wines from NI plants than those from I plants. In contrast, total hydroxybenzoic acid content was not significantly different in the wines obtained from both irrigation regimes in any of the years.

The *trans*-caftaric acid was by far the major acid (55–64 %), followed by the *trans*-coutaric acid (16–23 %).

The season affected the content of all the determined non-flavonoid compounds. The wines produced in 2017 had higher non-flavonoid compounds content than those from the 2016 and 2018 seasons.

In general, the seasonal effect was the dominant factor that explained the variation in the analysed phenolic compounds, whereas the treatment and treatment × season interaction only accounted for small fractions of the observed variation and explained none of the variation in hydroxybenzoic acids.

## 6. Effects of water deficit on proanthocyanidins

The water deficit affected proanthocyanidin content and the percentage of epicatechins (Table 5). The wines from NI grapes exhibited a higher proanthocyanidins content in 2017, whereas the water deficit had no effect on the proanthocyanidin content of the wines in 2016 and 2018.

In 2016 and 2017, the wines from NI grapes had a lower epicatechin concentration than those from I grapes; however, no differences were observed between treatments in 2018. In all the wines, the terminal units were primarily comprised of catechin, while epicatechin and epicatechin-gallate were found at lower quantities.

The season was the dominant factor that explained the variation in the concentration and composition of proanthocyanidins, except for mean degree of polymerisation of proanthocyanidins. Concretely, the season affected the proanthocyanidin content and the percentages of catechin, epicatechin and epicatechin-gallate terminal subunits. The 2017 wine had significantly higher proanthocyanidin concentrations than those from 2016 and 2018. The wines produced in 2018 had higher percentages of catechins than those produced in 2016 and 2017, whereas the wines from 2016 and 2017 had higher percentages of epicatechin and epicatechin-gallate than those from 2018.

The treatment x season interaction affected the proanthocyanidin content and the percentages of catechin, epicatechin and epicatechin-gallate terminal subunits of proanthocyanidins.

## 7. Effects of water deficit on sensory properties

All of the wines achieved low or medium scores for the evaluated descriptors using the sensory analysis scale (Figure 3). However, in all seasons, the wines from NI grapes were perceived to have higher colour intensity and aromatic intensity than those from I grapes (Figure 3A, B and C). In the 2016 and 2017 seasons, the wines from the NI plants received higher marks for mature fruit, persistence and global evaluation (Figure 3A and B).

Moreover, in 2016, the wines from NI plants received higher marks for the volume descriptor than those from I plants. In 2018, with the exception of colour and aromatic intensity, the panelists did not report any noticeable differences in the sensory attributes for the wines from the NI and I treatments; this was probably because the chemical changes in the wines were not significant enough to lead to sensory changes in the wines, or because less significant differences were detected related to the characteristics of the 2018 vintage wines from NI and I grapes.

# DISCUSSION

## 1. Effects of water deficit on vine performance and grape composition

Water deficit induces complex physiological regulation within grapevines and mainly affects plant growth. Indeed, water deficit has been reported to negatively affect the vegetative growth of vine trunks, shoots and leaves (Keller *et al.*, 2016). Moreover, water deficit greatly impacts the size and metabolic adjustment of grapes; the reduction in total yield depends on the severity, duration and timing of the water deficit (Intrigliolo *et al.*, 2012; Mirás-Avalos and Intrigliolo, 2017). We analysed the effects of sustained water deficit on established *V. vinifera* L. 'Callet' vines during the entire vegetative period in three consecutive seasons. In the first two seasons, water deficit significantly reduced the grape yield by decreasing the bunch weight (Table 1). However, in 2018, the intense and persistent water shortage during the previous two years may have induced a reduction in bud fertility (Buttrose, 1974); water deficit had a greater effect on the bunch number rather than the bunch weight, and thus resulted in a significant reduction in grape yield in 2018.

The water deficit also influenced the rate of sugar accumulation (Table 2) by increasing the TSS in NI plants compared with I plants; this is in accordance with other studies (Gambetta *et al.*, 2020). However, water deficit did not affect the berry weight in this study (Table 1), which is similar to the results of Shellie (2010), which showed similar berry weight in all irrigation treatments; thus for the same berry weight the NI vines had higher TSS than the I vines (Intrigliolo and Castel, 2010). These results indicate that berry weight variation is established early in the season (Gray and Coombe, 2009) when few differences between I and NI plants are observed.

## 2. Water deficit influences wine composition

In each of the three studied seasons the effects of water deficit on the oenological parameters of the wines were different. In general, reports on the impact of water deficit on wine quality parameters have described conflicting results: in some studies (Intrigliolo and Castel, 2010; Santesteban *et al.*, 2011) a decrease in titratable acidity (TA) under water deficit conditions has been observed, whereas others have reported no impact on TA and pH (Acevedo-Opazo *et al.*, 2010). On the other hand, Intrigliolo and Castel (2009) observed an increment in the pH of must as a result of irrigation. In the present study, we concluded that the only detrimental effect of withholding water on oenological parameters was an increase in the alcohol content during the 2016 and 2017 seasons and an increase in volatile acidity (VA) during the driest season (2018) compared to irrigated vines. However, withholding water had no observable impact on pH (Table 2).

With the exception of the wines obtained in 2017, the TA values of the Callet wines in this study were lower than reported TA values for varietal red wines after alcoholic fermentation (Martínez-Pinilla *et al.*, 2012; Mulet *et al.*, 1992). The VA values obtained after alcoholic fermentation confirmed suitable winemaking with an absence of microbial alterations. Similarly, the obtained wines exhibited lower ethanol contents after alcoholic fermentation than other varietal red wines (Martínez-Pinilla *et al.*, 2012), but they showed similar values to Callet wines (Mulet *et al.*, 1992).

## 3. Water deficit influences wine colour parameters and the total polyphenol index

The water deficit affected the TPI and all the evaluated colour parameters, except for the L* value. Similarly, Lizama *et al.* (2021) observed a detrimental effect of the irrigation treatments on the TPI and CI values. Furthermore, in agreement with our findings, Romero *et al.* (2013) observed that wines from vines subjected to a water deficit exhibited lower CI and higher CIELab parameters. The effects of irrigation on wine phenolics and colour composition reported in this trial may be due to the direct effects on the phenolic composition of the grape skins (Lizama *et al.*, 2021) rather than a dilution effect (higher skin-to-pulp-ratio) given the similar berry sizes observed in the irrigated and non-irrigated treatments.

Regardless of the treatment (and with the exception of the 2017 wines), the wines had similar colour intensity (CI) to previously reported values for Callet wines (Escalona *et al.*, 2006; Mulet *et al.*, 1992). The TPI values in this study were also in agreement with the normal values for Callet wines (Escalona *et al.*, 2006). The colour hue values ranged from 0.87 to 1.29. Wine hue is a measure of wine tint and indicates the development of the orange colour during ageing, with young wines showing values below 1 (0.5–0.7) and aged wines reaching an upper limit of around 1.2–1.3 (Skendi *et al.*, 2020). The literature reports that the hue is affected by winemaking procedures and that $O_2$-treated wines show higher hue values compared to the non-treated control wines (McRae *et al.*, 2015). Therefore, these results suggest that the analysed wines had undergone a certain degree of oxidation.

The season was a factor that strongly affected all of the colour characteristics and TPI of the wines: the wines produced from grapes in 2017 had the highest CI and TPI values. The CI values of the wines from 2016 and 2018 were abnormally low for red wines and similar to previously reported values for rosé wines (Sam *et al.*, 2021) and Callet red wines (Escalona *et al.*, 2006; Mulet *et al.*, 1992). In all the wines, MAC was considerably higher than CC and BSC, and MAC contributed an average of 56 % to the wine colour.

The treatment x season interaction affected the CI, hue, CC and TPI values.

It is interesting to note that the seasonal effect was the dominant factor of variation for all colour parameters and TPI, whereas the treatment and treatment × season accounted for a small fraction of the observed variation.

## 4. Water deficit influences wine monomeric phenolics

The water deficit affected all anthocyanin content, except for delphinidin-3-acglc, cyanidin-3-acglc, and cyanidin-3-cmglc (Table 4). The higher anthocyanin content of the wines from NI grapes may be a result of water stress-induced changes in the expression of genes and transcription factors involved in the phenylpropanoid pathway, which increase the concentration of phenolic compounds in grapes, such as anthocyanins and proanthocyanidins (Cáceres-Mella *et al.*, 2017; Deluc *et al.*, 2009). Moreover, Ojeda *et al.* (2001) and Chacón-Vozmediano *et al.* (2021), among others, have also reported higher concentrations of phenolic compounds in wines obtained from water-stressed plants than those obtained from well-watered plants due to a reduction in the berry weight and a higher skin to pulp ratio. Therefore, as proposed by Poni *et al.* (2018), Regulated Deficit Irrigation (RDI) may be a good strategy for increasing anthocyanins content, and therefore the colour of wines, such as Callet, from grapes with low phenolic content. In general, moderate water deficit has been reported to increase the concentrations of these compounds in red grapes by improving berry quality. However, these positive effects have been reported to decrease or even disappear when a certain water-deficit threshold has been surpassed, (Ojeda *et al.*, 2002).

In this trial, the content of several anthocyanins and the total anthocyanin content were lower in the wines produced in the 2016 season than those of 2017 and 2018. However, regardless of the season, while the total concentrations of monomeric anthocyanins in red wines produced from Callet were lower than the reported values for red varietal wines (Garde-Cerdán *et al.*, 2021; Martínez-Pinilla *et al.*, 2012), they were within the usual range of values for rosé wines (He *et al.*, 2012; Puértolas *et al.*, 2011). Malvidin-3-glc was the main anthocyanin found in wines, representing 48 % to 60 % of anthocyanin content, and its derivatives were also the main acetylated and coumaroylated forms of anthocyanins, which is typical for *Vitis vinifera* red wines (Martínez-Pinilla *et al.*, 2012).

Water deficit invariably affected the concentrations of flavonols and flavanols, depending on the year. In 2018, when larger differences in flavonols and flavanols between NI and I plants were observed (Table 1), higher contents of most individual flavonols, with the exception of free isorhamnetin, were present in wines from NI vines than in those from I plants. The greater reduction in total yield as a consequence of water deficit in 2018 (up to 22 %), compared to the reductions obtained in 2016 and 2017 (15 and 17 %, respectively), may explain the greater differences in the concentration of individual flavonols in the wines in 2018. In addition, water deficit may reduce canopy growth in NI

plants, and thus potentially increase the exposure of bunches to sunlight (Castellarin *et al.*, 2007). Flavonol biosynthesis is particularly responsive to light and UV exposure (Teixeira *et al.*, 2013); therefore, the inconsistencies in flavonols observed between the years could be related to the differences in the impact of water deficit on the canopy structure.

The obtained Callet wines exhibited similar flavonol contents to other red wines (Martínez-Pinilla *et al.*, 2012). Moreover, free quercetin was the main flavonol in the wines; quercetin and its glycosides are the main flavonols in almost all grape varieties (Mattivi *et al.*, 2006).

With respect to non-flavonoid phenolic compounds, the wines from NI grapes had higher total hydroxycinnamic acid content than the wines from the I grapes in all seasons. Niculcea *et al.* (2015) also reported that deficit irrigation increased the hydroxycinnamic acid content compared to well-watered Tempranillo grapes. In all the analysed wines, the *trans*-form of the acids were present at higher concentrations than the *cis* isomers, which has also been reported for other red wine varieties (Ginjom *et al.*, 2011).

## 5. Water deficit influences wine proanthocyanidins

A general increase in the mean degree of polymerisation and the proanthocyanidin content was observed in NI wines compared to I wines in 2017 (Table 5); similar effects on degree of polymerisation have been observed in other studies (Cáceres-Mella *et al.*, 2017; Ollé *et al.*, 2011). However, no differences in proanthocyanidin content as a result of the different degrees of water deficit were found in 2016 and 2018, indicating contrasting results between years. Herrera *et al.* (2015) reported that water deficits increased the proanthocyanidin content of Merlot berry skins. In contrast, other studies have reported water deficit to have little or no effect on the composition of proanthocyanidins (Pérez-álvarez *et al.*, 2021). This indicates that different grapevine varieties may respond differently to imposed water deficit, with different molecular families affected either positively or negatively (Pinasseau *et al.*, 2017).

In this trial, with the exception of the mean degree of polymerisation of proanthocyanidins (mDP), the season was the dominant factor explaining the variation in the concentration and composition of proanthocyanidins; the season affected proanthocyanidin content and the percentages of catechin, epicatechin and epicatechin-gallate terminal subunits. The proanthocyanidin concentration of all the wines was significantly higher in 2017 than in 2016 and 2018, although the proanthocyanidin contents in this study were lower than the values reported for red varietal wines (Martínez-Pinilla *et al.*, 2012). In all wines, the terminal units were primarily comprised of catechin, while epicatechin and epicatechin-gallate were present at lower quantities. Catechin is the primary terminal subunit in grape skin, while epicatechin and epicatechin-gallate are found at much lower quantities (Monagas *et al.*, 2003), suggesting that grape skins rather than grape seeds mainly contributed to the increases in proanthocyanidins in 2017.

## 6. Water deficit influences sensory properties

Globally, the vine water regime positively influenced the sensory characteristics of the Callet wines, with the exception of the year 2018, when the changes in the wine characteristics of the NI vines were less perceptible. This indicates that the higher water deficit experienced by the NI plants (compared with 2016 and 2017) may have negatively affected the aromatic potential of the wine.

The colour and aroma of the wines from NI vines were higher in intensity than in the wines from I vines. The NI wines also had higher colour intensities and total polyphenol indices than the wines from I grapes (Table 3). Therefore, the judges were able to perceive the physicochemical differences induced by the water deficit in the sensory analysis of the wines.

Moreover, the wines from the NI treatment in 2016 and 2017 received higher scores for the following descriptors: mature fruit, persistence and global evaluation (Figure 3A and B). The fact that the mature fruit score was higher for the wines from the NI grapes indicates a higher concentration of these fruit aroma compounds. Accordingly, water deficit has been reported to increase the concentration of C13-norisoprenoids and terpenes (Song *et al.*, 2012) by modulating structural and regulatory genes involved in the biosynthesis of volatile compounds (Deluc *et al.*, 2009). However, a lower water supply does not always have a positive effect on the sensory properties of wines (Trigo-Córdoba *et al.*, 2014). Therefore, regulated deficit irrigation (RDI) may be a better strategy to improve wine quality than either full irrigation or no irrigation treatments. Balint & Reynolds (2014) studied the effect of different irrigation strategies on cv. Cabernet Sauvignon aroma descriptors and reported that 100 % water replacement was not recommended at any phenological stage. However, 50 % and 25 % water replacement had overall positive effects on fruit composition and wine varietal typicity.

The differences in the persistence descriptor observed in the sensory analysis could be due to the higher alcohol content and TPI of wines from NI grapes in the 2016 and 2017 seasons (Tables 2 and 3).

## CONCLUSION

To conclude, the water deficit reduced crop yield by up to 22 %, 17 % and 15 % in 2018, 2017 and 2016 respectively. However, regardless of the season, the imposed water deficit mostly increased the fruit sugar content at harvest and the TPI and colour components of the wines. Generally, a continuous water deficit increased phenolics content; however, the water regime had a lower effect on the total concentration of proanthocyanidins. The changes in wine phenolics were season-dependent, indicating that different growing seasons are associated with specific biosynthetic effects that alter the phenolic content and, potentially, the extraction and retention of phenolic compounds in wine. Over the three years of this study, the NI wines were perceived to have higher colour intensity - which was related to a higher anthocyanin concentration and aromatic intensity - than the wines produced from the I regime. Overall, withholding water seemed to enrich the phenolic composition of the wines, with the additional advantage of reducing water usage. In order to confirm the apparent benefits of withholding water in a further study, it would be necessary to carry out regulated deficit irrigation during the drier years, similar to 2018 in the present study, which had less than 170 mm of precipitation during the spring and summer months.

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