**Phenolic composition of Tempranillo Blanco (*Vitis vinifera* L.) grapes and wines after biostimulation through a foliar seaweed application**

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## Abstract

**BACKGROUND**: Seaweeds are defined as novel elicitors in many crops, allowing to trigger the synthesis of secondary metabolites in different plant tissues. Currently, phenolic composition of Tempranillo Blanco grapes and wines is unpublished. This trial aimed to study the effects of an *Ascophyllum nodosum* seaweed extract applied to Tempranillo Blanco grapevines, at a low (Ld) and high (Hd) dosages, on grape and wine phenolic compounds during two consecutive seasons (2017-2018).

**RESULTS**: The results showed that catechin was the most abundant phenolic compound in Tempranillo Blanco grapes and wines. Season affected weight of 100 berries and some enological parameters. Catechin and flavonols concentration in grapes was increased after Hd applications to grapevines, independently of the season. The concentration of hydroxycinnamic and hydroxybenzoic acids in wines was affected by vintage, probably due to oxidation reactions, as well as pinking phenomena, whereas stilbenes content in wines was conditioned by the affection of cryptogamic diseases in grapes.

**CONCLUSIONS**: Therefore, seaweeds might to act as elicitor of several phenolic compounds in grapes, enhancing the content of some phenolic compounds in wines.

**KEYWORDS:** *Ascophyllum nodosum,* flavonoids, elicitation, resveratrol, stilbenes

**INTRODUCTION**

Tempranillo Blanco (*Vitis vinifera* L.) was originated from a natural mutation of a Tempranillo grapevine, which was discovered in 1988 in an ancient vineyard in the location of Murillo de Río Leza (La Rioja, Spain). It has been reported that a catastrophic genome rearrangement in Tempranillo caused the hemizygous deletion of 313 genes, including a loss of the functional copy for the myeloblastosis transcription factors required for anthocyanin pigmentation in the berry skin.1 This has led to the loss of the berry pigmentation and a low gamete viability, which compromises its fruit set and productivity.1 Respect to its enological potential, grapes and wines obtained from Tempranillo Blanco grapevines present high amounts of certain organic acids and total polyphenol index compared to other white grapevine varieties.2 These physico-chemical properties found in Tempranillo Blanco can be suitable, both for the production of young white wines, and for another type of barrel aging wine.2 Due to these particularities, Tempranillo Blanco production has been recently authorized by the Regulatory Council of Rioja Appellation (D.O.Ca. Rioja). This white grape variety is nowadays used by some D.O.Ca. Rioja certified wineries to elaborate fruity wines, particularly with intense citrus and tropical fruits characteristics.3

 Phenolic compounds play a key role on grape and wine quality since they are responsible for sensory attributes such as color, astringency and bitterness.4 These compounds are commonly classified into non-flavonoid (hydroxycinnamic acids, hydroxybenzoic acids and stilbenes) and flavonoid compounds (anthocyanins, flavonols and flavanols). Wines elaborated from red grapevine varieties contain a higher level of phenolic compounds than the white ones, since they are fermented under the presence of skins and seeds.5 However, the presence of phenolic compounds in white cultivars is of main importance since they are more susceptible to oxidation reactions, and in the presence of iron, they can react with oxygen to produce quinones and hydrogen peroxide, both of which continue the oxidation process.6,7 Additionally, hydroxycinnamic acids are the most abundant phenols in white musts and wines, which are enzymically oxidized during grapes crashing and subsequently prone to initiate browning phenomena in musts.7

 White grapevine varieties are more susceptible to fungal diseases than red grapevine varieties.8 Stilbenes are phytoalexins that are synthetized as glucosides in grapevines, in response to cryptogrammic diseases affection.9,10 Due to this, resveratrol derivatives can be considered as stress markers.11 Stilbenes are found mainly in grape skins, and therefore a greater content of these compounds may be release in wines.7,11 Some external stimuli such as ultrasonication treatments, ultra violet irradiation, macronutrients, fungicides and elicitors, have allowed to increase the stilbene concentration, including other phenolic compounds, in grape skins, musts and wines.12

During the last decade, the study of phenolic compounds is increasing due to their importance about the protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.13 Consumption of flavonols have been linked to protective effects against several specific cancers, including leukemia and pancreatic, breast, cervical, prostate, uterine, and urinary tract cancers.14 Stilbenes exhibit anti-inflammatory and cardioprotective potential by inhibiting the expression of inflammatory mediators and the monocyte adhesion to vascular endothelial cells.14,15 Moreover, catechin consumption has many beneficial properties for human health such as anti-cancer, anti-obesity, anti-diabetic, anti-cardiovascular, anti-infectious, hepatoprotective, and neuroprotective effects.16

 Different studies suggest that seaweed extracts can behave as novel elicitors, triggering the accumulation of secondary metabolites, mainly phenolic compounds in several plant crops, including grapevines.17-20 However, most of the studies about the effects of seaweed applications on grapevines are focused on its effects on plant physiology, productivity and some secondary metabolism of grapes or musts than in grape and wine quality in terms of phenolic composition.20-23 Due to this, the aim of this work was a) to characterize Tempranillo Blanco grape and wine phenolic composition and b) to study the effect of foliar applications of an *Ascophyllum nodosum* seaweed fertilizer to Tempranillo Blanco grapevines, at two different dosages, on grape and wine phenolic composition during two consecutive seasons (2017-2018).

**MATERIALS AND METHODS**

**Plant material**

A field study was conducted in an experimental vineyard of Tempranillo Blanco (*Vitis vinifera* L.) located in La Grajera, Logroño, La Rioja, in North of Spain (42º26’26.23’’ North Latitude 2º30’51.25’’ West Latitude; 447 m above sea level) during 2017 and 2018 seasons. The vineyard was planted in 2002 in an East-West orientation. At planting, the grapevines were grafted onto 110 Richter rootstock and they were trained to a vertically shoot positioned system (3.0 × 1.1 m) with a plant density of 3,030 plants ha-1. The vineyard was equipped with a drip irrigation system using 2.3 L h-1 drippers, to assure plant water needs. An annual irrigation of 65 L per vine was applied in 2017 and 50 L per vine was applied in 2018, both performed in a single supply at mid-august in each season. The vineyard productivity in 2017 was close to 5,000 kg ha-1, whereas in 2018 was about to 7,000 kg ha-1.

**Seasonal information**

An automatic weather station (AWS) was used to obtain climatic data, which was provided by the Agroclimatic Information Service of La Rioja Government (SIAR). During 2017 growing season (April to September), the accumulated rainfed and the reference evapotranspiration (ETo) were 180 mm and 893 mm, respectively. Along this period, maximum, average and minimum temperatures were 36.5 ºC, 18.7 ºC and -0.3 ºC, respectively whereas the average relative humidity (RH) was 60.1 %. During 2018 growing season, the accumulated rainfed and ETo were 354 mm and 790 mm, respectively. Along this period, maximum, average and minimum temperatures were 37.0 ºC, 18.3 ºC and 1.9 ºC, respectively whereas average relative humidity was 69.4 %.

 Annual ETo and precipitations reached along 2017 season was 1,210 mm and 376 mm, respectively whereas these same climatic variables recorded in 2018 season were 1,061 mm and 672 mm, respectively. Annual accumulated radiation was 46,019 W m-2 (3,976 MJ m-2) and 44,028 W m-2(3,804 MJ m-2) in 2017 and 2018 seasons, respectively. During the warmest month (July), ETo was 167 mm and 169 mm along the 2017 and 2018 seasons, respectively. Average radiation during this period was 285.5 W m-2 (24.7 MJ m-2) and 292.7 W m-2 (25.2 MJ m-2) in 2017 and 2018 seasons, respectively.

**Treatments**

A commercial fertilizer (Crop Plus, Adama, Santiago de Chile, Chile) based on an *Ascophyllum nodosum* seaweed extract was applied at two different dosages in an aqueous solution: a low dose (Ld) at 0.25 % (v v-1) and a high dose (Hd) at 0.50 % (v v-1), as well as a control treatment. The solutions were prepared using Tween 80 (Sigma-Aldrich, Madrid, Spain) as wetting agent, dissolved in water at a dosage of 1mL L-1. Control plants were sprayed with water with Tween 80 alone.

 Nitrogen composition of the extract was analyzed by HPLC according to the methodology exposed by Garde-Cerdán et al.24 Total nitrogen composition of the extract was 2.47 g N L-1, of which 1.34 g N L-1corresponds to NH4+ and the rest of the following amino acids: 4.81 (aspartic acid), 8.72 (glutamic acid), 716.68 (glycine), 16.99 (*ß*-alanine), 22.18 (*α*-alanine), 309.07 (proline), 4.77 (valine), 35.87 (methionine), 1.60 (isoleucine), 1.50 (leucine), 1.15 (phenylalanine), 3.83 (ornithine), and 4.15 (lysine), expressed in mg N L-1. Additionally, the product label states that the fertilizer contains other active ingredients such as sulphur (4.5 % w v-1), copper (1.3 % w v-1), iron (1.7 % w v-1), manganese (1.4 % w v-1), and zinc (3.0 % w v-1).

 Two applications of control and the two dosages of the seaweed extract were carried out to Tempranillo Blanco grapevines. The first application was carried out at veraison stage (at BBCH 81), and the second was sprayed one week after the first application. 200 mL of control and each seaweed treatments covered both sides of the grapevine canopy. The foliar applications were performed in triplicate and were arranged in a complete randomized block design along to the plot, considering seven Tempranillo Blanco vines per replicate.

All the grapes from each biological replicate were manually harvested close to 13 % (v v-1) of probable alcohol (at BBCH 89). Immediately before harvest, a random set of 200 grape berries per replicate were collected and frozen at −20 °C in order to analyze their phenolic composition. All the bunches from the nine replicates were mechanically destemmed and crushed, separately in an experimental winery, and classical parameters were evaluated in the obtained musts.

**Winemaking**

An experimental pneumatic press to a pressure of 3.5 bar was used to extract the white must without skins and seeds. Nine tanks of 12 L were filled with the must from each replication. Then, 50 mg of SO2 kg-1 of must was added in each tank to prevent oxidations and inhibit the development of undesirable microorganisms. Subsequently, the tanks were placed during one day into a cold room (7 ± 1 ºC) to favor settling. The must supernatant was introduced in 10 L tanks to be inoculated with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, St Simon, France) at a dosage of 25 g hL-1to carry out the alcoholic fermentation. All vinifications were performed at 17 ± 1 ºC in a temperature-controlled room. Alcoholic fermentation was daily followed by determining must temperature and density and was considered finished when the must reached lower than 2.5 g L-1 of residual sugar. Once decanted, the wines were introduced into 5 L tanks and aliquots of each wine were taken, and immediately were frozen and stored at −20 °C until the analyses of phenolic compounds. After this, the wines were bottled and stored at 18 ± 2 ºC for the subsequent sensorial analysis.

**Grape and wine classical parameters**

Probable alcohol in grapes and alcohol degree in wines, pH in grapes and wines and total acidity in grapes and wines were analyzed according to the methodologies exposed by the OIV.25 Malic acid in grapes and wines and residual sugar content were analyzed using the enzymatic equipment Miura One (TDI, Barcelona, Spain). Yeast assimilable nitrogen (YAN) was calculated according to the methodology exposed by Garde-Cerdán et al.24 Since treatments were performed in triplicate, the results of these classical parameters are shown as the average of three analyzes (n = 3).

**Extraction of phenolic compounds in grapes**

Phenolic compounds extraction in grapes was performed based on the methodology exposed by Niculcea et al.26 Berry samples were homogenized using an Ultra Turrax grinder mixer (IKA, Staufen, Germany) at 18,000 rpm for 2 min. Then, 1 g of the homogenate was transferred into a centrifuge tube of 15 mL, and 10 mL of 50 % v v-1 aqueous ethanol solution (pH 2.0) was added. The extraction was carried out in an ultrasonic bath (Bandelin, Berlin, Germany). Then, the tube was centrifuged at 2,300 g for 15 min at 10 ºC (Heraeus Megafuge 16/16R, ThermoFisher Scientific, Massachusetts, USA) to obtain the grape extract, and the supernatant was filtered through 0.45 μm membranes (PVDF Syringe filters, Proquinorte, Bilbao, Spain) and was placed into 2 mL screw amber vials.

**Phenolic compounds analysis**

Phenolic compounds in grapes and wines were analyzed according to the methodology exposed by González-Lázaro et al.27 using a high-performance liquid chromatographer (HPLC), in a 1100 Agilent liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a photodiode array detector (DAD). Flavonols, flavanols, hydroxybenzoic acids, hydroxycinnamic acids and stilbenes were analyzed by HPLC-DAD with a direct injection of 25 μL of sample previously filtered through 0.45 μm membranes. Separation was achieved in an LiChrospher 100 RP-18 (Scharlab, Barcelona, Spain) particle size 5 μm (250 mm x 4 mm) column protected with a guard column of the same material. The solvents used were: (A) 50 mM (NH4H2PO4 at pH 2.6), (B) acetonitrile/solvent A (80:20 % v v-1), and (C) 200 mM (*o*-phosphoric acid at pH 1.5), establishing the following gradient: isocratic 0 % B and 0 % C during 5 min, from 0 to 8 % B in 12 min, from 8 to 14 % B and 0 to 86 % C in 5 min, from 14 to 18 % B and 86 to 82 % C in 7 min, from 18 to 21 % B and 82 to 79 % C in 26 min, from 21 to 33 % B and 79 to 67 % C in 15 min, from 33 to 50 % B and 67 to 50 % C in 8 min, from 50 to 80 % B and 50 to 0 % C in 8 min, at a flow of 1 mL min-1. Spectra were recorded from 250 nm to 600 nm.

Phenolic compounds were identified according to the retention times of pure compounds and the UV–Vis characteristics obtained from authentic standards. Quantification was made using DAD chromatograms recorded at 360 nm (flavonols), 280 nm (hydroxybenzoic acids and flavanols), 310 nm (stilbenes), and 320 (hydroxycinnamic acids). Since most of the individual phenolic compounds were not commercially available as reference standards, quantification of phenolic compounds in grapes (mg kg-1) and in wines (mg L-1) was made according to the calibration graphs of the most similar compounds by an external standard calibration curve (R2 > 0.991), according to the reported by González-Lázaro et al.27 All treatments were performed in triplicate, so the results for phenolic compounds in grapes and wines correspond to the average of three analyses (n = 3).

**Sensory analysis**

Fourteen expert judges performed the panel in the sensory analysis of Tempranillo Blanco wines. They were nine females and five males with an age ranging between 21 and 44 years old. Wine sensory analysis was carried out 5 months after bottling. Two bottles of each replicate of 750 mL (a total of 18 bottles) were taken from the storage room and placed into a cold chamber (at 5 ± 1 ºC) one day before the wine sensory evaluation to decant the solids formed to the bottom of the bottle. The wines were evaluated in a comparative way, using the blind tasting system. The tasting sheet used was the one approved by the OIV28 with a scale of 40 (insufficient) to 100 (excellent). The analysis also included a quantitative evaluation of six attributes or descriptors such as sweetness, acidity, bitterness, alcoholic, astringency and balance. Panellists rated each attribute on a scale from 0 (absence) to 10 (maximum presence). All the tastings were made in transparent glasses at room temperature and in individual blocks. During the wine sensory analysis, the samples were presented in a random order.

**Statistical analysis**

Classical oenological parameters, phenolic compounds and sensory analysis were subjected to a variance analysis (one-way ANOVA) in the Statgraphics Centurion XVI.I software (Warrento, Virginia, USA). Differences between samples were compared using the Duncan test (*p* ≤ 0.05). A multiple factor analysis (MFA) was also performed considering classical parameters, phenolic compounds and sensory analysis quantified in grapes and wines. MFA was also performed by Statgraphics Centurion XVI.I. Two principal component analyses (PCA) was performed using the concentration of each phenolic compound found in grapes and wines during both study seasons, using the InfoStat software (InfoStat, www.infostat.com.ar).

**RESULTS AND DISCUSSION**

**Classical parameters**

Table 1 shows the grape and wine classical parameters of the different samples in 2017 and 2018 seasons. The weight of 100 berries was not affected by the seaweed treatments, but season factor considerably affected it value, according to the multiple analysis (MFA) performed. Additionally, season factor affected pH, total acidity, malic acid and yeast assimilable nitrogen (YAN) in grapes (Table 1). These seasonal differences are probably due to the fact that accumulated rainfall in 2018 was higher than in 2017, whereas reference evapotranspiration (ET0) was similar in both seasons. Therefore, the grapevines in 2017 had less water to supply to the atmospheric demand, resulting in a lower weight of berries and an earlier maturity than the grapevines in 2018, affecting the accumulation of organic acids and nitrogen compounds. van Leeuwen et al.29 reported that water deficit stress anticipated shoot growth slackening, limited berry weight and enhanced berry anthocyanin content. Garde-Cerdán et al.30 reported that the accumulation of amino acids in Grenache grapes was strongly related to the degree of grape maturity. Generally, the treatments did not affect must and wine enological parameters with the exception of pH in the musts in 2018 season (Table 1). Thus, the high dosage of the seaweed application (Hd) increased the pH compared to control and the low dosage of the seaweed treatment (Ld). Sabir et al.31 reported that a seaweed extract (*Ascophyllum nodosum*), applied to *Vitis vinifera* L. cv. Narince, did not affect soluble solids, total acidity and pH in grapes. These results were also reported by Frioni et al.20 who studied the effects of an *A*. *nodosum* biostimulants applied to grapevines on ripening dynamics and fruit quality of Sangiovese, Pinot Noir and Cabernet Franc varieties. None of the factors affected the content of the classical parameters in wines (Table 1). Volatile acidity values in wines were lower than 0.6 g L-1, which is commonly perceived as a spoilage character on wine. Therefore, it seems to be that the seaweed applications to grapevines scarcely affects grape and wine enological parameters, whereas climatic conditions widely affect enological parameters of grapes at harvest.

**Effects of seaweed applications to grapevines on grape phenolic compounds**

Table 2 shows the phenolic compounds found in grapes (mg kg-1) from control and treated grapevines with a low (Ld) and high dosage (Hd) of a seaweed extract in 2017 and 2018 seasons. Catechin was the only flavanol identified and the most abundant phenolic compound found in Tempranillo Blanco grapes, with concentrations ranged from 72.9 to 236.3 mg kg-1. Rodríguez Montealegre et al.5 reported that catechin concentration in different white grape varieties ranged from 120 (in Viogner) to 500 mg kg-1 (in Gewurztraminer). Based on this work, Tempranillo Blanco grapes showed lower catechin concentration than Chardonnay, Moscatel, Gewurztraminer and Riesling, and higher concentration of this flavanol than Sauvignon Blanc and Viogner. Catechin is the dominant flavanol monomer in grape skin and it is not detected in the pulp of either red or white grapes.32 Respect to the treatments, Hd seaweed foliar applications increased catechin concentration respect to control and Ld samples in both study seasons (Table 2). Leucoanthocyanidin reductase (LAR) involves the biosynthesis of leucocyanidin to catechin and then enzymatically converting catechin to epicatechin.33 Additionally, anthocyanidin reductase (AR) involves the conversion of cyanidin into catechin.34 Seaweed contains a wide range of polysaccharides that can act as elicitors in horticulture.19 Horbowicz et al.35 suggested that methyl jasmonate (an abiotic elicitor) probably increases activity of the leucocyanidin reductase or anthocyanidin reductase in seedlings of common buckwheat (*Fagopyrum esculentum*). Due to this, it is probable that seaweed applications to grapevines at high dosage might to trigger the activation of LAR or AR enzymes, inducing the synthesis of catechin in grapes. According to the multiple factorial analysis (MFA), treatment and season affected catechin content in grapes (Table 2). During 2018, the rainfall was higher than in 2017, and certain grape bunches were affected by *Botrytis cinerea*. Catechin concentration tended to decrease in 2018, probably due to the fact that flavanols are broken down by the fungus activity.36 Additionally, annual accumulated radiation was higher in 2017 than in 2018 season. Contradictory results about the effects of the environmental factors on flavanol biosynthesis in grapes have been reported by certain authors. Downey et al.37 reported that shading had no significant effects on the levels of condensed tannins in skins or seeds of ripe fruit, while Koyama et al.38 showed that cluster shading partially decreased proanthocyanidins content in grapes. Contrary to this, post-veraison sunlight exposure increased anthocyanin and flavanol levels in grapes skins compared to shading grapes.39

Quercetin-3-*O*-glucoside (glc) and quercetin-3-*O*-glucuronide (glcU) were the most abundant flavonols found in Tempranillo Blanco grapes and their concentration varied from 4.3 to 10.1 mg kg-1and from 4.9 to 18.4 mg kg-1, respectively (Table 2). Isorhamnetin-3-*O*-galactoside (gal)+glc were the least abundant flavonols found in Tempranillo Blanco grapes, and their concentrations ranged from 0.8 to 2.4 mg kg-1. Castillo-Muñoz et al.40 showed that quercetin-3-*O*-glc and quercetin-3-*O*-glcU were the most abundant flavonols in grapes from several white grapevine varieties. According to our results, glucosylated fraction was the main derivatives for each of the aglycone-type flavonols, with the exception of quercetin-3-*O*-glcU in some grape samples (Table 2). Quercetin-type flavonols dominated the flavonol profile of Tempranillo Blanco grapes, accounting for 67.7 to 77.8 % of total flavonols, with a mean value of 72.1 ± 13.1 %. Kaempferol-type flavonols were the second in abundance, accounting for 16.2 to 27.0 % of total flavonols, with a mean value of 22.7 ± 2.0 %. Isorhamnetin-type flavonols were the minor form, accounting for 4.4 to 6.2 %, with a mean value of 5.2 ± 0.8 %. These results are similar to those exposed by Rodríguez Montealegre et al.5 and Castillo-Muñoz et al.40 in different white grape varieties, such as Airén, Chardonnay, Gewürztraminer, Moscatel de Alejandría, Pedro Ximénez, Riesling, Sauvignon Blanc, Torrontés, Ugni Blanc, Verdejo, and Viogner. The lack of expression of the enzyme flavonoid 3’,5’-hydroxylase in white varieties limits the synthesis of flavonols to quercetin, kaempferol and isorhamnetin derivatives, whereas red varieties can synthetize also myricetin, laricitrin and syringic derivatives due to the presence of this enzyme.41 In 2017 season, Hd treatment increased the concentration of all the flavonols, including its total concentration compared to control (Table 2). These increments in the concentration of the individual flavonols ranged from 43 to 69 %. In 2018 season, Hd treatment increased the concentration of all flavonols, except querating-3-glc, including its total concentration compared to control and Ld treatment whereas this treatment presented lower concentration of quercetin-3-*O*-glc compared to control. In addition, in 2018, both seaweed treatments increased kaempferol-type flavonols. The improvements in the concentration of the individual flavonols ranged from 112 to 308 %, compared to control, except the content of quercetin-3-*O*-glc, which increased close to 42 %. According to the MFA (Table 2), treatment affected the concentration of all flavonols, except quercetin and isorhamnetin-type flavonols. Season affected the concentration of quercetin-3-*O-*glc, quercetin-3-*O*-glcU, isorhamnetin-3-*O*-gal+glc, isorhamnetin-type flavonols, and total flavonols. Treatment and season interaction affected the concentration of most of the flavonols, including its total concentration and kaempferol-type flavonols, except quercetin-3-*O*-glc and total quercetin content. Flavonols accumulation in grapes appears to occur prior to veraison, reaching its maximum content in this phenological stage, and its biosynthesis occurs only in the grape skin.36 The concentration of individual flavonols increased due to abiotic elicitor applications to Monastrell grapevines.42 Therefore, it is possible that a high dosage of the seaweed applied to grapevines enhance the flavonol synthase activity, allowing the synthesis of individual flavonols. These compounds are important antioxidants molecules allowing to protect the musts and wines against enzymatic browning and can contribute to the human healthy due to its importance facing several types of cancers.7,14

Hydroxycinnamic acids (HCAs) are phenolic acids composed by a conjugated double bond between the phenolic ring and the carboxylate group.7 These compounds are found in the grape pulp primarily as their tartrate ester.7 *trans*-Caftaric, *cis*-coutaric, and caffeic acids were the most abundant HCAs found in Tempranillo Blanco grapes and their concentration varied from 0.5 to 2.8, 0.6 to 2.7 and 0.5 to 1.3 mg kg-1, respectively. The least abundant HCAs was *trans*-coutaric acid and its concentration varied from 0.3 to 0.6 mg kg-1. Seaweed application to the grapevines scarcely affected HCAs concentration in grapes. In 2017 season, Hd samples presented higher caffeic acid concentration than Ld samples (Table 2). In 2018, control samples showed the highest concentration of *cis*-coutaric acid, while Hd samples had the highest concentration of caffeic acid. According to the MFA (Table 2), treatment affected *trans*-caftaric acid, caffeic acid and total hydroxycinnamic acids content whereas season affected *trans*-caftaric acid, *cis*-coutaric acid and total hydroxycinnamic acids content. The interaction between treatment and season did not shown significant differences. The differences in HCAs concentration according to treatment and season can be attributed to the fact that the degree of must oxidation might to affect the concentration of HCAs, even in thawed samples, which are substrates for polyphenol oxidase activity in grapes.36

Benzoic acids such as vanillic, gallic, syringic and salicylic acids appear to be bound to the cell walls.43 Ours results reported that syringic acid was the only one hydroxybenzoic acid found in Tempranillo Blanco grapes, and its content ranged from 1.2 to 2.5 mg kg-1 (Table 2). In 2017 season, Hd samples showed higher syringic acid content than Ld samples, whereas in 2018, Ld samples had the lowest content of this hydroxybenzoic acid. According to the MFA (Table 2), treatment and season affected syringic acid content in grapes. Tempranillo Blanco presents lower vegetative mass, growth and stomatal density than Tempranillo variety.44 These conditions might to influence the effectiveness of seaweed foliar applications, since to enter into the leaf, foliar fertilizers must first penetrate throughout the cuticular membrane.45

**Effects of seaweed applications to grapevines on wine phenolic compounds**

Table 3 shows the phenolic composition of wines (mg L-1) made from control and treated Tempranillo Blanco grapevines with a low (Ld) and high dose (Hd) of a seaweed extract in 2017 and 2018 seasons. The most abundant phenolic compounds in Tempranillo Blanco wines were catechin, *trans*-caftaric acid, gallic acid, *trans*-coutaric acid, and *cis*-coutaric acid. The phenolic profile of white wines is dominated by gallic, caftaric and coutaric acids, and there are little amounts of flavanols due to the fact that white wines are vinified without skins and seeds.7 Catechins and hydroxycinnamic acids are the main compounds responsible for coloration in white wines, which are initially colorless but can be oxidized either enzymatically or chemically to form yellow and brown products.46 Catechin concentration in Tempranillo Blanco wines ranged from 30.6 to 48.8 mg L-1 (Table 3). Wide differences in catechin concentrations in white wines are reported by different authors, ranging from not detected to 143 mg L-1.47-49 In 2017 season, Ld wine samples showed the highest catechin content. In 2018 season, Hd wine samples presented higher catechin content than control wine samples. According to the multiple factorial analysis (MFA) (Table 3), all the factors affected catechin content in wines. The differences in catechin content in wines according to treatment, season and the interaction of these factors can be attributed to oxidation reactions that involve the catechin in these steps. Also, the catechin concentration in musts could have been affected by the action of cryptogamic diseases, affecting by consequent the concentration of catechin in wines.

Hydroxycinnamic acids (HCAs) has a relevant importance on wine quality, since these compounds, are the main cause of browning in white wines and the origin of the 4-ethylphenol and 4-ethylguaiacol formation.50 HCAs were the most important family of phenolic compounds and its total concentration ranged from 36.9 to 43.6 mg L-1. As in grapes, the seaweed foliar applications to grapevines had little effects on HCAs in wines (Table 3). In 2017 season, Ld samples showed higher content of *cis*-coutaric and ferulic acids than control samples, whereas Hd samples had higher content of ferulic acid than control samples, and lower content of this HCA than Ld samples. In 2018 season, Hd samples showed the highest *cis*-coutaric acid content, whereas control samples had the highest *trans*-fertaric acid content. According to the MFA (Table 3), the treatment only affected the *cis*-coutaric acid content, whereas the season affected the content of most of the HCAs, except *trans*-caftaric acid, *trans*-coutaric acid and total HCAs content. Treatment and season interaction influenced the content of *cis*-coutaric and ferulic acids. The content of HCAs during the oxidation process decreases, seemingly unrelated to the extent of wine browning, whereas flavanols content in wines is related to the degree of browning at the end of the oxidation process.51 Enzymatic browning appears to follow coupled oxidation reactions where hydroxycinnamate quinones oxidize catechol groups on the flavanols to quinones, regenerating hydroxycinnamates.7 Moreover, hydroxycinnamates have the ability to form co-pigments with anthocyanins and might to contribute to bitterness and astringency.52 Due to these reasons, it is possible that HCAs were more affected by winemaking than by the treatment applied to the grapevines.

Hydroxybenzoic acids (HBAs) are phenolic compounds with a general structure C6-C1. Gallic and syringic acids were the only HBAs found in Tempranillo Blanco wines (Table 3). Gallic acid was not found in grapes, but it was quantified in wines, varying its concentration from 10.8 to 25.1 mg L-1 (Table 3). Gallic acid is produced in wine by the hydrolysis of the gallate esters found in condensed and hydrolysable tannins.7 In 2017, Hd samples showed the highest content of gallic acid and total HBAs. In 2018, seaweed foliar treatments did not affect wine HBAs content. According to the MFA (Table 3), treatment affected the content of gallic acid, whereas season affected the content of all HBAs, including its total content. Similar to the HCAs, the acids with catechol or galloyl functionality are also susceptible to oxidation reactions.7

As it was above mentioned, stilbenes are phytoalexins that are synthetized as glucosides in grapevines, in response to cryptogrammic diseases affection, UV-B radiation, among others.13 Stilbenes were scarcely found in wines produced in 2017 and a high level of stilbenes were found in the wines produced in 2018. In 2018 season, the most abundant stilbenes found in Tempranillo Blanco wines were *trans*-resveratrol and *trans*-piceid and their concentration ranged from 0.52 to 0.60 and 0.36 to 0.57 mg L-1, respectively (Table 3).These results are similar to those exposed in Tempranillo wines53,54 and in wines from white grapevine varieties.55,56 According to the MFA (Table 3), season affected the concentration of all stilbenes, including its total concentration, whereas treatment and the interaction of the two factors did not influence the content of the stilbenes in the wine samples. Stilbenes are accumulated in grapevine leaves and grape skins in a glycosylated form in response to biotic and abiotic stresses,13 which can be release by yeast activity in wines. As was above mentioned, the rainfall was higher in 2018 than in 2017season and certain grape bunches were affected by *Botrytis cinerea*. It is possible that due to these conditions, season factor influenced a high diseases pressure to grapevines, affecting the concentration of stilbenes.

**Principal component analysis (PCA) in grapes and wines**

PCA was performed with the aim to classify the different samples, according to phenolic compounds found in grapes (Figure 1a) and wines (Figure 1b) from control (C) and treated grapevines with two different dosages of a seaweed application: Low dosage (Ld) at 0.25 % (v v-1) and high dosage (Hd) 0.50 % (v v-1) during the 2017 and 2018 seasons. Respect to the grape phenolic compounds, PC1 explained 65.8 % of the variance and PC2 explained 21.9 % of the variance, representing 87.7 % of all variance. PC1 was strongly correlated with quercetin-3-*O*-gal, quercetin-3-*O*-glcU, kaempferol-3-*O*-gal, kaempferol-3-*O*-glc, isorhamnetin-3-*O*-gal+glc and caffeic acid PC2 was correlated with *trans*-caftaric and *cis*-coutaric acids. Grape samples were separated by treatment rather by season. C-2017 samples were inversely related to syringic acid content, while C-2018 samples were positively related with *cis*-coutaric acid content. Ld-2017 and Ld-2018 samples were inversely related to the concentration of flavonols and caffeic acid. Hd-2017 treatment was positively related with catechin and quercetin-3-*O*-glc concentrations while Hd-2018 treatment was positively related with the content of isorhamnetin-3-*O*-gal+glc, quercetin-3-*O*-glcU, quercetin-3-*O*-gal and caffeic acid. Elicitors described by different authors are diverse in nature including polysaccharides, oligosaccharides, peptides, proteins and lipids.57 Elicitor activity is associated with polysaccharide fraction of the seaweed elaborated extracts.57 Polysaccharides purified from seaweeds as well as derived oligosaccharides have the ability to trigger plant defense responses.19,57 To our knowledge, there is little information available in literature about the effects of seaweed application to grapevines. Frioni et al.20 and Salvi et al.23 reported that seaweed applications to grapevines improved skin anthocyanins accumulation and total phenolic content in grape berries. Frioni et al.58 reported that the increasing of anthocyanins and phenolic concentrations in grapes after seaweed applications are related to specific modulation of genes involved in the flavonoid metabolic pathways. Additionally, these authors showed that grapevines treated with an *A*. *nodosum* fertilizer were less affected by gray mold compared to untreated grapevines. Taskos et al.22 reported that seaweed applications to grapevines induced to an increase in yield, the number of berries and anthocyanin extractability in berry skins, whereas their application decreased phenol content in grapes. Similar results were reported by Gutiérrez-Gamboa et al.59 respect to productive parameters after seaweed applications to Carmenère grapevines. Therefore, it seems to be that the effectiveness of biotic and abiotic elicitors on flavonoid and non-flavonoid concentration in grapes is mostly dependent of treatments rather than season or edaphoclimatic conditions of each site.60

Despite that the precipitations conditioned the weight of 100 berries and certain enological parameters of Tempranillo Blanco grapes in both study seasons (Table 1), Hd treatment improved the concentration of catechin and flavonols in grapes, independently of the season. These results are of crucial importance for the viticultural management due to the importance of these compounds as antioxidant against enzymatic browning of musts and wines and their implication in avoiding the risk of cancer, coronary diseases, neurodegenerative diseases, among others widely reported by different authors.7,14-16,50,51

Respect to the wines (Figure 1b), PC1 explained 77.4 % of the variance and PC2 explained 10.3 % of the variance, representing 87.7 % of all variance. PC1 was strongly correlated with catechin, *cis*-caftaric acid, caffeic acid, *p*-coumaric acid, hydroxybenzoic acids and stilbenes PC2 was only correlated with *cis*-coutaric acid. C-2017 and Ld-2017 were positively related to *cis*-caftaric acid content and inversely related to *trans*-resveratrol, *p*-coumaric and syringic acids content. Ld-2018 was positively related to syringic and *p*-coumaric acids content. C-2018 was positively related to *trans*-resveratrol content and inversely related to *cis*-caftaric acid content. Hd-2017 samples were positively related to gallic acid content and inversely related to *trans*-caftaric acid, ferulic acid, *trans*-piceid and *cis*-piceid content, whereas Hd-2018 was positively related to *trans*-caftaric acid, ferulic acid, *trans*-piceid and *cis*-piceid content and inversely related to gallic acid content. Therefore, wine samples were separated by season rather by treatments.

**Descriptive sensory analysis**

Table 4 shows sensory evaluation of wines made from control and treated grapevines with a low (Ld) and a high (Hd) dosage of an *A. nodosum* seaweed fertilizer in 2017 and 2018 seasons. Additionally, Figure 2 shows the score of gustatory attributes of these wines. Total evaluation of wines was bellow to 75 points, that is considered as “very good” wines according to the scale used by OIV.28 Among the wines, there were not differences on view, odor, taste and harmony attributes (Table 4). According to gustatory phase of evaluated wines, in 2017 the judges did not perceive differences among the wines, whereas in 2018, the judges perceived the control wines as the most balanced and acids (Figure 2). In 2018 season, there were no differences among the wines samples on the enological parameters analyzed. Additionally, the 2017 wine samples were perceived as more acids and alcoholic than the 2018 wine samples (Figure 2). Although there were no statistical differences in alcoholic degree of wines produced in 2017 and 2018, there is a slight tendency to that alcoholic degree reached during 2017 were higher than 2018 (Table 1). This fact could condition the perception of the judges on the alcoholic character of the wines.

 A relevant aspect mentioned by the judges during the sensory evaluation of 2018 wine samples is that most of the analyzed Tempranillo Blanco wines were perceived with an unusual salmon-red blush color. This undesirable sensation is attributed to pinking phenomenon, that is produced exclusively in bottled wines produced from white grapevine varieties.61 Oxidative changes due to the presence of oxygen could lead to produce pinking phenomenon.62 However, the compounds associated to this are of a limited knowledge. Andrea-Silva et al.63 reported that the origin of pinking phenomenon in white wines are anthocyanins, mainly malvidin-3-*O*-glc. Tempranillo Blanco originated from a natural mutation of Tempranillo, which conducted a loss of berry coloration due to the loss of the functional copy for the MYB transcription factors required for anthocyanin pigmentation in the berry skin.1 Asomatic variation for berry skin color in white varieties, generating pink or red colored berries, had been associated with the presence of a Gret1 retrotransposon in the promoter region of VvmybA1, a Myb gene whose expression is associated to skin coloration.64,65

**CONCLUSIONS**

Catechin was the most abundant phenolic compound found in Tempranillo Blanco grapes and wines. The flavonol profile of Tempranillo Blanco grapes was dominated by quercetin-type, following by kaempferol-type and isorhamnetin-type flavonols. Seaweed foliar application at a high dosage to Tempranillo Blanco increased the concentration of catechin and most of the individual flavonols in grapes in 2017 and 2018 seasons. Hydroxycinnamic acids in grapes and wines were scarcely affected by the seaweed foliar applications to Tempranillo Blanco grapevines. The concentration of hydroxycinnamic and hydroxybenzoic acids was probably affected by sample oxidations reactions, whereas stilbenes content in wines was conditioned by the affection of cryptogamic diseases in grapes. According to sensory analysis, most of the judges found pinking phenomenon in Tempranillo Blanco wines made in 2018 season, probably due to that these wines were more prone to oxidations than the 2017 wine samples. In this way, judges perceived little differences among the wines, in which 2017 wines were perceived more acids and alcoholic than 2018. The results suggest that seaweeds can act as biotic elicitors to trigger the accumulation of phenolic compounds, mainly of catechin and flavonols in Tempranillo Blanco grapevines in two consecutive seasons. This is the first report that allowed to characterize the phenolic composition of grapes and wines in this variety and how the foliar applications of seaweed affected the flavonoid and non-flavonoid phenolic compounds in its grapes and wines.

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**Figure Captions**

**Figure 1**. Principal component analysis (PCA) performed with the concentration of individual phenolic compounds quantified in grapes (a) and in wines (b) obtained from untreated (C) and treated grapevines with an *Ascophyllum nodosum* commercial fertilizer at two dosages: Low dosage (Ld) at 0.25 % v v-1 and high dosage (Hd) at 0.50 % v v-1. S1 corresponds to 2017 season, while S2 corresponds to 2018 season. Nomenclature abbreviation: Q, quercetin K, kaempferol I, isorhamnetin gal, galactoside glc, glucoside glcU, glucuronide.

**Figure 2**. Gustatory attribute scores of wines obtained from untreated (control) and treated grapevines with an *Ascophyllum nodosum* commercial fertilizer at two dosages: Low dosage at 0.25 % v v-1 and high dosage at 0.50 % v v-1 in 2017 and 2018 seasons. Footnote: For each gustatory attribute, different letters indicate significant differences between treatments (*p* ≤ 0.05).

**Table 1.** Classical parameters of Tempranillo Blanco grapes and wines from untreated grapevines (control) and treated with seaweed foliar applications at different dosages: Low dosage (Ld) at 0.25 % (v v-1) and high dosage (Hd) at 0.50 % (v v-1), in two seasons, 2017 and 2018.

|  |  |  |  |
| --- | --- | --- | --- |
|   | **2017** | **2018** | **Multifactorial analysis** |
| ***Grape*** | **Control** | **Ld** | **Hd** | **Control** | **Ld** | **Hd** | **T** | **S** | **TxS** |
| Weight of 100 berries (g) | 144.03±9.39a | 140.20±3.46a | 140.90±13.80a | 198.87±39.91a | 187.54±6.55a | 192.21±14.47a | NS | \*\*\* | NS |
| Probable alcohol (% v v-1) | 13.06±0.21a | 13.09±0.04a | 12.76±0.83a | 12.90±0.66a | 12.51±0.44a | 12.24±0.59a | NS | NS | NS |
| pH | 3.53±0.04a | 3.55±0.02a | 3.56±0.12a | 3.32±0.03a | 3.30±0.03a | 3.39±0.03b | NS | \*\*\* | NS |
| Total acidity (g L-1)a | 5.08±0.42a | 5.00±0.21a | 5.08±0.41a | 6.38±0.50a | 6.54±0.53a | 5.80±0.09a | NS | \*\*\* | NS |
| Malic acid (g L-1) | 2.11±0.27a | 2.17±0.12a | 2.24±0.07a | 2.96±0.44a | 3.06±0.48a | 2.71±0.09a | NS | \*\*\* | NS |
| YAN (mg N L-1) | 170.51±8.46a  | 207.56±29.02a  | 231.17±61.55a  | 362.37±69.44a  | 348.85±33.00a  | 359.18±24.33a  | NS | \*\*\* | NS |
| ***Wine*** |  |  |  |  |  |  |  |  |  |
| Alcoholic degree (% v v-1) | 13.20±0.10a | 13.20±0.10a | 12.65±0.57a | 13.03± 0.12a | 12.83±0.31a | 12.70±0.60a | NS | NS | NS |
| Total acidity (g L-1)a | 6.51±0.17a | 6.59±0.14a | 6.58±0.35a | 6.51±0.11a | 6.68±0.57a | 6.09±0.23a | NS | NS | NS |
| pH | 3.32±0.03a | 3.34±0.04a | 3.34±0.10a | 3.32±0.05a | 3.25±0.05a | 3.32±0.04a | NS | NS | NS |
| Malic acid (g L-1) | 1.13±0.11a | 1.07±0.10a | 0.98±0.09a | 1.64±0.26a | 1.67**±**0.31a | 1.44±0.05a | NS | NS | NS |
| Volatile acidity (g L-1)b | 0.40±0.06a | 0.40±0.06a | 0.50±0.15a | 0.39±0.09a | 0.41±0.11a | 0.42±0.04a | NS | NS | NS |

All the parameters are given with their standard deviation (n = 3). For each parameter and season, different letters indicate significant differences between treatments (*p* ≤ 0.05). aAs g L-1 of tartaric acid. bAs g L-1 of acetic acid. YAN: yeast assimilable nitrogen. T: Treatment, S: Season, and TxS: Interaction between treatment and season. Statistically significant at \**p* ≤ 0.05, \*\**p* ≤ 0.01 and \*\*\**p* ≤ 0.001, NS: Not significant.

**Table 2.** Phenolic compounds (mg kg-1) in grapes from untreated (control) and treated grapevines with different dosages of a seaweed application: Low dosage (Ld) at 0.25 % (v v-1) and high dosage (Hd) at 0.50 % (v v-1), in two seasons, 2017 and 2018.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **2017** | **2018** | **Multifactorial analysis** |
| ***Flavanol*** | **Control** | **Ld** | **Hd** | **Control** | **Ld** | **Hd** | **T** | **S** | **TxS** |
| Catechin | 159.18±43.31a | 120.36±22.26a | 236.27±28.63b | 98.57±3.75a | 72.86±21.06a | 190.02±4.33b | \*\*\* | \*\* | NS |
| ***Flavonols*** |  |  |  |  |  |  |  |  |  |
| Quercetin-3-*O*-gal | 1.58±0.54a | 1.23±0.18a | 2.46±0.25b | 1.64±0.34a | 1.15±0.24a | 3.83±0.31b | \*\*\* | \* | \* |
| Quercetin-3-*O*-glcU | 6.32±2.34a | 4.89±0.36a | 10.55±1.50b | 6.75±1.53a | 5.09±2.30a | 18.39±1.55b | \*\*\* | \* | \* |
| Quercetin-3-*O*-glc | 7.05±2.51ab | 5.75±0.33a | 10.06±1.26b | 6.30±0.94b | 4.27±0.12a | 8.96±0.20c | \*\* | NS | NS |
| Kaempferol-3-*O*-gal | 1.18±0.43a | 1.03±0.18a | 1.99±0.39b | 0.71±0.06a | 0.89±0.27a | 2.90±0.72b | \*\*\* | NS | \* |
| Kaempferol-3-*O*-glc | 3.35±1.17a | 2.91±0.48a | 5.72±1.13b | 2.34±0.29a | 2.51±1.10a | 9.57±2.24b | \*\*\* | NS | \* |
| Isorhamnetin-3-*O*-gal+glc | 0.91±0.21a | 0.80±0.06a | 1.42±0.08b | 1.14±0.17a | 0.91±0.28a | 2.42±0.14b | \*\*\* | \*\*\* | \*\* |
| Total quercetin | 14.94±5.39a | 11.87±0.87a | 23.07±3.01b | 14.68±2.81a | 10.51±2.66a | 31.17±2.06b | \*\*\* | NS | NS |
| Total kaempferol | 4.52±1.61a | 3.93±0.66a | 7.72±1.52b | 3.05±0.35a | 3.40±1.37a | 12.47±2.96b | \*\*\* | NS | \* |
| Quercetin type (%) | 73.34±26.44a | 71.47±5.23a | 71.64±9.36a | 77.76±14.86a | 70.91±17.98a | 67.67±4.47a | NS | NS | NS |
| Kaempferol type (%) | 22.20±2.19a | 23.69±0.92a | 23.96±2.12a | 16.18±0.45a | 22.93±1.94b | 27.07±4.38b | \*\* | NS | \* |
| Isorhamnetin type (%) | 4.47±1.05a | 4.84±0.34a | 4.40±0.24a | 6.06±0.89a | 6.16±1.88a | 5.26±0.30a | NS | \* | NS |
| **Total flavonols** | 20.37±7.17a | 16.61±1.38a | 32.20±2.08b | 18.88±2.83a | 14.82±4.32a | 46.06±5.16b | \*\*\* | \* | \* |
| ***Hydroxycinnamic acids*** |  |  |  |  |  |  |  |  |  |
| *tran*s-Caftaric acid | 0.96±0.32a | 0.49±0.14a | 0.74±0.12a | 2.83±0.93a | 1.26±0.49a | 2.57±0.18a | \* | \*\*\* | NS |
| *cis*-Coutaric acid | 0.85±0.28a | 0.59±0.09a | 1.10±0.52a | 2.71±0.63b | 1.52±0.00a | 1.44±0.10a | NS | \*\* | NS |
| *trans*-Coutaric acid | 0.57±0.19a | 0.37±0.06a | 0.60±0.08a | 0.53±0.24a | 0.29±0.04a | 0.49±0.04a | NS | NS | NS |
| Caffeic acid | 0.83±0.26ab | 0.51±0.02a | 1.02±0.01b | 0.75±0.02a | 0.59±0.16a | 1.29±0.00b | \*\*\* | NS | NS |
| **Total hydroxycinnamic acids** | 2.93±0.84a | 1.95±0.32a | 3.46±0.69a | 6.82±1.66a | 3.66±0.38a | 5.79±0.32a | \* | \*\*\* | NS |
| ***Hydroxybenzoic acid*** |  |  |  |  |  |  |  |  |  |
| Syringic acid | 1.51±0.33ab | 1.20±0.24a | 1.78±0.10b | 2.48±0.37b | 1.27±0.23a | 2.43±0.13b | \*\*\* | \*\* | NS |

Nomenclature abbreviation: gal, galactoside glc, glucoside glcU, glucuronide. All the parameters are given with their standard deviation (n = 3). For each parameter and season, different letters indicate significant differences between treatments (*p* ≤ 0.05). T: Treatment, S: Season, and TxS: Interaction between treatment and season factors. Statistically significant at \**p* ≤ 0.05, \*\**p* ≤ 0.01 and \*\*\**p* ≤ 0.001, respectively. NS: Not significant.

**Table 3.** Phenolic compounds (mg L-1) in wines obtained from untreated (control) and treated grapevines with different dosages of a seaweed application: Low dosage (Ld) at 0.25 % (v v-1) and high dosage (Hd) at 0.50 % (v v-1), in two seasons, 2017 and 2018.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **2017** | **2018** | **Multifactorial analysis** |
| ***Flavanol*** | **Control** | **Ld** | **Hd** | **Control** | **Ld** | **Hd** | **T** | **S** | **TxS** |
| Catechin | 42.16±2.06a | 48.78±1.47b | 44.67±1.88a | 30.59±3.67a | 33.04±2.61ab | 37.25±2.12b | \*\* | \*\*\* | \* |
| ***Hydroxycinnamic acids*** |  |  |  |  |  |  |  |  |  |
| *cis*-Caftaric acid | 1.97±0.14a | 1.93±0.34a | 1.83±0.04a | 1.40±0.23a | 1.49±0.17a | 1.50±0.15a | NS | \*\*\* | NS |
| *trans*-Caftaric acid | 27.45±3.72a | 25.48±9.95a | 22.48±2.72a | 27.14±7.16a | 28.41±2.71a | 26.76±2.92a | NS | NS | NS |
| *cis*-Coutaric acid | 3.58±0.04a | 3.86±0.15b | 3.70±0.11ab | 3.16±0.36a | 3.29±0.17a | 3.85±0.04b | \*\* | \*\* | \* |
| *trans*-Coutaric acid | 7.38±0.56a | 6.94±1.74a | 6.16±1.28a | 7.32±2.54a | 7.64±0.30a | 8.06±0.74a | NS | NS | NS |
| Caffeic acid | 1.94±0.05a | 1.95±0.08a | 2.03±0.10a | 1.62±0.09a | 1.73±0.13a | 1.85±0.16a | NS | \*\*\* | NS |
| *trans*-Fertaric acid | 0.07±0.01a | 0.07±0.01a | 0.09±0.02a | 0.06±0.01b | 0.06±0.00a | 0.05±0.00a | NS | \*\* | NS |
| *p*-Coumaric acid | 0.19±0.05a | 0.21±0.04a | 0.22±0.02a | 0.61±0.07a | 0.50±0.14a | 0.64±0.21a | NS | \*\*\* | NS |
| Ferulic acid | 0.37±0.02a | 0.48±0.00c | 0.40±0.02b | 0.52±0.02a | 0.45±0.03a | 0.51±0.09a | NS | \*\* | \*\* |
| **Total hydroxycinnamic acids** | 42.95±4.39a | 40.90±12.03a | 36.91±4.22a | 41.84±10.26a | 43.58±3.00a | 43.22±3.64a | NS | NS | NS |
| ***Hydroxybenzoic acids*** |  |  |  |  |  |  |  |  |  |
| Gallic acid | 18.50±0.93a | 18.72±0.16a | 25.05±4.34b | 10.80±3.37a | 11.77±1.28a | 11.84±2.44a | \* | \*\*\* | NS |
| Syringic acid | 0.51±0.03a | 0.46±0.06a | 0.49±0.04a | 1.25±0.07a | 1.24±0.10a | 1.25±0.03a | NS | \*\*\* | NS |
| **Total hydroxybenzoic acids** | 19.01±0.96a | 19.18±0.11a | 25.54±4.33b | 12.05±3.41a | 13.01±1.25a | 13.09±2.44a | NS | \*\*\* | NS |
| ***Stilbenes*** |  |  |  |  |  |  |  |  |  |
| *trans*-Piceid | 0.07±0.01a | 0.05±0.01a | 0.04±0.01a | 0.36±0.12a | 0.46±0.06a | 0.57±0.22a | NS | \*\*\* | NS |
| *cis*-Piceid | 0.03±0.01a | 0.05±0.01a | 0.04±0.01a | 0.13±0.01a | 0.14±0.03a | 0.16±0.03a | NS | \*\*\* | NS |
| *trans*-Resveratrol | 0.05±0.01a | 0.06±0.02a | 0.06±0.02a | 0.61±0.10a | 0.52±0.06a | 0.70±0.18a | NS | \*\*\* | NS |
| *cis*-Resveratrol | 0.03±0.01a | 0.02±0.00a | 0.02±0.01a | 0.13±0.03a | 0.09±0.01a | 0.09±0.01a | NS | \*\*\* | NS |
| **Total stilbenes** | 0.18±0.03a | 0.16±0.05a | 0.15±0.02a | 1.11±0.13a | 1.21±0.12a | 1.53±0.44a | NS | \*\*\* | NS |

All the parameters are given with their standard deviation (n = 3). For each parameter and season, different letters indicate significant differences between treatments (*p* ≤ 0.05). T: Treatment, S: Season, and TxS: Interaction between treatment and season factors. Statistically significant at \**p* ≤ 0.05, \*\**p* ≤ 0.01 and \*\*\**p* ≤ 0.001, respectively. NS: Not significant.

**Table 4**. Sensory evaluation of wines elaborated from untreated (control) and treated grapevines with different dosages of a seaweed application: Low dosage (Ld) at 0.25 % (v v-1) and high dosage (Hd) at 0.50 % (v v-1), in 2017 and 2018 seasons.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **2017** | **2018** | **Multifactorial analysis** |
|  |  | **Control** | **Ld** | **Hd** | **Control** | **Ld** | **Hd** | **T** | **S** | **TxS** |
| **View** | **Cleanness** | 3.64 ± 0.65a | 3.04 ± 0.80a | 3.00 ± 0.67a | 3.02 ± 0.70a | 3.31 ± 0.46a | 2.95 ± 0.21a | NS | NS | NS |
| **Color** | 7.04 ± 1.00a | 6.56 ± 1.71a | 6.07 ± 1.12a | 6.36 ± 0.75a | 6.52 ± 0.59a | 6.29 ± 0.14a | NS | NS | NS |
| **Smell** | **Intensity** | 5.39 ± 0.25a | 5.46 ± 0.69a | 5.27 ± 0.48a | 5.76 ± 0.55a | 5.57 ± 0.31a | 5.61 ± 0.36a | NS | NS | NS |
| **Frankness** | 4.07 ± 0.24a | 4.19 ± 0.46a | 3.80 ± 0.43a | 3.86 ± 0.40a | 3.54 ± 0.21a | 3.51 ± 0.11a | NS | \*\* | NS |
| **Quality** | 11.69 ± 0.76a | 11.61 ± 1.24a | 11.93 ± 0.44a | 11.88 ± 0.79a | 11.12 ± 0.53a | 11.22 ± 0.07a | NS | NS | NS |
| **Taste** | **Intensity** | 6.03 ± 0.56a | 5.85 ± 0.35a | 5.78 ± 0.48a | 5.95 ± 0.58a | 5.88 ± 0.04a | 6.00 ± 0.31a | NS | NS | NS |
| **Frankness** | 4.01 ± 0.42a | 4.10 ± 0.18a | 3.96 ± 0.06a | 3.90 ± 0.39a | 3.80 ± 0.04a | 3.71 ± 0.26a | NS | NS | NS |
| **Quality** | 15.94 ± 0.71a | 15.86 ± 1.27a | 15.14 ± 0.17a | 15.90 ± 1.59a | 15.29 ± 0.69a | 14.98 ± 0.29a | NS | NS | NS |
| **Persistence** | 6.09 ± 0.34a | 6.06 ± 0.34a | 6.10 ± 0.38a | 5.95 ± 0.37a | 5.95 ± 0.18a | 5.86 ± 0.29a | NS | NS | NS |
| **Harmony** | 8.85 ± 0.43a | 8.81 ± 0.32a | 8.60 ± 0.15a | 8.87 ± 0.42a | 8.78 ± 0.14a | 8.66 ± 0.12a | NS | NS | NS |
| **Total evaluation** | 72.74 ± 4.68a | 71.51 ± 7.08a | 69.67 ± 3.02a | 71.59 ± 5.18a | 69.52 ± 1.62a | 68.81 ± 1.57a | NS | NS | NS |

All the parameters are given with their standard deviation. For each parameter and season, different letters indicate significant differences between treatments (*p* ≤ 0.05). T: Treatment, S: Season, and TxS: Interaction between treatment and season factors. Statistically significant at \**p* ≤ 0.05, \*\**p* ≤ 0.01 and \*\*\**p* ≤ 0.001, respectively. NS: Not significant.

**Figure 1**.



**Figure 2**.

