

Repository Istituzionale dei Prodotti della Ricerca del Politecnico di Bari

On the use of Ethephon as abscising agent in cv. Crimson Seedless table grape production: Combination of Fruit Detachment Force, Fruit Drop and metabolomics

This is a pre-print of the following article

Original Citation:
On the use of Ethephon as abscising agent in cv. Crimson Seedless table grape production: Combination of Fruit Detachment Force, Fruit Drop and metabolomics / Rizzuti, A., Aguilera Sáez, L.M., Gallo, V., Cafagna, I., Mastrorilli, P., Latronico, M., Pacifico, A., Matarrese, A.M.S., Ferrara, G.. - In: FOOD CHEMISTRY. - ISSN 0308-8146. - ELETTRONICO. - 171:(2015), pp. 341-350. [10.1016/j.foodchem.2014.08.132]

Availability:
This version is available at http://hdl.handle.net/11589/810 since: 2021-03-10

Published version
DOI:10.1016/j.foodchem.2014.08.132

Publisher:

Terms of use:

(Article begins on next page)

27 November 2026

Elsevier Editorial System(tm) for Food Chemistry
Manuscript Draft

Manuscript Number:

Title: On the use of Ethephon as abscising agent in cv. Crimson Seedless table grapes production: a Combined Study based on Fruit Detachment Force, Fruit Drop and Metabolomics

Article Type: Research Article (max 7,500 words)

Keywords: Table Grapes; Crimson Seedless; Berry Loosening; Fresh-cut food; High Resolution Mass Spectrometry; Nuclear Magnetic Resonance; Principal Component Analysis; Covariance Analysis; Fruit Detachment Force

Corresponding Author: Dr. Antonino Rizzuti, Ph.D.

Corresponding Author's Institution: Polytechnic of Bari

First Author: Antonino Rizzuti, Ph.D.

Order of Authors: Antonino Rizzuti, Ph.D.; Luis M Aguilera-Saez, Dr; Vito Gallo, Ph.D.; Isabella Cafagna, Ph. D.; Piero Mastrorilli, Prof.; Mario Latronico, Prof.; Andrea Pacifico, Dr; Angela M Matarrese, Dr; Giuseppe Ferrara, Ph.D.

Abstract: The effect of 2-chloroethylphosphonic acid (Ethephon, in the following ETH) as abscising agent on cv. Crimson Seedless table grapes was investigated by means of Fruit Detachment Force (FDF) and Fruit Drop (FD) analyses combined with a metabolomic study carried out by High Resolution Mass Spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR) spectroscopy. The effectiveness of ETH as abscising agent was ascertained with ETH concentration ranging from 1.4 to 4.0 g/L in a two-year study. The ETH treatments caused berry drops higher than 40% and induced an increase of tartaric acid, procyanidin P2, terpenoid derivatives and peonidin-3-glucoside as well as a decrease of catechin and epicatechin. HRMS-NMR covariance analysis was carried out to correlate the fluctuations of tartaric acid NMR signals to those of MS peaks of the secondary metabolites affected by ETH treatments.

Cover Letter

POLITECNICO DI BARI

DIPARTIMENTO DI INGEGNERIA CIVILE, AMBIENTALE, DEL TERRITORIO, EDILE E DI CHIMICA

VIA E. ORABONA, 4 • 70125 BARI (ITALY)

Tel.: +39 0805963607 • Fax: +39 080 5963611

Dr. Antonino Rizzuti, Ph.D.

e-mail: antonio.rizzuti@poliba.it

Dear Editor,

please find attached our manuscript entitled:

"**On the use of Ethephon as abscising agent in cv. Crimson Seedless table grapes production: a Combined Study based on Fruit Detachment Force, Fruit Drop and Metabolomics**"

by

Antonino Rizzuti, Luis Manuel Aguilera-Saez, Vito Gallo, Isabella Cafagna, Piero Mastrorilli, Mario Latronico, Andrea Pacifico, Angela Maria Stella Matarrese, Giuseppe Ferrara

which we wish to be considered for publication in *Food Chemistry.*

This manuscript reports, for the first time, a study based on the combination of agronomical analyses (Fruit Detachment Force and Fruit Drop) and chemical analyses (Metabolomics carried out by Nuclear Magnetic Resonance spectroscopy and High Resolution Mass Spectrometry) aimed to evaluate the effects of Ethephon (ETH) as abscising agent in *cv.* Crimson Seedless table grapes production.

Along with the metabolomic approach, HRMS-NMR covariance analysis was carried out to gain important information on the effects of Ethephon on primary and secondary metabolite composition. In particular, it was found a correlation between fluctuations of tartaric acid NMR signals and those of MS peaks of the secondary metabolites affected by ETH treatments. These findings give a hint to consider a possible role of tartaric acid in secondary metabolic pathways.

The interest towards ETH application derives from the possibility to produce undamaged single berries suitable for the fresh-cut fruits market.

All the authors have contributed significantly and are in agreement with the content of the manuscript.

The paper is new, not declined by other journals, and it is not being considered for publication elsewhere.

Yours sincerely

Antonino Rizzuti

On the use of Ethephon as abscising agent in *cv.* Crimson Seedless table grapes production: a Combined Study based on Fruit Detachment Force, Fruit Drop and Metabolomics

Antonino Rizzuti, Luis Manuel Aguilera-Saez, Vito Gallo, Isabella Cafagna, Piero Mastrorilli, Mario Latronico, Andrea Pacifico, Angela Maria Stella Matarrese, Giuseppe Ferrara

Highlights

NMR and HRMS were used to characterize ETH treated grapes

ETH treatments caused berry drops higher than 40%

ETH treatments increase procyanidins, terpenoid derivatives and peonidin-glu contents

Tartaric acid is involved in senescence processes

*Manuscript
Click here to view linked References

On the use of Ethephon as abscising agent in *cv.* Crimson Seedless table grapes production: a Combined Study based on Fruit Detachment Force, Fruit Drop and Metabolomics

Antonino Rizzuti,*[a] *Luis Manuel Aguilera-Saez*,[a] *Vito Gallo*,[a,b] *Isabella Cafagna*,[a] *Piero Mastrorilli*,[a,b] *Mario Latronico*,[a,b] *Andrea Pacifico*,[c] *Angela Maria Stella Matarrese*,[c] *Giuseppe Ferrara*[c]

[a] Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica, Politecnico di Bari, Via Orabona, 4, I-70125, Bari, Italy

[b] Innovative Solutions S.r.l. – Spin off company of Politecnico di Bari, zona H, 150/B, 70015, Noci (BA), Italy

[c] Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy

* Corresponding author. Tel.: +39 080 5963607; fax: +39 080 5963611.

e-mail address: antonio.rizzuti@poliba.it

Abstract

The effect of 2-chloroethylphosphonic acid (Ethephon, in the following ETH) as abscising agent on *cv*. Crimson Seedless table grapes was investigated by means of Fruit Detachment Force (FDF) and Fruit Drop (FD) analyses combined with a metabolomic study carried out by High Resolution Mass Spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR) spectroscopy. The effectiveness of ETH as abscising agent was ascertained with ETH concentration ranging from 1.4 to 4.0 g/L in a two-year study. The ETH treatments caused berry drops higher than 40% and induced an increase

of tartaric acid, procyanidin P2, terpenoid derivatives and peonidin-3-glucoside as well as a decrease of catechin and epicatechin. HRMS-NMR covariance analysis was carried out to correlate the fluctuations of tartaric acid NMR signals to those of MS peaks of the secondary metabolites affected by ETH treatments.

Keywords

Table Grapes; Crimson Seedless; Berry Loosening; Fresh-cut food; High Resolution Mass Spectrometry; Nuclear Magnetic Resonance; Principal Component Analysis; Covariance Analysis; Fruit Detachment Force

1. Introduction

Fresh-cut fruits and vegetables are rapidly increasing popularity because of their high quality, attractiveness and numerous nutritional facts, and they are highly practical for a prompt consumption (Çelikkol & Türkben, 2012).

Table grape is a fruit well suited for consumption as fresh-cut product. The most important feature for its acceptance on markets is the lack of defects such as decay, cracking, stem browning, insect damage, grey mould infection and berry softness. Grape quality depends on many factors including pedoclimatic conditions, vineyard treatments, harvest time, cultivar, degree of ripening and phytosanitary conditions. After harvest, table grape undergoes water loss resulting in either stem drying or browning and berry softening. However, long lasting storage of grapes is obtained by post-harvest treatments and by using appropriate packages (Costa et al., 2011).

Italy is one of the greatest world producer and exporter of table grape and Puglia, a region in Southeastern Italy, is the most important Italian region for table grape production. Official data (ISTAT 2012) indicate a table grape cultivation on about 30,000 hectares (60% of the italian area under table grape cultivation), with a yield of 660,000 tons. The economical importance of table grapes worldwide is recognized by the extent of table grapes world market assessed at about 4

millions of tons and 5 billions of euros. ‘New countries’, such as Thailand, South Korea, China, Lithuania, are highly requiring table grapes. In this world situation, in 2012, Italian export of table grapes reached 590 million of euros with 482,000 tons, with a mean value of 1.22 €/kg of grape. Besides the traditional buyers of Italian table grapes (Germany, France, UK, Spain), an increasing demand is coming from countries such as Poland, Kingdom of Saudi Arabia and United Arab Emirates. This data suggest also a possible output of table grape as a fresh-cut fruit either to traditional markets (Europe) or to new countries.

Fresh-cut products (especially vegetables) are showing an interesting increased consumption in many countries, and it is worth pointing out the remarkable surplus that such products are achieving in terms of commercial value. In these perspectives, table grape as fresh-cut fruit could be sold in supermarkets as well as in vending machines located in schools and offices at a valuable price. In the production of fresh-cut grapes, harvesting is both a time and money consuming part. The application of fruit abscission (loosening) agents can contribute greatly toward enhancing the efficiency of harvesting. In fact, the application of abscission agents decreases the Fruit Detachment Force (FDF) required to separate berries from the stem thus facilitating mechanical harvesting of undamaged individual grape berries (Fidelibus, Cathline, & Burns, 2007). In particular, 2-chloroethylphosphonic acid (ETH), a commercial ethylene-releasing agent used as plant-growth regulator (Brown, 1997), has also been evaluated as a potential fruit loosening agent able to reduce the FDF in a wide range of fruit such as citrus (*Citrus sinensis L.*), grape (*Vitis vinifera L.*) (Christensen, 2000), olive (*Olea europaea L.*), and cherry (*Prunus avium L.)* (Malladi, Vashisth, & Johnson, 2012).

The influence of external factors (e.g., growth regulators) on fruit production can be evaluated by a metabolomic approach. Metabolomics aims at measuring the global, dynamic and metabolic response of the living systems to biological stimuli and provides information on a wide range of detectable chemical compounds contained in food products. Metabolomic studies usually involve Nuclear Magnetic Resonance (NMR) spectroscopy or High Resolution Mass Spectrometry (HRMS)

supported by multivariate statistical methods (Ali, Maltese, Fortes, Pais, Choi & Verpoorte, 2011; Bevilacqua, Triggiani, Gallo, Cafagna, Mastrorilli & Ferrara, 2012; Ferrara et al., 2013; Ferrara, Mazzeo, Netti et al., 2014; Gallo et al., 2014; Kim, Choi, & Verpoorte, 2010; Nicholson & Lindon, 2008; Schripsema, 2010; Wishart, 2008; Son et al., 2009; Sumner, Mendes, & Dixon, 2003). It is generally accepted that a single analytical technique seldom provides complete information on the metabolome and therefore a combined approach is desirable to gain a comprehensive view. In this respect, combination of NMR with HRMS is advisable as NMR easily provides information on molecules with relatively high concentration (typically, such molecules correspond to primary metabolites) while HRMS supplies precious information on molecules at low concentration (typically, such molecules constitute secondary metabolites) (Aliferis & Jabaji, 2010 Rizzuti, Caliandro, Gallo, Mastrorilli, Chita, & Latronico, 2013). Recently, our research has been focused on the evaluation of the effects of various plant-growth regulators on the metabolic profiles of table grapes. The positive effect of S-ABA on skin colour of *cv*. Crimson Seedless was ascertained, without any significant change in the profile of the primary metabolites (Ferrara et al., 2013). Moreover, the effects of gibberellic acid (GA_3) and 1-(2-chloropyridin-4-yl)-3-phenylurea (forchlorfenuron, CPPU) on amino acids composition of *cv*. Italia (Ferrara, Mazzeo, Matarrese et al., 2014) have been established.

In the present study, application of ETH on *cv*. Crimson Seedless was investigated with the aim to verify its effectiveness as abscising agent for producing undamaged single berries suitable for the fresh-cut fruits market. Furthermore, the effect of ETH on metabolic profile was evaluated by combination of NMR and HRMS measurements that were correlated to the Fruit Drop (FD) and Fruit Detachment Force (FDF) values.

2. Materials and Methods

All chemicals were of analytical reagent grade. Sodium hydroxide, sodium azide and formic acid were purchased from Sigma Aldrich (Milan, Italy). Acetonitrile, and methanol LC/MS grade and

isopropanol HPLC grade were purchased from VWR (Milan, Italy). Water was doubly deionised (resistivity: 18 MΩ·cm) with a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany).

2.1 Fruit materials and sample preparation

Table grape samples of *cv*. Crimson Seedless were collected for two consecutive years (2010 and 2011) in vineyards located in the countryside of Adelfia and Acquaviva delle Fonti, in the province of Bari (Puglia region). Vines were spaced 2.8 × 2.5 m, trained to an overhead trellis system with 5-6 fruiting canes/vine and drip irrigated (3,000-3,200 m^3/ha). Fertilizer additions, pest control and other vineyard operations (berry thinning, leaf removal, and lateral shoots thinning) were conducted according to local practices. A randomized block design was used with three blocks and three treatments, and each treatment in the block consisted of three grapevines selected for uniform vigour and with a similar crop load. The bunches were treated with increasing amount of ETH. During 2010, samples were submitted to ETH concentrations of 1.4 and 3.0 g/L (10T1 and 10T2, respectively) and compared with the control samples (10CTRL), while during 2011, 3.0 and 4.0 g/L (11T2 and 11T3, respectively) of ETH were used and the treated grapes were also compared with control samples (11CTRL). In 2010, ETH was applied on the 19th of September and the grapes were harvested on the 5th of October, whereas in 2011 ETH treatment was performed on the 23rd of September and harvest was done on the 7th of October. The bunches were sprayed by using a manual pump with care to wet whole bunches only when the fruits reached sufficient soluble solids content (at least 16 °Brix). After treatments, bunches were wrapped by a plastic net in order to collect berries dropping from pedicel before harvesting (see Figure 1). At harvest, the bunches were collected and carried to the laboratory in a thermal bag at 8-10 °C. Bunches were manually shaken and abscised berries were collected in order to be visually checked for integrity of (dry) stem scar and for the presence/absence of the pedicel. Fruit drop percentage was calculated as FD% = [(DB+SB)/TB]*100 where DB represents the weight of the berries spontaneously dropped into the

plastic net before harvest, SB represents the weight of the berries dropped after shaking of the bunches in the laboratory and TB represents the total weight of the bunch (sum of DB, SB and the weight of the berries still attached to the rachis). FDF measurements were performed on the berries still attached to the rachis by means of a mechanical detachment gauge (Somfy Tec, France).

Detached grape berries were stored at –20 °C until NMR and HRMS analyses. Before sample preparation, berries were defrosted for 40 minutes, then they were squeezed and centrifuged (15 min and 4000 rpm at room temperature) to obtain juices for NMR and HRMS measurements.

2.2 High Resolution Mass Spectrometry

100 µL of centrifuged juice was added to 1.0 mL of a solution containing CH_3OH, H_2O, HCOOH (in the volume ratio 70:30:1, respectively) and 30 µg of NaN_3. Fifteen replicates were prepared for each treatment.

Liquid chromatography was carried out with an Agilent High Performance Liquid Chromatography (HPLC) system (Agilent, Milan, Italy), equipped with a vacuum degasser (G1322A, Agilent), an autosampler (G1377A, Agilent), a quaternary pump and a thermostated column department, a reversed-phase C_{18} analytical column (HDB, 4.6 x 150 mm, particle size 5 µm, Agilent) protected by a guard cartridge of the same packing, and maintained at 25 °C. The HPLC device was connected online to a MicrOTOF-Q II mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with an Electrospray Ionization Source (ESI). The injection volume of the samples was 20 µL. The mobile phase, consisting of water with formic acid (0.1%) (A) and acetonitrile with formic acid (0.1%) (B), was pumped at 1.0 mL/min into the HPLC system with the following gradient elution program: 0-3 min, isocratic 95% A; 3-18 min, linear from 5 to 95% B; 18-20 min, isocratic 95% B; 20-21 min, linear from 95 to 5% B; 21-25 min, isocratic 5% B. A divert valve was used to remove substances (mainly polar primary metabolites) eluting during the initial three minutes thus allowing detection of the less polar part mainly consisting of secondary metabolites. The Time-Of-Flight (TOF) detector, used for accurate mass measurements, operated in

both negative (nebulizer gas, nitrogen, 4 bar; dry gas, nitrogen, 10 L/min, 200 °C; endplate offset –500 V; capillary voltage +3.5 kV; mass range 50-1000 *m/z*) and positive (nebulizer gas, nitrogen, 4 bar; dry gas, nitrogen, 10 L/min, 200 °C; endplate offset –500 V; capillary voltage –4.5 kV; mass range 50-1000 *m/z*) mode. External calibrations were made using a 100 L KD Scientific syringe pump with a reference solution made up of 10 μL of formic acid (98%), 10 μL of aqueous sodium hydroxide (1.0 M), 490 μL of *i*-propanol and 490 μL of deionized water. The raw-file data were collected as continuum mass spectra at a regular time interval (spectra rate of 1 spectrum/s with a rolling averages of 3). Mass spectra were processed using Data Analysis 4.0. The SmartFormula tool within DataAnalysis™ (Bruker Daltonik GmbH, Bremen, Germany) was used to obtain the elemental composition, errors and sigma values for each detected compounds. MS data were assigned to metabolites on the bases of accurate mass, isotopic distribution and fragmentation pattern in both positive and negative ion modes. Assignments were confirmed after comparison with literature data (Cantos, Espín, & Tomás-Barberán, 2002; Cavaliere, Foglia, Gubbiotti, Sacchetti, Samperi & Laganà, 2008; Cejudo-Bastante, Pérez-Coello, & Hermosín-Gutiérrez, 2010; Flamini, 2013; Godevac, Teševic, Veličković, Vujisica, Vajs & Milosavljević, 2010; Gollücke, Catharino, de Souza, Eberlin, & de Queiroz Tavares, 2009; Gómez-Romero, Zurek, Schneider, Baessmann, Segura-Carretero & Fernández-Gutiérrez, 2011; Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010; Guerrero et al., 2009; He et al., 2010; Kajdžanoska, Gjamovski, & Stefova, 2010; Kneknopoulos, Skouroumounis, Hayasaka, & Taylor, 2011; Moco et al., 2006; Rodríguez-Medina, Segura-Carretero, & Fernández-Gutiérrez, 2009; Sonni, Clark, Prenzler, Riponi, & Scollary, 2011; Sonni, Moore, et al., 2011; Stalmach, Edwards, Wightman, & Crozier, 2011; Stefova & Ivanova, 2011) and with on-line public metabolite databases (PubChem <http://pubchem.ncbi.nlm.nih.gov/>, Metlin <http://metlin.scripps.edu/> and Chemspider <http://www.chemspider.com>). Mass data for statistical analysis were generated by bucketing procedures of the mass spectra. Bucketing was performed using AMIX 3.9.13 software (BrukerBioSpin GmbH, Rheinstetten, Germany) applying the advanced bucketing mode with a *m/z*

displacement of 0.03 in the range of 50.50 and 1000.50 Da, and scaling the intensities of individual ions to total intensity recorded between 3.25 and 10.75 min. The buckets were used as variables for Principal Component Analysis (PCA) executed by AMIX 3.9.13.

2.3 Nuclear Magnetic Resonance

500 μL of centrifuged juice were added to 300 μL of 0.15 $\%_w$ sodium salt of (trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) in D_2O, 200 μL of oxalate buffer pH = 4.2 [pH value was reached after addition of 37% HCl to 100 mL of an aqueous solution containing $Na_2C_2O_4$ (0.25 M) and NaN_3 ($2.5 \cdot 10^{-3}$ M)]. Fifteen replicates were prepared for each treatment.

One-dimensional ^{1}H NOESY spectra were recorded on a Bruker Avance I 400 MHz spectrometer equipped with a 5 mm inverse probe and with an autosampler. ^{1}H NOESY spectra were acquired with 128 scans of 64 K data points with a spectral width of 8013 Hz, a pulse angle of 90°, an acquisition time of 4.09 s, a mixing time of 10 ms and a recycle delay of 3.0 s. Each spectrum was acquired using TOPSPIN 3.0 software (Bruker BioSpin GmbH, Rheinstetten, Germany) under an automatic procedure lasting approximately 22 min and consisting of sample loading, temperature stabilization for 5 min, tuning, matching, shimming and 90° pulse calibration. Free induction decays (FIDs) were Fourier transformed, the phase was manually corrected, the baseline was automatically corrected and the spectra were aligned by setting the TSP singlet to 0 ppm. NMR raw data were processed using TOPSPIN 3.0 and, unless otherwise stated, converted in regular rectangular buckets (0.04 ppm width) by AMIX 3.9.13. Multivariate statistical analysis of NMR data were performed using the AMIX 3.9.13.

2.4 Covariance analysis

The correlation among NMR and HRMS data vectors were studied by calculating the covariance matrix:

$$COV_{AB}(i,j) = \sum_k \left(\hat{y}_k(i) - \langle \hat{y}(i) \rangle\right)_A \left(\hat{y}_k(j) - \langle \hat{y}(j) \rangle\right)_B$$

where A and B stand for NMR and HRMS, respectively, $\hat{y}_k(i)$ is the pre-processed bucket of sample *k* and $\langle \hat{y}(i) \rangle$ is the average value calculated over the samples for the *i*-th variable. Calculation of the covariance matrix was performed using AMIX 3.9.13 with NMR and HRMS buckets generated in the advanced bucketing mode. The resulting covariance matrix was submitted to the combined covariance tool in AMIX 3.9.13.

3. Results and discussion

3.1 Fruit Drop and FDF analyses

The effect of ETH on fruit drop of treated bunches was significant in both 2010 and 2011 years. Berries abscised after the treatment with ETH presented a dry, corky scar at the abscission zone (pedicel/fruit interface) (Figure S1).

During 2010, drop of berries ranged from 40% (10T1) to 59% (10T2) for ETH treated grape whereas fruit drop of control berries was almost absent (Figure S2a). FDF measurements on berries still attached to the rachis indicated that untreated grapes were found slightly more resistant (10CTRL: 8.06 ± 2.07 N) with respect to treated ones (10T1: 6.16 ± 2.71 N; 10T2: 6.50 ± 2.48 N). No significant difference derived from the two concentrations of ETH used in the treatments.

During 2011, the percentage of berry drop following the application of ETH was comparable to values recorded in 2010 and was not different for the two treatments (11T2: 42%; 11T3: 46 %). Fruit drop value of control berries was again very low. FDF values measured for control grapes (11CTRL: 7.94 ± 2.08 N) were similar to those recorded for treated grapes (11T2: 8.04 ± 1.78 N; 11T3: 7.69 ± 1.70 N) (Figure S2b).

In all cases, detached berries showed dry stem scars which is a positive and desired response, especially in the view of a possible use of ‘Crimson Seedless’ berries as fresh-cut fruits. In general,

ETH treatments were effective, even at the lowest concentration of 1.4 g/L to abscise around 40% of berries by simple shaking.

3.2 Analysis of HRMS spectra

HRMS analysis permitted the identification of 43 compounds belonging to several metabolite classes including alcohols, organic acids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids), amino acid derivatives, flavonoids (flavanols, flavones and anthocyanins) and terpenoid glycosides. The lists of detected metabolites are reported in table 1 (detection in negative mode) and table S1 (detection in positive mode).

As shown in Tables 1 and S1, different phenolic compounds such as flavonols, flavanols, anthocyanins, hydroxybenzoic and hydroxycinnamic acids were identified by HRMS analysis. Such metabolites are considered healthy compounds due to their antioxidant activities (Flamini, 2013) and they constitute an added value for table grapes used as fresh-cut fruit products. Anthocyanins found in our grapes were only peonidin, petunidin and delphinidin glucosides. In fact, Crimson Seedless has a relatively low concentration of total anthocyanins (100-200, mg/kg of fresh weight) with respect to other red table grape cultivars, with the predominant anthocyanin being peonidin-3-glucoside (66-85% of the measured anthocyanins) (Cantos et al., 2002; Ferrara, Mazzeo, Netti et al., 2014).

Glutathionyl caftaric acid and indole-3-lactic acid hexose were the metabolites that gave the most intense peaks. Glutathionyl caftaric acid is produced rapidly by the enzymatic oxidation of the caftaric acid due to the crushing of the grape berries during the preparation of the samples (Singleton, Salgues, Zaya, & Trousdale, 1985). Indole-3-lactic acid hexose is a metabolite deriving from the tryptophan de-amination pathway which leads ultimately to indole-3-acetic acid (IAA) formation. IAA is the most abundant member of the auxin class of plant hormones and represents the inhibitor of ripening most studied for non-climacteric fruits such as grapes.

3.2.1 PCA applied to HRMS data

PCA was performed in order to find possible grouping of grape samples as a consequence of ETH treatments. Figure 2 shows PC1/PC2 *scores* and *loadings* plots obtained for grape samples harvested during 2010 and analysed by HRMS(–). The *scores* plot (Figure 2a) indicates that grape samples are differentiated into three groups with PC1 explaining 32.7% and PC2 15.6 % of the total variance. As ascertained by PC1/PC2 *loadings* plot (Figure 2b), the discriminating metabolites along PC1 were procyanidin P2 (*m/z* 577.1501), procyanidin C1 (*m/z* 865.2238), indole-3-lactic acid hexose and its $[2M\text{-}H]^-$ adduct (*m/z* 366.1288 and 733.2681), benzyl alcohol hexose-pentose (*m/z* 401.1560) and coumaric acid hexose (*m/z* 325.0960). In particular, samples 10T2 were characterized by a higher amount of procyanidins whereas indole-3-lactic acid hexose, benzyl alcohol hexose-pentose and coumaric acid hexose were found in larger amounts in 10T1 and 10CTRL samples.

Along PC2, samples 10CTRL were differentiated from 10T1 and 10T2 due to higher content of catechin/epicatechin (*m/z* 289.0779), glutathionyl-catechin compounds (*m/z* 594.1559) and coumaric acid hexoses (*m/z* 325.0960 and 145.0313). Moreover, 10T1 and 10T2 samples contained higher amount of tetrahydroabscisic acid hexose (*m/z* 429.2192), dihydrophaseic acid hexose (*m/z* 443.2038), peonidin-3-O-glucoside (*m/z* 461.1221), dihydroisorhamnetin hexoside (*m/z* 479.1316), myricetin derivative (*m/z* 481.1436), glutathionyl caftaric acid (*m/z* 616.1378) and glutathionyl caffeic acid derivative (*m/z* 646.17395).

The reduction of catechin/epicatechin as a consequence of ETH application is probably the result of the condensation of such flavanols, a clear symptom of ageing ('senescence') of the berry. This hypothesis is substantiated by the increase of procyanidin P2 and C1 (flavanols dimers) and of terpenoid derivatives such as dihydrophaseic acid hexose in treated berries. Dihydrophaseic acid (DPA) is an abscisic acid (ABA) catabolite (Owen, Lafond, Bowen, Bogdanoff, Usher & Abrams, 2009), which can suggest that large quantities of ABA had been produced and catabolized in the berry due to treatments with ETH, even though such treatments occurred at late stages. High

283 amounts of peonidin-3-O-glucoside in treated samples are in agreement with literature data
284 indicating that peonidin-3-glucoside content significantly increased after ETH application to
285 Crimson Seedless grapes (Human & Bindon, 2008).
286 PC1/PC2 *scores* and *loadings* plots obtained by mass data acquired in positive ion mode
287 [HRMS(+)] are shown in figures S3a and S3b, respectively. In this case, 10CTRL, 10T1 and 10T2
288 samples were differentiated along the PC1 (explaining 22.8% of the total variance) mainly due to
289 anthocyanin peonidin-3-O glucoside at *m/z* 463.1292 which was found in larger amount in the 10T2
290 samples and to indole-3-lactic acid hexose at *m/z* 385.1638 (in the form of its ammonia adduct),
291 found in larger amount in the 10CTRL samples. These results confirm those obtained with PCA
292 applied to HRMS(–) data.
293 In 2011 Crimson Seedless samples were treated using an ETH concentration of 3.0 g/L and 4.0 g/L.
294 In order to have a correspondence of ETH concentration with treatments carried out in 2010,
295 samples treated with 3.0 g/L will be indicated with 11T2 while samples treated with 4.0 g/L of ETH
296 will be denoted as 11T3. PCA applied to HRMS data of 11CTRL, 11T2 and 11T3 samples
297 indicated a lower grouping of the grapes thus suggesting that, during 2011, ETH treatments had a
298 lower influence on their metabolic profiles. These findings match those described above for FDF
299 and FD analyses.
300
301 *3.3 Analysis of NMR spectra*
302 ^{1}H NMR spectra of table grape samples collected during 2010 and 2011 allowed for straightforward
303 identification of primary metabolites, namely sugars, organic acids and amino acids.
304 In Figure S4, a typical spectrum of ‘Crimson Seedless’ berries is shown. In the portion between
305 10.0 and 6.0 ppm very weak signals attributable to phenolic and aromatic compounds are present.
306 The most intense signals, in the region from 6.0 to 2.5 ppm, are attributable to glucose, fructose,
307 tartaric acid and malic acid. In the region 2.5-0.0 ppm most signals derive from amino acids.
308 Signals attribution was made by comparison with spectra of authentic samples and with literature

data (Ali et al., 2011; Ferrara et al., 2013; Ferrara, Mazzeo, Netti et al., 2014; Gallo et al., 2014; Son et al., 2009).

In Table 2, metabolites identified in berries of 'Crimson Seedless' are listed and, for each signal, chemical shift (δ, ppm) and multiplicity are also reported.

3.3.1 PCA applied to NMR data

PCA applied to NMR data (generated by regular bucketing of the whole spectrum) indicated no substantial effects of the treatments on the primary metabolite profile of the grapes. In fact, for each year, scores of the treated samples were superimposable to those of control grapes. Considering both 2010 and 2011 production years, samples were clearly discriminated on the basis of the harvest, thus confirming that pedo-climatic conditions had a greater discriminating effect on the primary metabolites with respect to the treatments.

A deeper inspection of NMR spectra indicated that effects of the treatments could be appreciated, for samples collected during 2010, when only signals belonging to the organic acids (tartaric, malic and citric), proline and ethanol were considered. Thus, PCA was applied to variables generated by integration of NMR signals of such metabolites.

Figure 3 shows PC1/PC2 *scores* and *loadings* plots obtained for samples collected during 2010. *Scores* plot indicates that the three groups of grapes (10CTRL, 10T1, 10T2) are differentiated each other along PC1, which explains 75.2 % of the total variance. Control samples are located at positive PC1 values and treated samples move towards negative PC1 values as an effect of the increasing ETH concentration. As ascertained by *loadings* plot, the species mainly responsible for such a discrimination is tartaric acid.

NMR data related to samples collected during 2011 do not indicate substantial effects of ETH treatments on metabolic profiles of the grapes.

335 *3.4 HRMS-NMR covariance analyses*

336 It is known that application of ETH has no important effects on soluble solid content, total acidity,
337 pH, yield and berry weight whereas it affects composition of secondary metabolites (Szyjewicz,
338 Rosner & Kliewer, 1984). Our findings are in agreements with these results as indicated by NMR
339 for primary metabolites and by HRMS for secondary metabolites. Anyway, the dependence of
340 tartaric acid on ETH treatments for grapes harvested during 2010 raises the question whether ETH
341 affect the ripening level of the grapes or only metabolic pathways leading to berry senescence.
342 Tartaric acid biosynthesis begins with L-ascorbic acid (differently from other fruit acids), in
343 particular with the cleavage of a six-carbon intermediate between C4/C5 thus yielding tartaric acid
344 and glycoaldehyde (DeBolt, Cook & Ford, 2006). In our case, the application of ETH may stimulate
345 the catabolism of ascorbic acid (AA) thus leading to an increase of tartaric acid biosynthesis, since
346 ethylene stimulates plant senescence and consequently reduction of AA in leaves (Bartoli,
347 Simontacchi, Montaldi & Puntarulo, 1996) and probably in other plant organs. One of the most
348 important changes observed during plant senescence is the decline in antioxidant contents and the
349 increase in the steady state of reactive oxygen species and a reduction of AA is considered very
350 important for the plant antioxidant defence (Gergoff, Chaves & Bartoli, 2010). The decreases of AA
351 may be the consequence of ETH effect on either reducing the biosynthesis or on increasing the
352 degradation through reactions possibly leading to a slight increase of tartaric acid in the berry.

353 In order to find a possible link between tartaric acid and the secondary metabolites affected by ETH
354 treatments, HRMS-NMR covariance analysis was carried out. Covariance analysis indicates signals
355 belonging to metabolites characterized by correlated fluctuations of their intensities. Correlations
356 are positive when the metabolite content distributions are coherent and negative when the
357 metabolite content distributions are opposite.

358 Figure 4 shows the results obtained by correlation of NMR spectra with HRMS spectra recorded in
359 negative (Figure 4a) and positive ion mode (Figure 4b) for samples collected during 2010. Tartaric
360 acid is positively correlated to peonidin-3-O-glucoside, dihydroisorhamnetin hexoside and

procyanidin P2 and is negatively correlated to catechin/epicatechin, coumaric acid hexose, indole-3-lactic acid hexose, indole-3-lactic acid hexose adduct, benzyl alcohol hexose-pentose, glutathionyl catechin, glutathionyl caftaric acid and indole-3-lactic acid hexose adduct.

In other words, tartaric acid is positively correlated to compounds which were found more abundant in ETH treated samples and is negatively correlated to compounds characterizing control grapes. Variation of the tartaric acid concentration could be associated to possible different ripening levels of the grapes as an effect of ETH treatments. Actually, this is not the case. In this regard, it must be considered that ripening of the grapes is characterized by an increase of sugar amounts and a concomitant decrease of organic acids concentrations, especially tartaric acid. However, a recent work on Arabidopsis and spinach leaves showed that leaf AA biosynthesis and content is down-regulated by ethylene application (Gergoff et al., 2010) and AA is the key component for tartaric acid biosynthesis (DeBolt et al., 2006). Since NMR signal intensities of sugars were unaltered by ETH treatments and since tartaric acid was correlated to metabolites characterizing ETH treated samples, it can be concluded that ETH application at late stages does not affect ripening level of the grapes and tartaric acid is somehow involved in secondary metabolic pathways (as in leaves) which deserve deeper investigations.

4. Conclusions

This study demonstrates that ETH is an effective abscising agent giving satisfactory results in terms of berry drop ranging from 40% to 59% during 2010 and from 42% to 46% during 2011. Considering that Crimson Seedless is a cultivar unaffected by shatter problems, application of ETH to cultivars with shatter problems could be expected to increase the drop percentage. Moreover, drop percentages obtained in our study encourages a possible use of Crimson Seedless as fresh-cut fruit.

As usual in horticultural studies, also in our case different results were obtained for each year. In particular, effects of ETH applications on FDF values and on metabolic profile were more

pronounced during 2010. Concerning primary metabolite composition, tartaric acid content was found slightly higher in ETH treated grapes. Among the secondary metabolites, accumulation of procyanidins and terpenoid derivatives in the treated samples indicated that senescence processes are favored by ETH.

Finally, HRMS-NMR covariance analysis allowed to correlate NMR tartaric acid signal to mass peaks of secondary metabolites discriminating ETH treated samples. Moreover, such covariance study indicated that the variations of tartaric acid quantities in the grapes are related to ETH treatments and are not due to possible different ripening levels.

Acknowledgements

Regione Puglia is greatfully aknowledged for financial support in the framework of the call "Reti di Laboratori pubblici di ricerca – Apulian Food Fingerprint, Project n.68". We thank Polytechnic of Bari for a research fellowship to AR.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http:

403 **References**

404 Ali, K., Maltese, F., Fortes, A. M., Pais, M. S., Choi, Y. H., & Verpoorte, R. (2011). Monitoring
405 biochemical changes during grape berry development in Portuguese cultivars by NMR
406 spectroscopy. *Food Chemistry*, *124*(4), 1760–1769.

407 Aliferis, K. A., & Jabaji, S. (2010). ^{1}H NMR and GC-MS metabolic fingerprinting of
408 developmental stages of Rhizoctonia solani sclerotia. *Metabolomics*, *6*(1), 96–108.

409 Bartoli, C. G., Simontacchi, M., Montaldi, E. & Puntarulo, S. (1996). Oxidative stress, antioxidant
410 capacity and ethylene production during ageing of cut carnation (Dianthus caryophyllus)
411 petals. *Journal of experimental botany*, *47*, 595–601.

412 Bevilacqua, V., Triggiani, M., Gallo, V., Cafagna, I., Mastrorilli, P., & Ferrara, G. (2012). An
413 Expert System for an Innovative Discrimination Tool of Commercial Table Grapes. In D.-S.
414 Huang, J. Ma, K.-H. Jo, & M. M. Gromiha (Eds.), *Intelligent Computing Theories and*
415 *Applications* (pp. 95–102). Springer Berlin Heidelberg.

416 Brown, K. M. (1997). Ethylene and abscission. *Physiologia Plantarum*, *100*(3), 567–576.

417 Cantos, E., Espín, J. C., & Tomás-Barberán, F. A. (2002). Varietal differences among the
418 polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. *Journal of*
419 *Agricultural and Food Chemistry*, *50*(20), 5691–5696.

420 Cavaliere, C., Foglia, P., Gubbiotti, R., Sacchetti, P., Samperi, R., & Laganà, A. (2008). Rapid-
421 resolution liquid chromatography/mass spectrometry for determination and quantitation of
422 polyphenols in grape berries. *Rapid Communications in Mass Spectrometry*, *22*(20), 3089–
423 3099.

424 Cejudo-Bastante, M. J., Pérez-Coello, M. S., & Hermosín-Gutiérrez, I. (2010). Identification of
425 New Derivatives of 2-S-Glutathionylcaftaric Acid in Aged White Wines by HPLC-DAD-
426 ESI-MS^{n}. *Journal of Agricultural and Food Chemistry*, *58*(21), 11483–11492.

427 Çelikkol, I., & Türkben, C. (2012). Effects of postharvest applications on berry quality, microbial
428 population and morphological (epicuticular wax) deterioration of ready-to-eat table grapes.
429 *Journal of Food, Agriculture and Environment*, *10*(3-4), 213–220.
430 Christensen, L. P. (2000). *Raisin Production Manual*. UCANR Publications: Oakland, California.
431 Costa, C., Lucera, A., Conte, A., Mastromatteo, M., Speranza, B., Antonacci, A., & Del Nobile, M.
432 A. (2011). Effects of passive and active modified atmosphere packaging conditions on
433 ready-to-eat table grape. *Journal of Food Engineering*, *102*(2), 115–121.
434 DeBolt, S., Cook, D. R. & Ford C. M. (2006). L-tartaric acid synthesis from vitamic C in higher
435 plants. *Proceedings of the National Academy of Sciences, 103*, 5608–5613.
436 Ferrara, G., Mazzeo, A., Matarrese, A. M. S., Pacucci, C., Pacifico, A., Gambacorta, G.,
437 Mastrorilli, P. (2013). Application of Abscisic Acid (S-ABA) to "Crimson Seedless" Grape
438 Berries in a Mediterranean Climate: Effects on Color, Chemical Characteristics, Metabolic
439 Profile, and S-ABA Concentration. *Journal of Plant Growth Regulation*, 1–15.
440 Ferrara, G., Mazzeo, A., Matarrese, A. M. S., Pacucci, C., Punzi, R., Faccia, M., Trani, A.,
441 Gambacorta, G., (2014). Use of abscisic acid (S-ABA) and sucrose for improving color,
442 anthocyanin content and antioxidant activity of 'Crimson Seedless' grape berries. Australian
443 Journal of Grape and Wine Research, *in press*.
444 Ferrara, G., Mazzeo, A., Netti, G., Pacucci, C., Matarrese, A. M. S., Cafagna, I., Mastrorilli, P.,
445 Vezzoso, M., Gallo, V. (2014). Girdling, gibberellic acid and forchlofenuron: effects on
446 yield, quality and metabolic profile of table grape cv Italia, American Journal of Enology
447 and Viticulture, *in press*.
448 Fidelibus, M. W., Cathline, K. A., & Burns, J. K. (2007). Potential Abscission Agents for Raisin,
449 Table, and Wine Grapes. *HortScience*, *42*(7), 1626–1630.
450 Flamini, R. (2013). Recent Applications of Mass Spectrometry in the Study of Grape and Wine
451 Polyphenols. *ISRN Spectroscopy*, *2013*, 1–45.

452 Gallo, V., Mastrorilli, P., Cafagna, I., Nitti, G. I., Latronico, M., Longobardi, F., Minoja, A. P.,
453 Napoli, C., Romito, V. A., Schäfer, H., Schütz, B., Spraul, M. (2014). Effects of
454 Agronomical Practices on Chemical Composition of Table Grapes evaluated by NMR
455 Spectroscopy. *Journal of Food Composition and Analysis, in press*.
456 Gergoff, G., Chaves, A., Bartoli, C. G. (2010). Ethylene regulates ascorbic acid content during
457 dark-induced leaf senescence. *Plant science*, *178*, 207–212.
458 Godevac, D., Teševic, V., Veličković, M., Vujisica, L., Vajs, V., & Milosavljević, S. (2010).
459 Polyphenolic compounds in seeds from some grape cultivars grown in Serbia. *Journal of the*
460 *Serbian Chemical Society*, *75*(12), 1641–1652.
461 Gollücke, A. P. B., Catharino, R. R., de Souza, J. C., Eberlin, M. N., & de Queiroz Tavares, D.
462 (2009). Evolution of major phenolic components and radical scavenging activity of grape
463 juices through concentration process and storage. *Food Chemistry*, *112*(4), 868–873.
464 Gómez-Romero, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Metabolite profiling
465 and quantification of phenolic compounds in methanol extracts of tomato fruit.
466 *Phytochemistry*, *71*(16), 1848–1864.
467 Gómez-Romero, M., Zurek, G., Schneider, B., Baessmann, C., Segura-Carretero, A., & Fernández-
468 Gutiérrez, A. (2011). Automated identification of phenolics in plant-derived foods by using
469 library search approach. *Food Chemistry*, *124*(1), 379–386.
470 Guerrero, R. F., Liazid, A., Palma, M., Puertas, B., González-Barrio, R., Gil-Izquierdo, Á., García-
471 Barroso, C., Cantos-Villar, E. (2009). Phenolic characterisation of red grapes autochthonous
472 to Andalusia. *Food Chemistry*, *112*(4), 949–955.
473 He, F., Mu, L., Yan, G.-L., Liang, N.-N., Pan, Q.-H., Wang, J., Reeves, M. J., Duan, C.-Q. (2010).
474 Biosynthesis of Anthocyanins and Their Regulation in Colored Grapes. *Molecules*, *15*(12),
475 9057–9091.

476 Human, M. A., & Bindon, K. A. (2008). Interactive effect of ethephon and shading on the
477 anthocyanin composition of Vitis vinifera L. cv. crimson seedless. *S. Afr. J. Enol. Vitic.*,
478 *29*(1), 50–58.
479 Kajdžanoska, M., Gjamovski, V., & Stefova, M. (2010). HPLC-DAD-ESI-MS^n identification of
480 phenolic compounds in cultivated strawberries from Macedonia. *Macedonian Journal of*
481 *Chemistry and Chemical Engineering*, *29*(2), 181–194.
482 Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants.
483 *Nature Protocols*, *5*(3), 536–549.
484 Kneknopoulos, P., Skouroumounis, G. K., Hayasaka, Y., & Taylor, D. K. (2011). New Phenolic
485 Grape Skin Products from Vitis vinifera cv. Pinot Noir. *Journal of Agricultural and Food*
486 *Chemistry*, *59*(3), 1005–1011.
487 Malladi, A., Vashisth, T., & Johnson, L. K. (2012). Ethephon and methyl jasmonate affect fruit
488 detachment in rabbiteye and southern highbush blueberry. *HortScience*, *47*(12), 1745–1749.
489 Moco, S., Bino, R. J., Vorst, O., Verhoeven, H. A., Groot, J. de, van Beek, T. A., Vervoort, J., de
490 Vos, C. H. R. (2006). A Liquid Chromatography-Mass Spectrometry-Based Metabolome
491 Database for Tomato. *Plant Physiology*, *141*(4), 1205–1218.
492 Nicholson, J. K., & Lindon, J. C. (2008). Systems biology: Metabonomics. *Nature*, *455*(7216),
493 1054–1056.
494 Owen, S.J., Lafond, M.D., Bowen, P., Bogdanoff, C., Usher, K. & Abrams, S.R. (2009). Profiles of
495 abscisic acid and its catabolites in developing Merlot grape (*Vitis vinifera*) berries. *American*
496 *Journal of Enology and Viticulture*, *60*, 277-284.
497 Rizzuti, A., Caliandro, R., Gallo, V., Mastrorilli, P., Chita, G., & Latronico, M. (2013). A combined
498 approach for characterisation of fresh and brined vine leaves by X-ray powder diffraction,
499 NMR spectroscopy and direct infusion high resolution mass spectrometry. *Food Chemistry*,
500 *141*(3), 1908–1915.

Rodríguez-Medina, I. C., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2009). Use of high-performance liquid chromatography with diode array detection coupled to electrospray-Qq-time-of-flight mass spectrometry for the direct characterization of the phenolic fraction in organic commercial juices. *Journal of Chromatography. A*, *1216*(23), 4736–4744.

Schripsema, J. (2010). Application of NMR in plant metabolomics: techniques, problems and prospects. *Phytochemical Analysis*, *21*(1), 14 – 21.

Singleton, V. L., Salgues, M., Zaya, J., & Trousdale, E. (1985). Caftaric Acid Disappearance and Conversion to Products of Enzymic Oxidation in Grape Must and Wine. *American Journal of Enology and Viticulture*, *36*(1), 50–56.

Son, H.-S., Hwang, G.-S., Kim, K. M., Ahn, H.-J., Park, W.-M., Van Den Berg, F., Hong, Y.-S, Lee, C.-H. (2009). Metabolomic Studies on Geographical Grapes and Their Wines Using ^{1}H NMR Analysis Coupled with Multivariate Statistics. *Journal of Agricultural and Food Chemistry*, *57*(4), 1481–1490.

Sonni, F., Clark, A. C., Prenzler, P. D., Riponi, C., & Scollary, G. R. (2011). Antioxidant action of glutathione and the ascorbic acid/glutathione pair in a model white wine. *Journal of Agricultural and Food Chemistry*, *59*(8), 3940–3949.

Sonni, F., Moore, E. G., Clark, A. C., Chinnici, F., Riponi, C., & Scollary, G. R. (2011). Impact of glutathione on the formation of methylmethine- and carboxymethine-bridged (+)-catechin dimers in a model wine system. *Journal of Agricultural and Food Chemistry*, *59*(13), 7410–7418.

Stalmach, A., Edwards, C. A., Wightman, J. D., & Crozier, A. (2011). Identification of (Poly)phenolic Compounds in Concord Grape Juice and Their Metabolites in Human Plasma and Urine after Juice Consumption. *Journal of Agricultural and Food Chemistry*, *59*(17), 9512–9522.

525 Stefova, M., & Ivanova, V. (2011). Analytical Methodology for Characterization of Grape and
526 Wine Phenolic Bioactives. In *Fruit and Cereal Bioactives: Sources, Chemistry, and*
527 *Applications* (pp. 409–427). CRC Press, Taylor & Francis Group.
528 Sumner, L. W., Mendes, P., & Dixon, R. A. (2003). Plant metabolomics: Large-scale
529 phytochemistry in the functional genomics era. *Phytochemistry*, 62, 818−836.
530 Szyjewicz, E., Rosner, N. & Kliewer, M. V. (1984). Ethephon ((2-Chloroethyl)phosphonic acid,
531 Ethrel, CEPA) in viticulture – A review. *Am. J. Enol. Vitic.*, *35*, 117-123.
532 Wishart, D. S. (2008). Quantitative metabolomics using NMR. *Trends in Analytical Chemistry*,
533 *27*(3), 228–237.

Tables

Table 1. Peak assignment of the metabolites found in 'Crimson Seedless' berries using HRMS in the negative-ion mode.

*Peak label**	*Retention Time [min]*	*[M-H]⁻ m/z*	*Fragments or adducts formed in the MS source*	*MS/MS ions*	*Error (mDa)*	*mSigma Value*	*Formula [M-H]⁻*	*Compound*
Alcohols and derivatives								
1	3.25	383.1560 (100)	-	-	-3.0	33.5	$C_{15}H_{27}O_{11}$	2,3-Butanediol pentoside hexoside
20	7.67	431.1613 (100)	-	-	-5.3	3.3	$C_{19}H_{27}O_{11}$	Benzyl alcohol dihexose
27	8.13	401.1508 (100)	269.1061 (2)	-	-5.4	8.9	$C_{18}H_{25}O_{10}$	Benzyl alcohol hexose-pentose
31	8.29	415.1663 (100)	-		-5.3	24.7	$C_{19}H_{27}O_{10}$	Benzyl alcohol disaccharide derivative
38	8.77	415.1675 (100)	-	-	-6.4	13.7	$C_{19}H_{27}O_{10}$	Benzyl alcohol disaccharide derivative
Hydroxybenzoic acids and derivatives								
2	3.66	331.0716 (100)	-	-	-2.5	30.4	$C_{13}H_{15}O_{10}$	Gallic acid hexose
5	5.39	315.0755(100)	-	-	-2.8	16.1	$C_{13}H_{15}O_{9}$	Dihydroxybenzoic acid hexose
6	6.66	299.0810 (100)	-	137.0217 (100)	-3.7	82.5	$C_{13}H_{15}O_{8}$	Hydroxybenzoic acid hexose
Organic acids and derivatives								
3	3.74	205.0374 (35)	111.0104 (100)	-	-2.0	44.5	$C_{7}H_{9}O_{7}$	Citric acid derivative
4	5.12	279.1119 (100)	-	-	-3.4	15.3	$C_{11}H_{19}O_{8}$	Hydroxyvaleric acid hexose
39	8.85	366.1285 (100)	733.2537 (24)	204.0706 (62); 186.0619 (74); 142.0688 (100)	-5.8	11.0	$C_{17}H_{20}NO_{8}$	Indole-3-lactic acid hexose

Hydroxycinnamic acids and derivatives								
7	6.81	616.1186 (100)	-	167.0157 (81); 149.0121 (100)	-9.6	5.5	$C_{23}H_{26}N_3O_{15}S$	Glutathionyl caftaric acid
9	6.98	646.1653 (100)	-	193.0002 (100)	-10.6	23.5	$C_{25}H_{32}N_3O_{15}S$	Glutathionyl caffeic acid derivative
10	7.12	616.1190 (100)	-	167.0212 (100); 149.0085 (85)	-10.0	4.8	$C_{23}H_{26}N_3O_{15}S$	Glutathionyl caftaric acid
11	7.25	646.1694 (100)	-	193.0004 (100)	-13.4	23.3	$C_{25}H_{32}N_3O_{15}S$	Glutathionyl caffeic acid derivative
14	7.37	341.0927 (100)	161.0280 (20)	161.0284 (49); 133.0324 (100)	-4.9	1.8	$C_{15}H_{17}O_9$	Caffeic acid hexose
19	7.59	327.1144 (100)	165.0593 (47)	-	-3.5	6.1	$C_{15}H_{19}O_8$	Hydroxyhydrocinnamic acid hexose
22	7.72	624.1629 (100)	-	192.9999 (100)	8.7	20.0	$C_{23}H_{34}N_3O_{15}S$	Glutathionylcaffeic acid derivative
24	7.99	325.0984 (100)	163.0445 (10); 145.0325 (38)	145.0389 (54); 117.0360 (81)	-5.3	5.8	$C_{15}H_{17}O_8$	Coumaric acid hexose
28	8.23	325.0983 (100)	163.0527 (6); 145.0322 (19)	145.0365 (54); 117.0364 (100)	-4.5	1.7	$C_{15}H_{17}O_8$	Coumaric acid hexose
29	8.23	355.1085 (100)	193.0557 (5)	160.0206 (100); 132.0208 (59)	-5.0	13.0	$C_{16}H_{19}O_9$	Ferulic acid hexose
32	8.36	355.1089 (100)	193.0567 (34)	160.0200 (100); 132.0208 (35)	-5.4	21.8	$C_{16}H_{19}O_9$	Ferulic acid hexose
Flavanols								
8	6.93	594.1474 (100)	-	321.0486 (100); 183.0161 (82); 167.0322 (71); 137.0225 (70); 125.0259 (65)	-7.5	22.4	$C_{25}H_{28}N_3O_{12}S$	Glutathionyl-catechin
12	7.28	594.1508 (100)	-	321.0501 (65); 183.0170 (92); 167.0386 (100); 137.0225 (87); 125.0265 (96)	-8.0	6.3	$C_{25}H_{28}N_3O_{12}S$	Glutathionyl-catechin
15	7.37	577.1403 (100)	-	-	-5.2	9.4	$C_{30}H_{25}O_{12}$	Procyanidin P2
16	7.42	594.1494 (100)	-	321.0504 (46); 183.0165 (38); 143.0484 (100); 125.0265 (50)	-9.4	7.9	$C_{25}H_{28}N_3O_{12}S$	Glutathionyl-catechin
18	7.54	577.1411 (100)	-	407.0830 (34); 289.0807 (45); 245.0844 (46); 125.0265 (100)	-5.9	2.6	$C_{30}H_{25}O_{12}$	Procyanidin P2
21	7.72	594.1506 (100)	-	321.0501 (46); 183.0153 (50); 143.0487 (100); 125.0265 (56)	-10.7	5.4	$C_{25}H_{28}N_3O_{12}S$	Glutathionyl-catechin
26	8.01	289.0751 (18)		-	-9.9	17.7	$C_{15}H_{13}O_6$	Catechin/Epicatechin
33	8.44	865.2069 (100)	289.0751	-	-8.4	18.8	$C_{45}H_{37}O_{18}$	Procyanidin C1

34	8.50	289.0763 (100)	125.0252 (11)	123.0430 (100); 109.0301 (55)	-4.3	11.0	$C_{15}H_{13}O_6$	Catechin/Epicatechin
37	8.67	865.2069 (100)	289.0751	-	-8.4	18.8	$C_{45}H_{37}O_{18}$	Procyanidin C1
Amino acids								
13	7.32	203.0859 (100)	-	-	-3.3	22.4	$C_{11}H_{11}N_2O_2$	Tryptophan
Terpenoid derivatives								
17	7.42	443.1988 (100)	-	-	4.1	9.3	$C_{21}H_{31}O_{10}$	Dihydrophaseic acid hexose
30	8.29	429.2187 (100)	-	205.1624 (81); 153.0971 (93); 113.0246 (100)	-6.9	15.4	$C_{21}H_{33}O_9$	Tetrahydroabscisic acid hexose
Flavonols								
23	7.84	481.1429	-	177.0176 (92); 151.0420 (91); 137.0275 (99); 109.0301 (51)	-6.5	5.4	$C_{22}H_{25}O_{12}$	Myricetin derivative
Anthocyanins								
25	7.99	461.1161 (100)	-	283.0299 (100)	-7.2	13.3	$C_{22}H_{23}O_{11}$ **	Peonidin-3-O glucoside
41	9.25	463.0959 (100)	-	301.0336 (27); 271.0309 (100); 255.0347 (54)	-7.7	6.1	$C_{21}H_{19}O_{12}$ **	Delphinidin-3-O-glucoside
43	9.63	477.1116 (100)	-	314.0507 (45); 285.0395 (59); 271.0312 (100); 243.0365 (92);	-7.7	33.9	$C_{22}H_{22}O_{12}$ **	Petunidin-3-O-glucoside
Flavanonols								
35	8.53	449.1138 (100)	-	-	-4.0	14.0	$C_{21}H_{21}O_{11}$	Taxifolin-deoxy hexoside A/B
36	8.60	479.1142 (100)	-	-	-6.8	27.8	$C_{22}H_{23}O_{12}$	Dihydroisorhamnetin hexoside
42	9.46	449.1126 (100)	-	-	-3.6	10.2	$C_{21}H_{21}O_{11}$	Taxifolin-deoxy hexoside A/B
Other metabolites								
40	9.05	450.1992 (100)	-	143.0468 (100); 128.0388 (49)				Gluthationyl derivative

* Peak label assigned according to overall temporal elution order.

** $[M-2H]^-$.

Table 2. Peak assignment of selected metabolites in 'Crimson Seedless' samples using ^{1}H-NMR.

Metabolite	*Group*	*Multiplicity*	*$\delta^{1}H$ (ppm)*
Acetic acid	CH_3	s	2.03
Alanine	CH_3	d	1.47
γ-Aminobutirric acid (GABA)	αCH	dd	3.04
Arginine	βCH_2	m	1.89
	γCH_2	m	1.68
Ethanol	CH_3	t	1.17
Fructose		d	4.10
		m	4.03
		m	3.99
α-Glucose	C^1H	d	5.22
β-Glucose	C^1H	d	4.63
	C^2H	dd	3.23
Isoleucine	CH_3	m	0.95
Lactic acid	CH_3	d	1.32
Leucine	γCH_3	t	0.96
Malic acid	βCH	dd	2.59*
	β'CH	dd	2.79*
	αCH	dd	4.38*
Proline		m	2.00
		m	2.33
Tartaric acid	CH	s	4.38*
Valine	γ'CH_3	d	0.99
	γCH_3	d	1.04

* Values variable in function of ion strength of the samples. (s = singlet; d = doublet; t = triplet; m = multiplet).

Figure Captions

Figure 1: Plastic net used to collect berries spontaneously dropping from pedicel before harvest.

Figure 2: a) PC1/PC2 scores plot and b) PC1/PC2 loadings plot obtained by HRMS data of samples collected during 2010 (HRMS detection in negative ion mode).

Figure 3: PC1/PC2 a) score and b) loadings plot obtained by NMR data of grape samples collected during 2010.

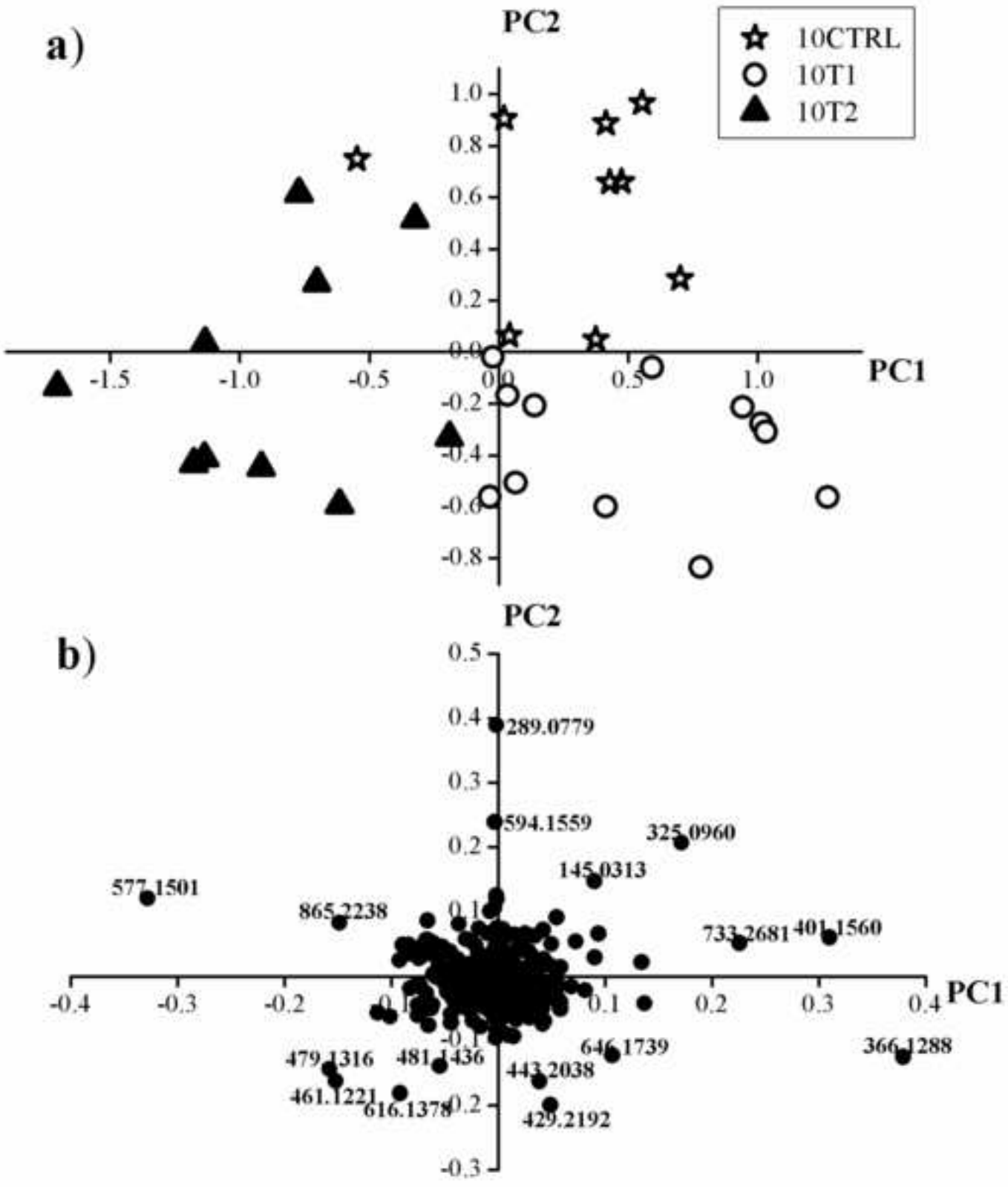
Figure 4: a) HRMS(-)-NMR and b) HRMS(+)-NMR covariance plot of grape samples collected during 2010.

Figure(1)

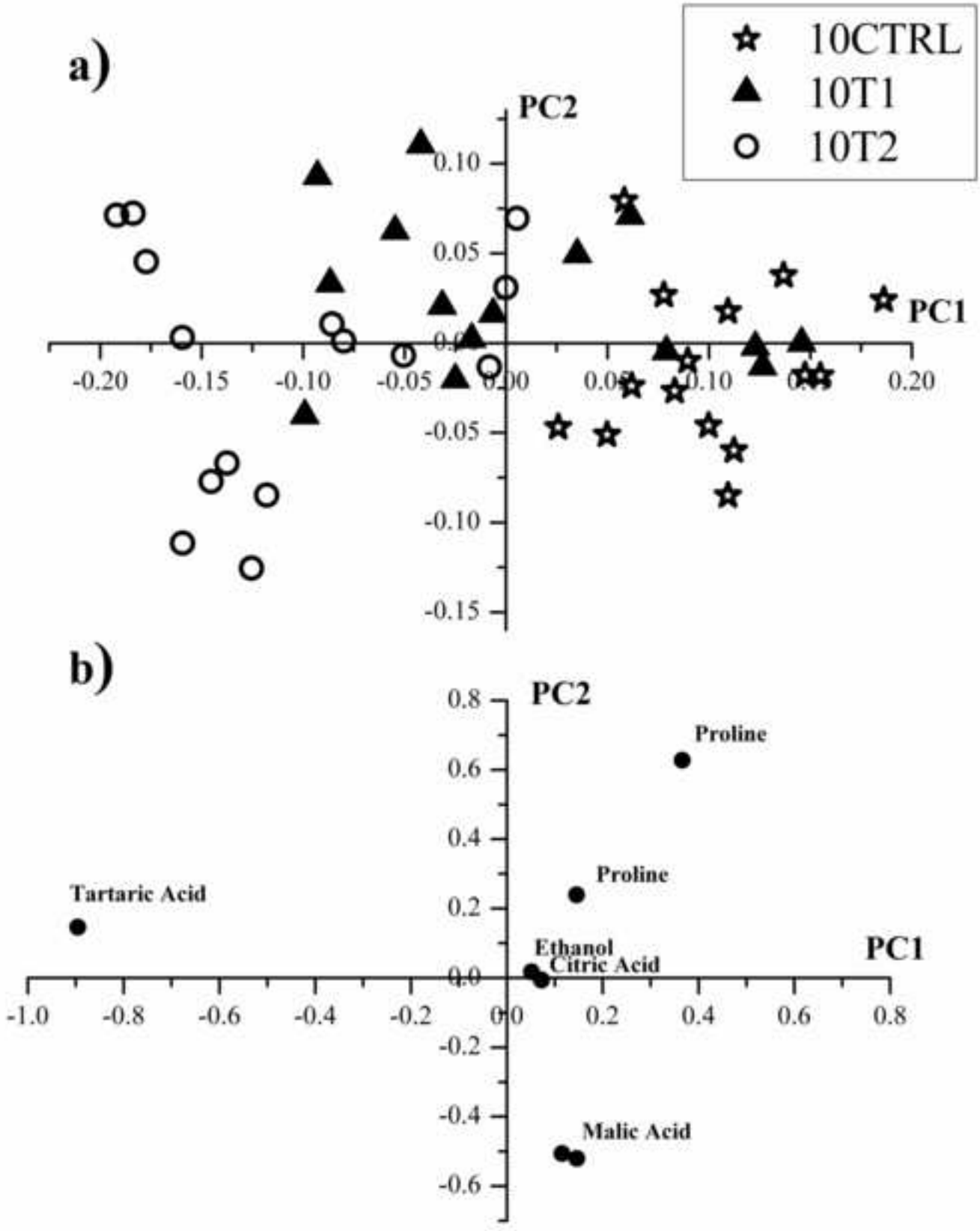
Click here to download high resolution image



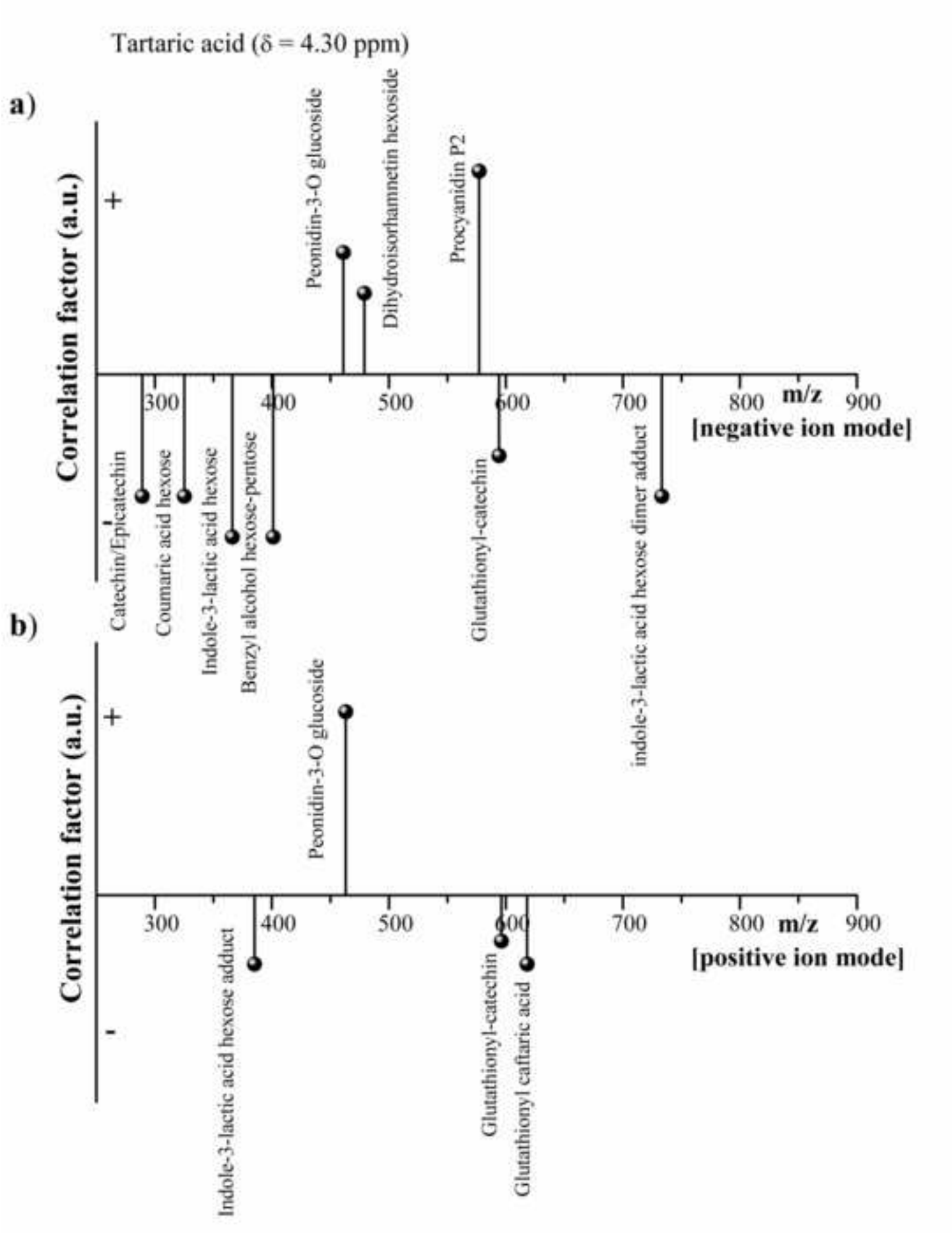
Figure(2)
Click here to download high resolution image



Figure(3)
Click here to download high resolution image



Figure(4)
Click here to download high resolution image



Supplementary Material

Click here to download Supplementary Material: Supplementary data.doc