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Abstract

In this study, during winemaking, was evaluated the influence of cultivar on bioactive compounds (organic acids, d-(+)-glucose, d-(−)-fructose, biogenic amines (BAs), anthocyanins, polyphenols and flavonoids) and antioxidant activity of Calabrian (Southern Italy) autochthonous grapes (Arvino, Gaglioppo, Greco Nero, Magliocco Canino, Magliocco Dolce and Nocera). Phenolic compounds increased from grapes to wine for all varieties. Arvino grapevine showed the highest DPPH radical scavenging activity, while a promising inhibition of the lipid peroxidation was observed with Greco Nero grapes. BAs were mostly formed during alcoholic fermentation and Arvino always showed the lowest BAs amounts, while Magliocco Canino generally exhibited the highest. Collectively, the results demonstrated that Calabrian autochthonous grapevines were rich in sugars, organic acids and phenolic compounds thus allowing the production of high quality wines.

Abbreviations

AA antioxidant activity

ABTS 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)

AF alcoholic fermentation

AGM agmatine

Bas biogenic amines

BHT butylated hydroxytoluene

CAD cadverine

DOC Denominazione Origine Controllata

DOCG Denominazione di Origine Controllata e Garantita

DPPH 2,2-diphenyl-1-picrylhydrazyl

ETH ethylamine

FRAP Ferric Reducing Ability Power

HIS histamine

FW fresh weight

HBA Total hydroxybenzoic acids

HPLC-DAD High-Performance Liquid Chromatography-Diode Array Detection

IC_{50} concentration giving 50% inhibition

IGT Indicazione Geografica Tipica

ISA isoamylamine

MET methylamine

MLA malolactic fermentation

PCA principal component analysis

PHE phenylethylamine

PUT putrescine

ROS reactive oxygen species

SPM spermine

SPD spermidine

SS standard scores

TA Total Anthocyanin Content

TEAC Trolox equivalent antioxidant capacity

TFC total flavonoids content

TPC total phenols content

TRY tryptamine

TYR tyramine

Keywords: Biogenic amines Organic acids Antioxidant activity Calabrian grapes PCA classification Winemaking

Introduction

Wine is a complex mixture of hundreds of molecules, some of them showing important biological properties, while others are mainly associated with its organoleptic characteristics (Saurina, 2010). Many conditions (i.e. genetic, agronomic, technological, storage, etc.) linked to each other by complex and multifactorial phenomena, affect both profiles and concentrations of bioactive compounds, either in grape or in wine.

Among wine chemical classes, polyphenols and biogenic amines (BAs) have been deeply investigated for their crucial influence on wine quality, safety and nutraceutical features (Rathi & Rajput, 2014). BAs generally originate in wine by microbial decarboxylation of amino acids and, while high concentrations of the former can cause undesirable physiological effects in sensitive humans, the seconds are precursors of aroma compounds and directly contribute to wine's smell, taste and appearance. BAs can be already present in grape berries or can be formed by the yeast during the alcoholic fermentation (AF). The other alternative in BAs production, is the action of lactic acid bacteria (LAB) involved in the malolactic fermentation (MLF). Ageing or storage of wine can contribute as well in BAs accumulation (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2008). Also the storage of grapes prior to crushing under non-sterile conditions, can influence BAs concentrations, suggesting that these compounds can be considered indicators of a lack of hygiene during the winemaking process or associated with poor sanitary conditions of grapes.

Some authors reported on the presence of BAs in different wine products (Anli & Bayram, 2009). A wide range of concentration was observed, starting from not-detected up to 130 mg L− 1 (Ancín-Azpilicueta et al., 2008), with the main amines being generally putrescine (PUT), hystamine (HIS), tyramine (TYR) and cadaverine (CAD). These are mainly the products of microbial decarboxylation of ornithine, histidine, tyrosine and lysine, respectively (Smit, du Toit, & du Toit, 2008), although PUT can also be formed via the arginine deiminase pathway from arginine (Mangani, Geurrini, Granchi, & Vincenzini, 2005). Many other BAs, such as phenylethylamine (PHE), agmatine (AGM),

tryptamine (TRY), isoamylamine (ISA), methylamine (MET), and ethylamine (ETH) have also been found in wine (Anli & Bayram, 2009). Finally, polyamines such as, PUT, spermine (SPE) and spermidine (SPD), can also be formed by the metabolisms of plants and thus already present in grapes. Phenolic compounds play a fundamental role in some sensory properties of grapes and wines (Guerrero, Puertas, Fernández, Palma, & Cantos-Villar, 2010). The main flavonoid compounds present in red wine include several classes, such as flavanols [(epi)catechin], flavonols (e.g., myricetin and quercetin) and anthocyanins (e.g., malvidin-3-glucoside), while non-flavonoid compounds present in wine are phenolic acids, phenols and stilbenes (Caridi et al., 2017, Krammerer and Carle, 2009). This class of compounds has been proved to exert important health effects, acting against cancer pathologies (Giovinazzo & Grieco, 2015) as well as reactive oxygen species (ROS) which are considered the main cause of different cardiovascular and neurodegenerative diseases.

The wine sector is a pillar of the Italian economy. Italy is the world's largest wine producer and the second largest exporter by volume after Spain; moreover, approximately one-third of Italy's wines are high quality products boasting the Controlled Appellation (DOC, DOCG and IGT). Although still far from the leading regions, Calabria (southern Italy), has gained attention during last decades, reaching 12 DOCs and 12 IGTs recognitions and hosting 174 grape varieties, 76 of them are unique to the region (IOV, 2016). Mostly used as blending grapes, in recent years, Calabrian indigenous cultivars have gone uphill to produce varietal wines as well, although the complete characterization of both the raw materials and the final products is still lacking. Because the composition of wine is greatly influenced, either by the grape cultivars or by the winemaking techniques, it therefore essential to know the chemical-physical characteristics of each wine, especially the ones obtained from monovarietal grapes.

In this context, the goal of the present study was the characterization of autochthonous Calabrian red grapes and wines (Arvino, Gaglioppo, Greco Nero, Magliocco Canino, Magliocco Dolce and Nocera) that, to the best of author's knowledge, have been never considered elsewhere. The evolution of different classes of bioactive compounds (organic acids, carbohydrates, polyphenols and BAs) and in

vitro antioxidant properties of the extracts, estimated using different assays (ABTS+, DPPHradical dot; β-carotene bleaching test and FRAP), was followed during winemaking. Moreover, in order to highlight differences among varieties, principal component analysis (PCA) was also applied to underline possible correlations among samples and different discriminating compounds.

Materials and methods

Chemicals and reagents

d-(+)-glucose, d-(−)-fructose, l-(+)-tartaric acid, l-(−)-malic acid, lactic acid, acetic acid, succinic acid and fumaric acid were purchased from Sigma-Aldrich Chem. Co. (Milwakee, WI, USA). BAs, SPE (tetrahydrochloride), SPD (trihydrochloride), PUT (dihydrochloride), HIM (dihydrochloride), TYR (hydrochloride), PHE (hydrochloride), dansyl-chloride, perchloric acid (60% w/w), ammonia (30%), ascorbic acid, butylated hydroxytoluene (BHT), β-carotene, chlorogenic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, sodium acetate buffer, sodium carbonate, sodium nitrite, Folin-Ciocalteau's reagent, Tween 20, potassium persulphate, linoleic acid, propyl gallate and Whatman filters (0.45 μm and 0.20 μm) were purchased from Sigma-Aldrich S.p.a. (Milan, Italy). Ultrapure water was obtained from Milli-Q System (Millipore Corp., Milford, MA, USA). SPE C18 cartridrges (0.5 g) were obtained from Supelco Inc. (Bellefonte, PA, USA). Gallic acid, (+)-catechin, caffeic acid (3, 4-dihydroxycinnamic acid), syringic acid (4-hydroxy-3, 5-dimethoxybenzoic acid), rutin (quercetin-3-O-rutinoside), trans-resveratrol, polydatin and quercetin were supplied by Extrasynthese (Genay-France).

Samples

A total of 18 samples (six berries, musts and wines) of autochthonous Calabria Vitis vinifera red grape varieties (Arvino, Gaglioppo, Greco Nero, Magliocco Canino, Magliocco Dolce and Nocera) were collected in September 2016 from a local producer (Azienda Agricola Donna Fidelia, Belvedere Marittimo, Cosenza, Italy). To limit variability of data and to emphasise the effect of grape variety,

in this study grapes, musts and wines were provided from the same factory and were harvested, stored and processed in the same way.

Grapes, harvested at technological maturation, were grown in the same vineyard undergoing the same agronomic practices to eliminate possible variations due to different soils, climatic conditions and viticulture procedures and vinified under the same controlled processes to avoid variations during the winemaking. Each grape varieties (50 kg) were destemmed, crushed and pressed and subjected to spontaneous AF. The fermentation-maceration process was carried out at a maximum temperature of 25 ± 2 °C and lasted 7 days. Wines were then run off and maintained at controlled wine cellar temperature for undergoing spontaneous MLF. After 1 month, wine samples were separated from lees, added with SO2 (10 g hL− 1) and then analyzed. In a standard procedure 0.5 kg of grapes and 250 mL of must and wine were collected for each variety in triplicate. All samples were immediately frozen with liquid nitrogen and stored at − 80 °C. The pH of each sample was measured before analysis. A microprocessor pH meter (Hanna Istruments, Eboli (SA), Italy), equipped with a combined glass-calomel electrode, was employed for pH measurements.

Polyphenols ultrasound extraction procedure

For the ultrasound assisted experiments, an ultrasonic water-bath (Branson model 3800-CPXH, Milan, Italy) was used. Sample (50 g) was mixed with 200 mL of ethanol/water (50:50 v/v) and an ultrasonic frequency of 40 kHz for 30 min was applied. After being extracted, the mixture was filtered under vacuum through Whatman filter, and the solvent was removed with a rotary vacuum evaporator. Each extraction was performed in triplicate.

Determination of d-(+)-glucose, d-(−)-fructose and organic acids

The sugars level in grape, must, and wine extracts was performed using a Knauer high liquid chromatography system (Asi Advanced Scientific Instruments, Berlin, Germany) equipped with a Knauer HPLC-Pump K-120 (Asi Advanced Scientific Instruments, Berlin, Germany), a Rheodyne

injection valve with loop of 20 μL and a Smartiline RI detector 2300. Elution was obtained on a VARIAN Meta Carb H Plus column (300 mm × 7.8 I.D., 5 μm). The column temperature was 55 °C and the flow rate was 0.25 mL min− 1. The mobile phase consisted of 0.01 N H2SO4 solution.

The HPLC analyses of organic acids were performed on a Knauer (Asi Advanced Scientific Instruments, Berlin, Germany) system equipped with two pumps Smartiline Pump 1000, a Rheodyne injection valve (20 μL) and a photodiode array detector UV/VIS equipped with a semi-microcell. Separation was obtained using an Acclaim OA column (250 mm × 4.0 I.D., 5 μm) at T = 30 °C. The mobile phase consisted of 100 mM Na2SO4 (pH = 2.65 with methanesulfonic acid) and the flow rate was 0.6 mL min− 1. Stock solutions of each standard, in different diluted concentration ranging from 0.2–2 g L− 1, were prepared in ultra-pure water provided by a Milli-Q system (Millipore Co., Bedford, MA). All solutions were filtered through 0.45 mm glass-microfiber GMF Whatman chromatographic filter (HAWP Millipore Co., Bedford), before analysis.

The data related to the concentration of d-(+)-glucose, d-(−)-fructose and organic acids are reported in Table 1.

Spectrophotometric analysis of phenols, flavonoids and anthocyanins

The total phenols content (TPC) was determined by the Folin-Ciocalteau's method. The absorbance was measured at 765 nm using a Perkin Elmer Lambda 40 UV/VIS spectrophotometer (Milan, Italy) after 2 h incubation at 25 °C. The total phenols content was expressed as mg chlorogenic acid equivalents per 100 g fresh weight (FW) for grape and must extracts and mg L− 1 for wine.

As regard the total flavonoids content (TFC) was recorded at 510 nm using a previously described method (Loizzo et al., 2017). Quercetin was chosen as standard and the level of total flavonoid content was expressed as mg quercetin equivalents per 100 g FW for grape and must extracts and mg L− 1 for wine.

The total monomeric anthocyanins content (TA) was determined using the pH-differential method (Loizzo et al., 2017). Results are expressed as mg of cyanidin3-O-glucoside equivalents per 100 g FW for grape and must extracts and mg L− 1 for wines.

RP-HPLC/DAD analysis of phenolic acids and flavonoids

Analysis was performed on a Knauer (Asi Advanced Scientific Instruments, Berlin, Germany) system equipped with two pumps Smartiline Pump 1000, a Rheodyne injection valve (20 μL) and a photodiode array detector UV/VIS equipped with a semi-microcell. The antioxidant compounds were separated on a TSK gel ODS-100 V (TOSOH Bioscience, Germany) column (250 mm × 3.0 I.D.; 3 μm). The column temperature was 30 °C and the flow rate was 0.5 mL min− 1. The mobile phase consisted of water/formic acid (99.9:0.1, v/v; solvent A) and acetonitrile/formic acid (99.9:0.1, v/v; solvent B) and the gradient profile was as follows: 0.01–20.00 min 5% B isocratic; 20.01–50.00 min, 5–40% B; 50.01–55.00 min, 40–95% B; 55.01–60.00 min 95% B isocratic. Target compounds belonging to different phenolic classes (gallic acid, (+)-catechin, caffeic acid, syringic acid, rutin, trans-resveratrol, polydatin and quercetin) were quantified. A standard mixture was prepared by adding an accurately weighed amount of each compound (100 mg) to a 100 mL volumetric flask and was brought to the mark with methanol (80:20). A calibration straight for each standard was obtained by analysing the standard solution diluted at different concentrations. All solutions were filtered through a 0.45 m millipore filter (GMF Whatman) and injected into the HPLC system to determine retention times. Identification and quantification were carried out based on recorded retention times in comparison with authentic standards at 280, 254, 330 and 305 nm. Analyses were performed in triplicate. Data processing were carried out using Clarity Software (Chromatography Station for windows). All extracts were dissolved in 10 mL of methanol and filtered through a 0.45 μm millipore filter (GMF Whatman) before HPLC/UV–Vis determination.

In vitro antioxidant activity

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test

ABTS test was used to investigate the radicals scavenging activity of samples (Sicari, Loizzo, Branca, & Pellicanò, 2016). A solution of ABTS radical cation (ABTS+) and potassium persulphate was prepared and left in the dark for 12 h before use. The ABTS+ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at λ = 734 nm by using a Perkin Elmer Lambda 40 UV/VIS spectrophotometer (Milan, Italy). A mixture of sample (25–500 μg/mL) and diluted ABTS+ solution was prepared and after 6 min the absorbance was measured at 734 nm. Ascorbic acid was used as positive control.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) test

The radical scavenging activity of samples was investigated by DPPH assay as previously described (Sicari et al., 2016). In DPPH test, a mixture of 0.25 mM DPPH and sample with a concentration from 25 to 1000 mg/mL was prepared and left at 25 °C for 30 min. Absorbance was read at 517 nm. The ABTS and DPPH radicals scavenging ability was expressed as inhibitory concentration 50% (IC50) value. Ascorbic acid was used as positive control.

FRAP test

The FRAP test was carried out as previously reported (Loizzo et al., 2017). In brief, sample (200 μL at a concentration of 2.5 μg/mL) was mixed with 1.8 mL of FRAP reagent. The absorbance at 595 nm was measured. FRAP value represents the ratio between the slope of the linear plot for reducing Fe3 +-TPTZ reagent by sample compared to the slope of the plot for FeSO4. BHT was chosen as positive control.

β-Carotene bleaching test

The β-carotene bleaching test was used to evaluate the protection of lipid peroxidation by grapes and must ultrasound assisted extracts and wine (Loizzo et al., 2017). Briefly, a mixture of linoleic acid,

Tween 20, β-carotene was prepared. After evaporation of solvent and dilution with water, the emulsion was added into tubes containing sample and incubated at 45 °C for 60 min. The measure was performed at 470 nm after 30 and 60 min of incubation. Results were expressed as IC50 values. Propyl gallate was used as positive control.

Relative antioxidant capacity index (RACI) calculation

Relative antioxidant capacity index (RACI), is a statistical application to evaluate the antioxidant ability of samples generated from different in vitro methods (Sun & Tanumihardjo, 2007). This value is a specific combination of data from different chemical methods with no unit limitation and no variance among methods. Therefore, it can be used as an integrated approach to evaluate and compare the antioxidant capacity of different samples. Thus, data obtained from TA, TFC, TPC, ABTS, DPPH, FRAP and β-carotene bleaching tests were used to calculate RACI values for our samples.

BAs extraction and purification

The identification of the BAs was performed by comparing the retention times of peaks in the samples to those of standard solutions and by addition of the suspected BA to the samples. Quantitative determination was accomplished by direct interpolation in the standard curves for each amine constructed for twelve concentration levels and three independent replicates (Restuccia et al., 2015). The extraction of BAs from grape and must samples was performed by adding 20 mL of perchloric acid 0.6 M to about 20.0 g of each sample (or grape and must spiked with standard solution), in a 50.0 mL test tube. The mixture was homogenized, centrifuged, filtered, collected in a plastic vial and purified by SPE as elsewhere reported (Restuccia et al., 2015). The eluting solution, dried up with nitrogen gas and the residue re-dissolved in a plastic test tube with 1.3 mL of ultrapure water. Recovery experiments were performed spiking, before the extraction procedure. BAs amount added to grape and must samples were of the same order of magnitude of the supposed BAs concentration

of each sample (1.0 mL of a standard solution mixture at concentration of 100 μg/mL). Dansylation of BAs was performed following the procedure of Restuccia et al. (2015).

LC analysis were performed with a Jasco PU-2080 instrument equipped with a Rheodyne 7725 injector with a 20 μL sample loop and a gradient pump (PU-2089 plus, Jasco Inc., Easton, MD, USA). The system was interfaced with an UV detector operating at $\lambda = 254$ nm (UV-2075, Jasco Inc., Easton, MD, USA). Data were collected and analyzed with an integrator Jasco-Borwin1. For LC-UV analysis, a reverse-phase C18 column (250 mm × 4.6 I.D., 5 μm) (Supelco Inc., Bellefonte, PA, USA) equipped with a C18 guard-pak (10 mm × 4.6 I.D., 5 μm) were used (Supelco Inc., Bellefonte, PA, USA). Two solvent reservoirs containing (A) purified water and (B) acetonitrile were used to separate all the amines with an HPLC elution programme which began with 3 min of isocratic programme A–B 50:50 (v/v) reaching after 20 min A–B 10:90 (v/v). Then 3 min of isocratic elution were carried out and 4 min further were necessary to restore again the starting conditions (A–B 50:50, v/v). Flow was kept constant at 1.2 mL min− 1, for a total analysis time of 30 min and a time interval of 10 min between two injections was applied (Spizzirri et al., 2013).

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± standard deviation (S.D.). Significance was performed using one-way analysis of variance (ANOVA) test, employing Duncan's multiple range test at significance level $p < 0.05$.

The concentration giving 50% inhibition (IC50) was calculated by nonlinear regression with the use of Prism Graph Pad Prism version 4.0 for Windows (Graph Pad Software, San Diego, CA, USA). The dose-response curve was obtained by plotting the percentage inhibition versus concentration. Differences within and between groups were evaluated by one-way analysis of variance test (ANOVA) followed by a multicomparison Dunnett's test compared with the positive control at significance level $p < 0.001$. To achieve the objective sets, the obtained data were processed using statistical procedures that tended to highlight any significant relationships among investigated

parameters. Studies of the Pearson's correlation coefficient (r) and linear regression, assessment of repeatability, calculation of average and relative standard deviation was performed using Microsoft Excel 2010 software. One-way ANOVA (Tuckey's test) and Principal Component Analysis (PCA) were applied by SPSS software for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Phenols, flavonoids and anthocyanins content

The amount of bioactive constituents in grapevine depends on genotypes, environmental factors and postharvest processing conditions. In particular, phenols may act in plant with a defence mechanisms to counter oxygen species and prevent cellular damages (Costa, Cosme, Jordão, & Mendes-Faia, 2014).The TPC of six grapevine from Calabria region (Italy) was investigated (Table 2). In grape samples TPC was in the range 2.94–114.77 mg chlorogenic acid equivalent per 100 g fresh weight (FW) for Greco Nero and Magliocco Canino. Similar values were obtained for musts (3.04–122.43 mg chlorogenic acid equivalent per 100 g FW for Greco Nero and Magliocco Dolce). Wine samples showed higher values, in the range 1530–2930 mg chlorogenic acid equivalent L− 1 for Magliocco Canino and Dolce, respectively. A TPC content, in the same order of magnitude, was found by Stratil, Kuban, and Fojtova (2008), that screened red wines from Serbia and Italy and by Tuberoso, Serreli, Congiu, Montoro, and Fenu (2017) for Sardinia wines.

Wines were also rich in flavonoids, with a TFC ranging from 580 to 3400 quercetin equivalent mg L− 1 for Magliocco Canino and Gaglioppo, respectively. Lower values were found in grapes (2.48–6.86 chlorogenic acid equivalent mg per 100 g FW for Greco Nero and Gaglioppo, respectively) and musts (3.99–8.73 quercetin equivalent mg per 100 g FW for Magliocco Dolce and Greco Nero, respectively).

Anthocyanins play a crucial role in the grape color and consequently of wine. Nocera wine showed the highest TA content with value of 28.33 mg L− 1 of cyanidin 3-glucoside equivalents. Greco Nero showed the highest TA content among investigated musts (0.55 mg of cyanidin 3-glucoside

342 equivalents per 100 g FW), while TA concentration of Gaglioppo grapes was the more relevant (0.57
343 mg of cyanidin 3-glucoside equivalents per 100 g FW).
344 Generally, it is difficult to compare our data with those reported in literature, since different units
345 were used to express the obtained results. Area of production, seasonal conditions, fruit size and
346 genotypes of grape cultivars also justified the differences among the data. Lower TPC but higher TA
347 content was found for vini novelli (red wines prepared by carbonic maceration) (Pellegrini et al.,
348 2000).
349
350 *Phenolic compounds content in grape, must and wine extracts*
351 Table 3 reported on typical phenolic profile in the investigated matrices. As can be noted, quercetin
352 was the most abundant compound in both grapes and musts, while hydroxybenzoic acids showed the
353 highest concentrations in wines, confirming previous results of Auw, Blanco, O'Keefe, and Sims
354 (1996). Biosynthetic pathways involved in flavonoid production in plant tissues are greatly influenced
355 by sunlight. In this sense, it should be normally expected that grapes highly exposed to daylight, as
356 grape samples evaluated in this study, are able to improve flavonol biosynthesis. Analysis of data also
357 underlined, that rutin and quercetin levels decreased during winemaking. A factor of great importance
358 in relation with wine quality, i.e. skin contact, appears to be a crucial determinant for the flavonol
359 profile. During contact of the pomace with the fermenting must, it was found that there is a gradual
360 extraction of both flavonol glycosides and aglycones, with peaks after a period of 8 to 14 days; this
361 is, however, accompanied by a decline during the following days (Table 3). Rutin, a glycoside bearing
362 a disaccharide, exhibited the highest decomposition passing from grapes to musts and some wines
363 did not show this compound after MLF. Flavonols are labile molecules and may be degraded upon
364 exposure to heat, enzymes, and oxidative chemical species (Makris & Rossiter, 2002). Besides,
365 flavonols are also able to react with anthocyanins, yielding a type of polyphenolic compound known
366 as co-pigments, and therefore the decrease in flavonols that is usually seen during winemaking could
367 also be attributed to the co-pigmentation phenomena.

On the contrary, a different trend was found for both hydroxybenzoic and caffeic acid (Francesca et al., 2014). The increased concentrations recorded for caffeic acid are a consequence of the partial hydrolysis of the corresponding tartrate ester, making the acid more available in the free form. Moreover, the fact that no decline in hydroxybenzoic and caffeic acid during winemaking was recorded, could be ascribed to the protective effect of lees with regard to oxidation phenomena. Literature data reported as chemical interactions between lees and polyphenols allow to reduce the oxygen consumption, whereas polyphenol species in the absence of lees consume higher oxygen amounts (Karathanos, Syrimbei, Chiou, Karathanos, & Makris, 2008).

For stilbene and catechin a regular trend could not be recognized. Interestingly, in Magliocco Canino must and wine trans-resveratrol showed the highest values (4.84 and 8.07 mg L− 1 respectively).

Antioxidant capacity of the extracts

Phenolic compounds demonstrated different biological activities, in particular antioxidant activity. They may prevent biomolecules from undergoing oxidative damage through free radical mediated reactions, can inhibit oxidizing chain reactions in several ways, including direct quenching of ROS, inhibition of enzymes, and chelation of metal ions such as Fe3 + and Cu+. Herein, we performed different in vitro antioxidant tests and the data are reported on Table 4. A concentration-effect relationship was found in all tests.

Generally, grapes are more active than musts and wines for all analyzed samples. In particular, Nocera grapes showed the highest ABTS+ radical scavenging activity with IC50 value of 25.02 μg/mL. Differently, Arvino grapes were the most active against DPPHradical dot radical. The bleaching of β-carotene is the consequence of hydro-peroxides formation from linoleic acid. The presence of phytochemicals with antioxidant activity in the reaction mixture hinders the rate of bleaching by neutralizing free radicals formed in the system. The best protection of lipid peroxidation was observed with Greco Nero grape and Arvino must extracts, with IC50 values of 26.18 and 33.17 μg/mL, respectively. Wine samples showed the highest antioxidant potential by using DPPH assays, with

IC50 values ranging from 28.50 to 989.67 μg/mL for Arvino and Nocera, respectively. All tested samples showed a lower Ferric Reducing Ability Power than the positive control BHT. A positive Pearson's correlation coefficient was found between grapes TPC and TFC, and ABTS+ (r equal to 0.77 and 0.79, respectively). Differently, in must a positive correlation was found between TPC and DPPH (r = 0.81) and TFC and FRAP (r = 0.84). No correlations were found between wine and TPC, TFC and TA content.

Several works reported the antioxidant properties of grapes and their derivatives. Our data are in agreement with those reported by Nile, Kim, and Keum (2015) founding a DPPH radical scavenging ability of grape extracts with percentage from 32.8 to 87.6 tested at 100 μg/mL. Lower radical scavenging potential was observed with ABTS test with percentage of inhibition from 11.1 to 74.5 for Chasselas Rouge and Flouxa, respectively.

Shelly, Lei, Janrong, Bruce, and Kequan (2009) compared the antioxidant activity of Norton (Vitis aestivalis), Cabernet Franc clone 1, and Cabernet Franc clone 313 (Vitis vinifera) grapes and all extracts exerted notable antioxidant activities. Significant ABTS+ radical scavenging was also observed with Bordô, followed by Concord, Merlot and Cabernet Sauvignon grape extracts (Burin, Ferreira-Lima, Panceri, & Bordignon-Luiz, 2014). The analysis of Cabernet Sauvignon wine also confirmed antioxidant potential of this cultivar, as well as vini novelli obtained by Cabernet, Corvina, Croatina and Uvarara grapes (Simonetti, Pietta, & Testolin, 1997). More recently, Tuberoso et al., 2017reported the antioxidant potential of the Italian monovarietal red wine Carignano showing higher values than those reported for red Cabernet-Sauvignon, Merlot and Syrah (Van Leeuw, Kevers, Pincemail, Defraigne, & Dommes, 2014).

The evaluation of foodstuff antioxidant properties should consider the combined effects of bioactive constituents. To this regard, the RACI of each sample was calculated as the mean of standard scores transformed from the raw data generated with different antioxidant tests, without difference in units and variances. Stepwise regression between RACI and different tests showed that a) each of the assays was selected as a significant variable with no single applied method being removed, b) each

test contributed the same weight in building RACI, and c) the regression was highly significant ($r = 1$, $p < 0.001$). Based on RACI calculation, wines showed a promising antioxidant potential with the following trend Magliocco Dolce > Gaglioppo ≥ Nocera > Greco Nero > Magliocco Canino > Arvino. Among grapes, Gaglioppo and Arvino displayed the highest values with 0.67 and 0.52, respectively. Interestingly, all investigated musts presented negative RACI values.

BAs in grapes, musts and wines

In Table 5 are reported the concentrations of BAs found in grape, must and wine samples. As can be seen, total BAs contents ranged in grapes from 8.1 mg kg− 1 (Arvino) to 36.4 mg kg− 1 (Magliocco Canino), with different profiles and concentrations depending on the sample. The observed differences among grapevines, can be related with types and amounts of amino acids, taking also into account the native bacteria present on grapes and their sanitary conditions. Considering single amines, it can be noted that, irrespective of the cultivar, SPE, SPD and PUT were always present and at higher concentrations, followed by TYR, HIS and PHE, detected more rarely and at lower level (Guo, Yang, Peng, & Han, 2015). This is not surprising as vegetal products generally contain natural polyamines. In particular, SPD and SPM originate from PUT, the latter being produced by decarboxylation of ornithine or more probably by metabolism of arginine, which is the most abundant amino acid in grape (Mangani et al., 2005). PUT has been also associated with poor sanitary conditions of grape, although it has been found that this amine can be present in fruits also without external microbial contamination.

As far as musts are concerned, after AF, total BAs concentrations raised ranging from 1.65 (Greco Nero) to 2.9 (Arvino) and accounting for no < 79% of the final BAs amounts in wines. This indicates that in the considered samples, the presence of BAs was linked to AF more than to MLF, as already reported by Wang, Ye, Zhu, Wu, & Duan, 2014

Almost the same order found in grapes could be recognized in musts, with Magliocco Canino showing the highest total BAs content (63.1 mg L− 1). All the amines were present in all samples (except PHE

446 in Magliocco Dolce), some appearing after AF, others increasing their quantities passing from grapes
447 to musts, each variety showing distinct BAs distributions and amounts.
448 BAs can be formed at the end of the AF as consequence of the normal metabolic processes of yeast
449 generally present in wines producing especially HIS, TYR, SPD, ETH, AGM, PHE and CAD (Wang
450 et al., 2014). TYR can also be released in musts as a consequence of the hydrolysis of
451 hydroxycinnamic amide compounds in grapes by the action of yeast. Moreover, during AF, amino
452 acid precursors can accumulate in musts as well, supporting a further BAs increase. Although there
453 is no general consensus about correlation between the concentration of the amino acid precursor and
454 the corresponding amine amount, as well as between the aminoacids in the medium and the total BAs
455 levels, this aspect should not be neglected (Martìnez-Pinilla, Guadalupe, Hernàndez, & Ayestaràn,
456 2013).
457 Considering BAs in wine samples, it can be underlined a further BAs accumulation after MLF.
458 Increasing factors from musts to wines are all close the value of 1.2, thus much lower of those found
459 passing from grapes to musts. The BAs present in the analyzed wines showed the same profiles
460 already described for the musts. In our study, after four weeks long MLF, amine concentrations were
461 found at relatively high levels, except for PHE which has been shown to increase in case of wine or
462 must spoilage, more than in relation with MLF (Konakovsky et al., 2011).
463 In general, red wines contain relatively high amounts of polyamines PUT, SPD and SPE as well as
464 the biologically active amines HIS and TYR as already reported by Soleas, Dam, Carey, and Goldberg
465 (1999) in Ontario wine, by Bover-Cid, Izquierdo-Pulido, Mariné-Font, and Vidal-Carou (2006) in
466 Spanish wines, and by La Torre et al. (2010) in a Sicilian white, red and sweet wines.
467 Total BAs amounts in wines were generally higher than those found in other studies (Tuberoso et al.,
468 2017). During MLF, BAs formation has been associated with species belonging to all four genera of
469 wine LAB. Oenococcus oeni is predominantly responsible for MLF, but species of Pediococcus and
470 Lactobacillus, generally associated with spoilage, may survive and grow during MLF, in particular if
471 the pH of the wine is above pH 3.5 (Du Toit, Engelbrecht, Lerm, & Krieger-Weber, 2011). This last

aspect should not be disregarded and is not rare to find. In fact, to meet the market demand, wines are generally less acid than the past. The ripening of the grape tends to be prolonged to the maximum possible, for increasing the extractability of the phenolic compounds and the concentration of the aroma precursors. For the same reason, grape skins are all processed in winery, producing higher levels of precursor amino acids that, after decarboxylation, allow the formation of BAs. Higher pH, implies that the number and type of microorganisms present during winemaking increases as its possibility to form BAs (Wang et al., 2014). As can be seen in Table 1, the pH values of the wines are not far from 3.5. However, Magliocco Canino showed the lower pH and the highest BAs quantity, probably in relation to its high BAs level already found in grapes.

Interestingly, it has also been reported that high concentrations of some grape and wine phenolic compounds can affect the BAs production by inhibiting LAB growth and metabolism (Alberto, Arena, & Manca de Nadra, 2007). The authors evaluated the effect of phenolic compounds on the growth of Lactobacillus hilgardii, a microorganism able to produce PUT from AGM. In particular, PUT production from AGM decreased in the presence of caffeic, vanillic and protocatechuic acids. Later, Galgano, Caruso, Condelli, and Favati (2012) confirmed that phenolic compounds seem to be a natural way of reducing PUT formation in wine, because these moieties can protect the cells against oxidative stress. Catechins were shown to target some enzymes of biogenic amine biosynthetic pathways, while the epigallocatechin gallate reduced HIS and PUT production, by inhibiting histidine decarboxylase and ornithine decarboxylase activities. Anyway, as can be seen from the data in Table 3, Table 5 a direct correlation between different phenols and BAs could not be recognized, as many different trends were found for each class of compounds in different samples, all contributing in a different manner to BAs increasing/reduction. In a more simple way, it can be recognized a general trend linking RACI and BAs total contents. Arvino and Gaglioppo are the only varieties possessing a positive RACI for grapes and thus showing the lower amounts of amines. On the contrary, all musts possess negative RACI values indicating a reduced antioxidant capacity. It's noteworthy that the highest increasing factors for BAs accumulation from grapes to must samples, are those of Arvino

498 and Gaglioppo (2.9 and 2.5 respectively), as they pass from positive to negative RACI values.
499 Moreover the wine samples showed all positive RACI values, confirming their excellent antioxidant
500 properties able to prevent the excessive BAs accumulation in the food matrices.
501 It has been demonstrated that HIS and TYR, but also other amines, may represent potential threats
502 for human health. Even though there are no accurate regulations, several countries are requiring BAs
503 analysis (Martuscelli, Arfelli, Manetta, & Suzzi, 2013). Switzerland was the only country that
504 established an official maximum limit (10 mg L− 1) for HIS in wines, removing it on imported wines
505 in 2011 while others countries recommended different upper limits (Germany 2 mg L− 1, Belgium 6
506 mg L− 1 and France 8 mg L− 1). As can be seen from Table 5, all wines exceeded these limits with
507 possible negative effects on commercial exchanges without corrective actions limiting HIS
508 accumulation during production.
509
510 **Principal component analysis (PCA) and correlations**
511 It has been reported that phenols fingerprints can be used to analyse grape varieties while amino
512 compounds are suitable markers to evaluate winemaking practices (Saurina, 2010).
513 Results were analyzed by a multivariate PCA method to reach a smaller number of artificial variables
514 accounting for most of the variance in the observed variables (D'Agostino et al., 2014). Grapes, musts
515 and wines obtained from six varieties were analyzed for the 28 parameters reported in the
516 experimental section. The obtained results are shown in Fig. 1a. In all considered samples, the first
517 five PC accounted for 100% of total variance. The Scree plot shows the variance of each component
518 in the dataset, used to determine how many components should be retained in order to explain a high
519 percentage of the variation in the data. As can be seen in Fig. 1a, most of the variance in grapes are
520 explained by PC1, PC2 and PC3. In particular, PC1 explains about 34%, PC2 about 25% and PC3
521 about 19% of the total variance. In musts, PC1 approximatively explains 41% of the total variance,
522 while PC2 and PC3 22% and 16%, respectively. Even in wines PC1, PC2 and PC3 explained a high
523 percentage of the total variance (34%, 27% and 17% respectively). The fourth and fifth component

524 (PC4 and PC5) explain a small percentage, while, the successive PCs can be considered as not
525 statistically significant. The analysis of the graphs (Fig. 1a) underlined possible differences and
526 analogies among wine varieties and evaluated variables.
527 Fig. 1b (grapes) showed that Magliocco Canino and Arvino are very close to each other and are
528 characterized by a high value in component 2, while the other variables are more scattered. Another
529 feature is the absence of groups: the variables are distributed evenly in space, with rare exceptions
530 (gallic acid-TFC-TPC group), where the variables appear very close. In grapes, the first principal
531 component increased with increasing SPM, TPC, TFC, ABTS and gallic acid, suggesting that these
532 parameters varied together. This component can be viewed as a measure of the antioxidant quality.
533 On the contrary, a negative correlation was observed with β-carotene bleaching test at 30 and 60 min,
534 as the first principal component increased with only decreasing these variables. PC2 increased with
535 rising of FRAP, trans-resveratrol, polydatin, glucose, α and tartaric acid, while a negative correlation
536 with the rutin content was found. Finally, PC3 was found to be positively correlated with PUT, SPD,
537 ΣBAs, DPPH, quercetin, increasing when PHE decreased.
538 As far as musts are concerned (Fig. 1b), Nocera is more isolated, while the other grapevines seem to
539 be closer, with values near to zero on all the three components. The Magliocco Dolce represented a
540 partial exception, showing negative values on PC1 and PC3. Variables are evenly distributed also for
541 must samples, although a group can be recognized as well (glucose, β-carotene bleaching test
542 evaluated at 60 min, TPC).
543 In the analysis of the loading variable clearly appeared a correspondence between PC1 and DPPH,
544 trans-resveratrol, glucose, fructose, gallic acid, malic and tartaric acids. In particular, positive
545 correlations were observed with DPPH, trans-resveratrol, glucose, fructose and malic acid, while
546 gallic and tartaric acids were negative related with PC1. PC2 increases with increasing FRAP,
547 syringic acid, rutin and quercetin, negatively correlated with polydatin. Finally, PC3 increased with
548 increasing HIS, SPD, total BAs, TA and with gallic acid descreasing.
549

The wines analyses displayed the isolation of Nocera, while the Magliocco Canino has a predominant component. In this study relatively high values in PC2 for all variables and the absence of particularly noticeable groups were recorded. The analysis of the loading variables clearly showed the positive correlation of PC1 with PUT, SPD, SPM, ΣBAs, FRAP, α, tartaric and malic acids, while PC2 increases with increasing of HIS, caffeic acid, syringic acid, polydatin and when TPC, TA, catechin and quercetin decrease. Finally, for PC3 a positive correlation was observed with SPD, gallic acid, glucose, fructose and a negative one with β-carotene bleaching test at 30 min and DPPH.

The generated models allowed discrimination between different cultivars as previously demonstrated also for Tempranillo and Graciano red wines by García-Marino, Hernández-Hierro, Santos-Buelga, Rivas-Gonzalo, and Escribano-Bailón (2011) and for Greek wines by Makris, Kallithraka, and Mamalos (2006).

Conclusions

In this study, Calabrian autochthonous grapevines were characterized during winemaking. Results evidenced relevant contents of sugars, organic acids and phenolic compounds demonstrating the potential of this region to produce high quality wines. BAs were accumulated during AF more than MLF. The correlation between RACI and ΣBAs seems to support the hypothesis that antioxidant properties of phenols actively contribute to limit the BAs accumulation.

The application of PCA demonstrated a certain degree of differences between grapes, musts and wines. In particular Magliocco Canino and Arvino were very close together in grapes and musts, while Nocera was more isolated in all analyzed plot.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1
Level of organic acids, glucose and fructose in grapes, musts and wines from Calabria autochthonous red grapes varieties.
Results are expressed as mean ± S.D. ($n = 3$), $p < 0.05$.

Sample	Cultivar	Sugar		Organic acid					pH
		D-(+)-glucose	D-(−)-fructose	L-(+)-tartaric acid	L-(−)-malic acid	L-(+)-lactic acid	Acetic acid	Succinic acid	
Grapes (g kg^{-1})	*Arvino*	321.7 ± 0.2	305.5 ± 0.4	5.34 ± 0.28	2.55 ± 0.11	*n.d.*	*n.d.*	*n.d.*	3.64
	Gaglioppo	293.5 ± 0.2	318.8 ± 0.2	4.91 ± 0.32	2.88 ± 0.08	*n.d.*	*n.d.*	*n.d.*	3.29
	Greco Nero	301.2 ± 0.2	310.2 ± 0.5	6.15 ± 0.33	2.02 ± 0.12	*n.d.*	*n.d.*	*n.d.*	3.65
	Magliocco Canino	401.7 ± 0.2	309.2 ± 0.5	12.05 ± 0.43	2.25 ± 0.03	*n.d.*	*n.d.*	*n.d.*	3.13
	Magliocco Dolce	336.7 ± 0.3	297.6 ± 0.3	13.33 ± 0.54	2.25 ± 0.08	*n.d.*	*n.d.*	*n.d.*	3.57
	Nocera	309.1 ± 0.2	305.7 ± 0.2	5.35 ± 0.30	2.15 ± 0.06	*n.d.*	*n.d.*	*n.d.*	3.22
Musts (g L^{-1})	*Arvino*	217.9 ± 0.2	116.0 ± 0.1	7.62 ± 0.18	*n.d.*	*n.d.*	1.15 ± 0.03	*n.d.*	4.02
	Gaglioppo	258.2 ± 0.2	244.6 ± 0.2	2.56 ± 0.08	3.90 ± 0.03	0.01 ± 0.01	0.04 ± 0.01	*n.d.*	3.65
	Greco Nero	207.3 ± 0.1	169.1 ± 0.1	7.35 ± 0.20	*n.d.*	*n.d.*	1.55 ± 0.04	*n.d.*	3.92
	Magliocco Canino	390.3 ± 0.2	333.4 ± 0.2	3.99 ± 0.18	4.80 ± 0.04	0.02 ± 0.01	0.19 ± 0.02	*n.d.*	3.74
	Magliocco Dolce	389.0 ± 0.2	378.0 ± 0.2	2.70 ± 0.14	2.70 ± 0.02	0.08 ± 0.02	0.32 ± 0.04	*n.d.*	3.81
	Nocera	272.4 ± 0.2	145.0 ± 0.2	5.99 ± 0,12	*n.d.*	*n.d.*	1.51 ± 0.04	*n.d.*	3.25
Wines (g L^{-1})	*Arvino*	3.5 ± 0.1	19.7 ± 0.1	2.82 ± 0.05	1.58 ± 0.02	3.61 ± 0.04	0.38 ± 0.03	1.14 ± 0.05	3.37
	Gaglioppo	9.1 ± 0.1	12.0 ± 0.1	4.33 ± 0.04	1.64 ± 0.05	3.33 ± 0.04	0.46 ± 0.02	1.69 ± 0.02	3.44
	Greco Nero	8.2 ± 0.1	11.7 ± 0.2	3.52 ± 0.03	1.77 ± 0.02	5,16 ± 0.05	0.34 ± 0.03	1.43 ± 0.06	3.49
	Magliocco Canino	27.5 ± 0.2	8.1 ± 0.1	7.14 ± 0.04	2.67 ± 0.04	2.64 ± 0.06	0.62 ± 0.05	1.48 ± 0.06	3.15
	Magliocco Dolce	7.8 ± 0.1	5.7 ± 0.1	5.32 ± 0.05	2.24 ± 0.07	3.55 ± 0.05	0.39 ± 0.03	1.77 ± 0.03	3.49
	Nocera	33.5 ± 0.1	22.1 ± 0.1	3.14 ± 0.05	2.23 ± 0.08	4.31 ± 0.02	0.25 ± 0.03	1.81 ± 0.01	3.27

n.d: not detected or below limit of quantitation.

Table 2
Total phenols, flavonoids and anthocyanin content of grapes, must and wines from selected grapes cultivars from Calabria region. Results are expressed as mean ± S.D. ($n = 3$), $p < 0.05$.

		TPC[a]	TFC[b]	TA[c]
Grapes	*Arvino*	3.98 ± 0.6	2.96 ± 0.2	0.25 ± 0.02
	Gaglioppo	107.17 ± 8.5	6.86 ± 1.3	0.57 ± 0.05
	Greco Nero	2.94 ± 0.2	2.48 ± 0.1	0.32 ± 0.04
	Magliocco Canino	114.77 ± 5.1	6.30 ± 2.4	0.38 ± 0.01
	Magliocco Dolce	80.24 ± 6.4	5.03 ± 0.9	0.23 ± 0.02
	Nocera	3.40 ± 0.2	2.70 ± 0.3	0.41 ± 0.03
Musts	*Arvino*	5.49 ± 0.4	4.21 ± 0.4	0.42 ± 0.01
	Gaglioppo	50.21 ± 7.2	4.53 ± 0.8	0.45 ± 0.07
	Greco Nero	3.04 ± 0.1	8.73 ± 0.2	0.55 ± 0.06
	Magliocco Canino	46.91 ± 3.4	5.19 ± 2.7	0.49 ± 0.06
	Magliocco Dolce	122.43 ± 7.9	3.99 ± 1.1	0.42 ± 0.06
	Nocera	6.41 ± 0.3	5.20 ± 0.3	0.47 ± 0.04
Wines	*Arvino*	2600 ± 11.4	3120 ± 10.6	22.44 ± 1.1
	Gaglioppo	2800 ± 15.3	3400 ± 13.1	18.37 ± 2.2
	Greco Nero	2400 ± 10.8	2414 ± 11.5	26.28 ± 1.2
	Magliocco Canino	1530 ± 10.2	580 ± 7.6	20.21 ± 3.1
	Magliocco Dolce	2930 ± 12.7	750 ± 8.3	28.26 ± 2.3
	Nocera	2800 ± 14.2	2975 ± 12.8	28.33 ± 1.5

TPC: total phenols content; TF: total flavonoids content; TA: total antocyanins content.

[a] mg chlorogenic acid equivalents *per* 100 g FW for grape and must extracts and mg L^{-1} for wine.

[b] mg quercetin equivalents *per* 100 g FW for grape and must extracts and mg L^{-1} for wine.

[c] mg cyanidin 3-glucoside equivalents *per* 100 g FW for grape and must extracts and mg L^{-1} for wine.

Table 3
Total level of bioactive compounds in grapes, musts and wines from Calabria autochthonous red grapes varieties. Results are expressed as mean ± S.D. ($n = 3$), $p < 0.05$.

Sample	Cultivar	Gallic acid	(+)-Catechin	Caffeic acid	Syringic acid	Rutin	*trans*-Resveratrol	Polydatin	Quercetin	ΣHBA	Stilbene	Flavonols
Grapes (mg kg^{-1})	*Arvino*	3.27 ± 0.06	110.57 ± 0.18	5.47 ± 0.08	31.07 ± 0.12	30.14 ± 0.06	1.93 ± 0.21	10.25 ± 0.09	257.66 ± 0.51	34.34 ± 0.18	12.18 ± 0.30	287.80 ± 0.57
	Gaglioppo	11.64 ± 0.15	103.05 ± 0.09	5.48 ± 0.09	28.93 ± 0.20	31.11 ± 0.07	4.66 ± 0.11	4.99 ± 0.08	221.21 ± 0.30	40.57 ± 0.35	9.65 ± 0.19	224.32 ± 0.37
	Greco Nero	5.18 ± 0.04	33.47 ± 0.15	4.27 ± 0.07	8.97 ± 0.08	35.12 ± 0.14	2.43 ± 0.18	8.57 ± 0.07	339.47 ± 0.39	14.15 ± 0.12	11.00 ± 0.25	374.59 ± 0.53
	Magliocco Canino	10.03 ± 0.06	66.77 ± 0.11	7.12 ± 0.11	18.33 ± 0.23	23.55 ± 0.12	5.33 ± 0.10	11.29 ± 0.17	306.11 ± 0.48	28.36 ± 0.29	16.62 ± 0.27	329.66 ± 0.60
	Magliocco Dolce	11.08 ± 0.08	78.24 ± 0.17	7.58 ± 0.07	22.12 ± 0.17	25.17 ± 0.09	6.53 ± 0.14	7.75 ± 0.06	234.33 ± 0.37	33.20 ± 0.25	14.28 ± 0.20	259.50 ± 0.46
	Nocera	6.28 ± 0.06	201.22 ± 0.12	6.24 ± 0.10	33.01 ± 0.17	44.41 ± 0.07	1.88 ± 0.13	8.13 ± 0.11	307.16 ± 0.24	39.28 ± 0.23	10.01 ± 0.24	351.57 ± 0.31
Musts (mg L^{-1})	*Arvino*	42.51 ± 2.14	70.55 ± 0.33	10.55 ± 0.28	48.24 ± 0.24	0.36 ± 0.04	1.34 ± 0.10	9.97 ± 0.06	55.24 ± 0.13	90.75 ± 2.38	11.13 ± 0.16	55.60 ± 0.17
	Gaglioppo	46.96 ± 0.20	338.14 ± 1.76	13.44 ± 0.15	53.89 ± 0.12	7.47 ± 0.08	2.75 ± 0.18	6.44 ± 0.08	180.68 ± 0.33	100.85 ± 0.32	9.19 ± 0.26	188.15 ± 0.41
	Greco Nero	29.24 ± 1.80	88.41 ± 0.14	7.88 ± 0.10	53.24 ± 0.40	0.37 ± 0.02	2.37 ± 0.15	10.14 ± 0.05	98.06 ± 0.11	82.44 ± 2.20	12.51 ± 0.20	98.43 ± 0.13
	Magliocco Canino	54.20 ± 0.53	74.07 ± 0.10	5.57 ± 0.10	73.31 ± 0.28	3.11 ± 0.08	4.84 ± 0.16	8.12 ± 0.07	214.12 ± 0.26	127.51 ± 0.81	12.96 ± 0.23	217.23 ± 0.34
	Magliocco Dolce	39.12 ± 0.47	109.41 ± 1.22	10.18 ± 0.10	52.47 ± 0.33	1.95 ± 0.07	3.25 ± 0.10	3.33 ± 0.05	368.73 ± 0.37	91.59 ± 0.11	6.58 ± 0.15	370.68 ± 0.44
	Nocera	42.13 ± 0.77	125.20 ± 1.17	10.55 ± 0.13	58.33 ± 1.40	6.67 ± 0.05	3.51 ± 0.20	1.81 ± 0.04	278.41 ± 0.28	100.46 ± 2.17	5.32 ± 0.24	285.08 ± 0.33
Wines (mg L^{-1})	*Arvino*	81.80 ± 0.18	62.36 ± 0.30	47.01 ± 0.13	87.30 ± 1.12	1.16 ± 0.12	6.11 ± 0.08	11.61 ± 0.30	41.34 ± 0.87	169.10 ± 1.30	17.72 ± 0.38	42.50 ± 0.99
	Gaglioppo	72.04 ± 0.33	55.53 ± 0.61	31.57 ± 0.28	97.33 ± 0.40	4.22 ± 0.35	1.52 ± 0.08	5.38 ± 0.17	37.21 ± 0.80	169.37 ± 0.73	6.90 ± 0.25	41.43 ± 1.15
	Greco Nero	95.13 ± 0.28	28.93 ± 0.11	47.07 ± 0.18	166.36 ± 1.05	3.40 ± 0.44	5.83 ± 0.26	12.54 ± 0.34	30.05 ± 0.24	261.49 ± 1.33	21.37 ± 0.60	33.45 ± 0.68
	Magliocco Canino	76.24 ± 0.37	52.11 ± 0.14	43.82 ± 0.24	127.92 ± 0.88	*n.d*	8.07 ± 0.18	6.44 ± 0.22	56.02 ± 0.18	204.16 ± 1.25	8.51 ± 0.40	56.02 ± 0.18
	Magliocco Dolce	66.43 ± 0.41	112.53 ± 0.27	38.29 ± 0.17	72.42 ± 0.28	*n.d*	7.11 ± 0.27	4.25 ± 0.15	44.31 ± 0.70	138.85 ± 0.69	12.36 ± 0.42	44.31 ± 0.70
	Nocera	95.10 ± 0.25	132.82 ± 0.24	16.18 ± 0.11	44.22 ± 0.33	*n.d*	3.37 ± 0.14	2.10 ± 0.10	39.03 ± 0.45	139.32 ± 0.58	5.47 ± 0.24	39.03 ± 0.45

n.d: not detected or below limit of quantitation.
ΣHBA: total hydroxybenzoic acids = gallic acid + syringic acid; Stilbene: *trans*-resveratrol + polydatin; flavonols: quercetin + rutin.

Table 4
Antioxidant activity of grapes, must and wines from selected grapes cultivars from Calabria region. Results are expressed as mean ± S.D. of $n = 3$ measurements.

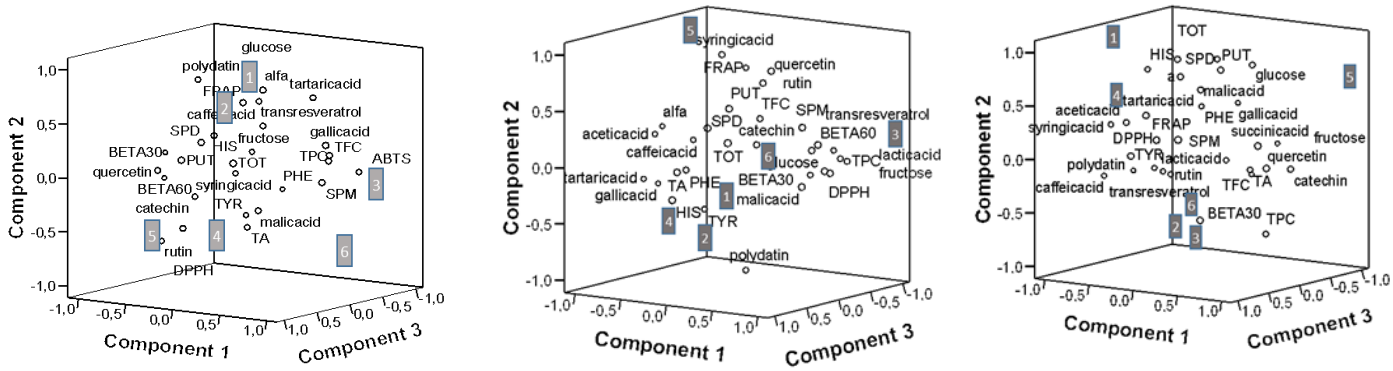
		ABTS IC_{50} (μg/mL)	DPPH test IC_{50} (μg/mL)	β-Carotene bleaching test IC_{50} (μg/mL)		FRAP test μM Fe(II)/g at 2.5 mg/mL	RACI
				30 min	60 min		
Grapes	*Arvino*	86.68 ± 4.2	31.91 ± 1.7	78.79 ± 2.5	48.83 ± 2.0	23.83 ± 1.1	0.52
	Gaglioppo	173.25 ± 4.2	61.94 ± 1.3	2.15% (at 100 μg/mL)	3.14%(at 100 μg/mL)	22.94 ± 1.0	0.67
	Greco Nero	67.63 ± 3.8	62.50 ± 3.7	26.18 ± 2.7	55.39 ± 2.4	21.35 ± 2.3	−0.70
	Magliocco Canino	108.91 ± 3.7	54.58 ± 3.6	13.60% (at 100 μg/mL)	6.99% (at 100 μg/mL)	28.96 ± 1.3	−0.61
	Magliocco Dolce	174.04 ± 4.5	62.00 ± 3.5	28.88% (at 100 μg/mL)	10.91% (at 100 μg/mL)	18.72 ± 1.1	−0.64
	Nocera	25.02 ± 1.5	176.05 ± 3.5	54.34 ± 2.6	31.08 ± 2.3	22.77 ± 2.1	−0.70
Musts	*Arvino*	12.61% (at 500 μg/mL)	33.11 ± 2.5	33.17 ± 1.2	43.05% (at 100 μg/mL)	2.68 ± 0.9	−0.66
	Gaglioppo	38.48% (at 500 μg/mL)	854.54 ± 8.8	94.65 ± 4.7	46.89% (at 100 μg/mL)	7.33 ± 0.8	−0.67
	Greco Nero	15.79% (at 500 μg/mL)	115.99 ± 5.8	43.09% (at 100 μg/mL)	18.11% (at 100 μg/mL)	NA	−0.70
	Magliocco Canino	17.11% (at 500 μg/mL)	952.14 ± 8.8	39.81% (at 100 μg/mL)	15.63% (at 100 μg/mL)	NA	−0.67
	Magliocco Dolce	32.42% (at 500 μg/mL)	921.17 ± 8.8	55.61 ± 5.3	57.70 ± 5.9	3.87 ± 0.9	−0.61
	Nocera	21.01% (at 500 μg/mL)	166.67 ± 8.8	48.95% (at 100 μg/mL)	38.23% (at 100 μg/mL)	9.77 ± 0.9	−0.70
Wines	*Arvino*	4.94% (at 500 μg/mL)	28.50 ± 2.2	12.01% (at 100 μg/mL)	17.14% (at 100 μg/mL)	NA	0.39
	Gaglioppo	14.84% (at 500 μg/mL)	948.38 ± 9.2	94.24 ± 4.4	26.85% (at 100 μg/mL)	10.64 ± 0.9	1.57
	Greco Nero	7.03% (at 500 μg/mL)	765.39 ± 7.4	31.46% (at 100 μg/mL)	20.33% (at 100 μg/mL)	NA	1.25
	Magliocco Canino	7.24% (at 500 μg/mL)	775.62 ± 6.7	43.05% (at 100 μg/mL)	22.93% (at 100 μg/mL)	12.91 ± 2.8	0.54
	Magliocco Dolce	10.21% (at 500 μg/mL)	775.71 ± 7.6	87.25 ± 5.7	28.10% (at 100 μg/mL)	8.36 ± 2.8	1.68
	Nocera	5.37% (at 500 μg/mL)	50.06% (at 1000 μg/mL)	41.37% (at 100 μg/mL)	43.28% (at 100 μg/mL)	NA	1.57
Positive control	*Ascorbic acid*	1.7 ± 0.4	5.0 ± 0.8	–	–	–	–
	BHT	–	–	–	–	63.2 ± 4.3	–
	Propyl gallate	–	–	1.0 ± 0.04	1.0 ± 0.03	–	–

NA: not active; DPPH test: One-way ANOVA $***p < 0.0001$ followed by a multicomparison Dunnett's test: $***p < 0.001$ compared with ascorbic acid. Antioxidant Capacity Determined by Radical Cation ($ABTS^+$): One-way ANOVA $***p < 0.0001$ followed by a multicomparison Dunnett's test: $***p < 0.001$ compared with ascorbic acid. Ferric Reducing Ability Power (FRAP): One-way ANOVA $***p < 0.0001$ followed by a multicomparison Dunnett's test: $**p < 0.001$ compared with BHT. β-Carotene bleaching test: One-way ANOVA $***p < 0.0001$ ($r^2 = 0.999$) followed by a multicomparison Dunnett's test: $**p < 0.001$ compared with propyl gallate.

Table 5
BAs content in grapes, musts and wines from Calabria autochthonous red grapes varieties. Results are expressed as mean ± S.D. ($n = 3$), $p < 0.05$.

Sample	Cultivar	Biogenic amines						
		PHE	PUT	HIS	TYR	SPD	SPM	ΣBAs
Grapes (mg kg^{-1})	*Arvino*	1.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	2.0 ± 0.1	8.1 ± 0.1
	Gaglioppo	2.1 ± 0.1	1.0 ± 0.1	*n.d.*	1.8 ± 0.1	3.4 ± 0.1	8.1 ± 0.2	16.4 ± 0.1
	Greco Nero	*n.d.*	2.1 ± 0.1	5.7 ± 0.1	9.3 ± 0.2	2.1 ± 0.1	7.5 ± 0.2	26.7 ± 0.2
	Magliocco Canino	*n.d.*	6.3 ± 0.2	5.7 ± 0.2	*n.d.*	13.6 ± 0.2	10.8 ± 0.2	36.4 ± 0.2
	Magliocco Dolce	*n.d.*	1.5 ± 0.1	*n.d.*	3.1 ± 0.1	1.6 ± 0.1	15.3 ± 0.2	21.5 ± 0.2
	Nocera	*n.d.*	6.8 ± 0.1	*n.d.*	*n.d.*	10.0 ± 0.2	6.5 ± 0.2	23.3 ± 0.2
Musts (mg L^{-1})	*Arvino*	2.8 ± 0.1	3.9 ± 0.1	5.7 ± 0.2	5.0 ± 0.2	3.2 ± 0.1	3.1 ± 0.1	23.7 ± 0.1
	Gaglioppo	3.7 ± 0.1	8.5 ± 0.1	7.2 ± 0.2	3.3 ± 0.1	5.7 ± 0.2	13.5 ± 0.3	41.9 ± 0.2
	Greco Nero	1.4 ± 0.1	4.2 ± 0.1	9.2 ± 0.2	14.0 ± 0.2	3.4 ± 0.1	11.8 ± 0.2	44.0 ± 0.2
	Magliocco Canino	2.2 ± 0.1	12.0 ± 0.2	8.9 ± 0.2	1.5 ± 0.1	21.0 ± 0.3	17.5 ± 0.2	63.1 ± 0.3
	Magliocco Dolce	*n.d.*	5.4 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	2.9 ± 0.1	20.4 ± 0.2	36.6 ± 0.2
	Nocera	2.3 ± 0.1	10.8 ± 0.2	6.2 ± 0.2	1.6 ± 0.1	15.0 ± 0.2	10.9 ± 0.2	46.8 ± 0.2
Wines (mg L^{-1})	*Arvino*	3.0 ± 0.1	8.2 ± 0.2	6.5 ± 0.2	5.3 ± 0.2	3.6 ± 0.2	3.4 ± 0.2	30.0 ± 0.2
	Gaglioppo	3.9 ± 0.1	14.5 ± 0.2	8.5 ± 0.2	3.6 ± 0.1	6.0 ± 02	13.8 ± 0.2	50.3 ± 0.2
	Greco Nero	1.5 ± 0.1	11.0 ± 0.2	12.0 ± 0.2	14.5 ± 0.2	3.5 ± 0.1	11.9 ± 0.2	54.4 ± 0.2
	Magliocco Canino	3.5 ± 0.2	16.2 ± 0.2	12.3 ± 0.2	2.1 ± 0.1	21.8 ± 0.2	18.2 ± 0.2	74.1 ± 0.2
	Magliocco Dolce	*n.d.*	10.4 ± 0.2	4.3 ± 0.2	4.5 ± 0.2	3.1 ± 0.2	21.0 ± 0.2	43.3 ± 0.2
	Nocera	2.5 ± 0.1	15.0 ± 0.2	7.5 ± 0.1	2.1 ± 0.1	15.9 ± 0.2	11.5 ± 0.2	54.5 ± 0.2

n.d.: not detected or below limit of quantitation. PHE β-phenylethylamine, PUT putrescine, HIS histamine, TYR tyramine, SPD spermidine, SPM spermine.



Grapes **Musts** **Wine**

Fig. 2. Principle component analysis (PCA) biplot of different vine variety: [1] Magliocco canino, [2] Arvino, [3] Magliocco dolce, [4] Greco nero, [5] Nocera and [6] Gaglioppo.