Metabolic profile comparison of fruit juice from certified sweet cherry trees (Prunus avium L.) of Ferrovia and Giorgia cultivars: A preliminary study

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Abstract

Sweet cherries are widely appreciated for fresh consumption as well as for production of juices, jams, jelly fruits and alcoholic beverages. The sweet cherry intake (as fresh fruit and related products) is extensively encouraged for their taste and nutritional qualities, due to the presence of water-soluble (C, B) and fat-soluble (A, E and K) vitamins, carotenoids, polyphenols and minerals, as well as glucose and fructose. However the market often endorses the consumption of a particular sweet cherry cultivar (as for most of vegetables) essentially for organoleptic and/or external appearance rather than nutraceutical qualities. In order to evaluate the potential difference in the nutritional quality of fruits, 56 sweet cherry juice samples from certified trees (Prunus avium L.) of two cultivars (30 from Ferrovia and 26 from Giorgia), grown in the same pedoclimatic Apulian region, were analyzed by 1H NMR spectroscopy and Multivariate Analysis (MVA). Interestingly, despite the usually lower commercial value with respect to the Ferrovia, Giorgia cultivar shows higher content of malic acid and phenolic compounds with important well known nutraceutical properties such as antioxidant activity and stimulating metabolism.

Graphical abstract

An untargeted 1H NMR based MVA comparison of the metabolic profiles of two typical Apulian sweet cherry cultivars, Ferrovia and Giorgia.
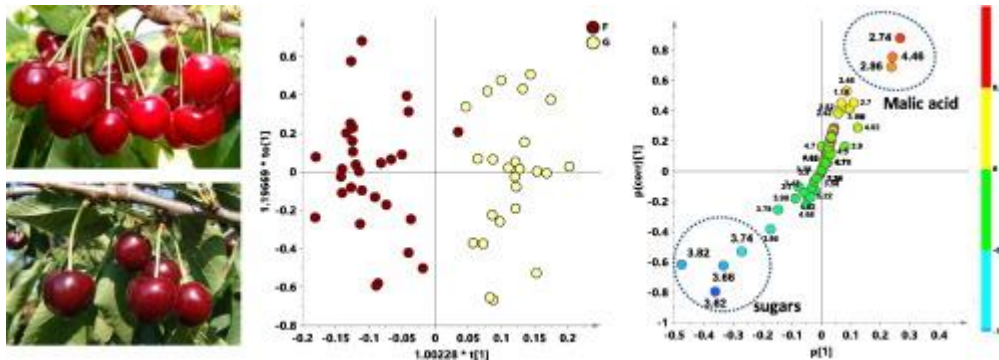
Keywords

Sweet cherryFerroviaGiorgiaNMR spectroscopyPCAOPLS-DA

1. Introduction

Sweet cherries are among the most popular temperate fruits and are widely appreciated for fresh consumption, production of juices, jams, jelly fruits and alcoholic beverages. In numerous production areas, sweet cherries are the first fresh fruits of the season, consumed mainly as no processed (Usenik, Fabčič, & Štampar, 2008). Sweet cherries are rich in various nutrients and phytochemicals also with important antioxidant activity. The organic acids and sugar content (the main soluble constituents of berries in general) determines the organoleptic properties of cherries such as ripeness and taste, or even represent an index of consumer acceptability (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012). In particular, the high content of sugars (glucose and fructose) determines sweetness that, together with skin color, are known to influence sweet cherry consumer acceptance both as fresh fruit and related

products (Crisosto, Crisosto, & Metheney, 2003). The presence of organic acids, in particular malic acid, also causes the acidity of the juice, keeping the organoleptic properties stable and avoiding fermentation processes (Xie, Ye, Donghong, & Ying, 2011). On the other hand, various phenolic compounds such as hydroxycinnamic and hydroxybenzoic acid and their derivatives, anthocyanins, flavonols, procyanidinis contribute to total antioxidant activity, as reported in literature (Usenik et al., 2008, Longobardi et al., 2013, Ballistreri et al., 2013, Mikulic-Petkovsek et al., 2012, Goulas et al., 2015, Crupi et al., 2014). It has been also demonstrated that sweet and sour cherry consumption can reduce the risk of many diseases outbreak as cancer, arthritis and neurovegetative afflictions (Ballistreri et al., 2013 and reference therein). In addition, other important components of sweet cherry are represented by hydrosoluble (C and B) and liposoluble (A, E and K) vitamins, carotenoids and minerals (Calcium, Magnesium, Phosphorus and Potassium) (Longobardi et al., 2013, Crupi et al., 2012).

In the world, the sweet cherry production is estimated at about 2294.455 t, according to the FAO dataset (FAO, 2013). With 131.175 t of sweet cherries, Italy is one of the top five producers in the world, following Turkey, the top worldwide producer, (494.325 t), United States of America, (301.205 t), Iran, (200.00 t) and followed by Uzbekistan (100.00) (FA0, 2013). In Italy sweet cherries are a very important commercial fruit. The Apulia Region is the top producer with about 62% of harvesting area (18.500 ha) and 33% of product (38.152 t) on a total of 114.738 t of 2014 Italian production (ISTAT, 2014). Among sweet cherry Italian cultivars, Apulian Ferrovia and Giorgia are considered the most representative, together with Bigarreau, Black and Anella (Longobardi et al., 2013). Ferrovia is the typical Apulian cultivar, originating from the area of Bari but actually diffused in various other geographical regions, Italian (as Verona, Veneto Region) or not Italian, as Greece (Vavoura, Badeka, Kontakos, & Kontominas, 2015), due to its adaptability to new environment and the exquisite characteristic of fruits (Istituito Sperimentale di Frutticoltura, Provincia di Verona, 2015). Giorgia cultivar has been obtained from a crossing by G. Bargioni, in 1964. Giorgia is actually considered a reference cultivar in Italy due essentially to: i) medium-early maturation of its fruits; ii) adaptability to different pedoclimatic conditions; iii) abundant and stable fructification (Istituito Sperimentale di Frutticoltura, Provincia di Verona, 2015).

Despite Ferrovia and Giorgia are considered the most representative Apulian sweet cherry cultivars, a comparison of their 1H NMR metabolic profiles has never been performed. In a recent work (Longobardi et al., 2013), a 1H NMR based MVA fingerprinting was used for the comparison of two geographical origins for several undifferentiated Italian cultivars. Among these cultivars, Ferrovia and Giorgia were considered and treated as a single Apulian class in order to characterize different geographical origins. In the present work, the differences in the metabolic profiles and potential nutritional quality of the two different cultivars (Giorgia and Ferrovia) were evaluated for the first time by 1H NMR-based MVA methods.

2. Materials and methods

2.1. Sample collection

A total of 90 sweet cherry fruits (nine groups each consisting of ten cherries evenly sampled from each tree) were harvested from four Giorgia and five Ferrovia cultivar nine years old trees (nursery certified) in the same pedoclimatic area (Conversano, South East of Bari Province, Apulian Region). Six technical replicates were obtained from each of the Ferrovia groups, reaching a total of 30 Ferrovia juice samples. In order to obtain a comparable number of Giorgia juice samples six replicates were obtained from two of the four Giorgia groups and seven replicates from the other two, reaching a total number of 26 Giorgia juice samples. A total of 56 juice samples (30 from Ferrovia and 26 from Giorgia) were therefore prepared from the collected fruits. The fruits were harvested at commercial maturity, in the year 2015, from the third week of May (Giorgia) to the third week of June (Ferrovia). The collected fruit samples were cooled in a few hours and transported to laboratory assuring the maintenance of the cold chain. Subsequently, cherries were washed with water, kept frozen and stored at − 20 °C in a freezer.

2.2. Sample preparation for NMR analysis

Cherries were defrosted and, after removal of pit, juices were obtained by squeezing with a 23 mm Potter-Elvehjem homogeneizer (Kontes Glass Co., Vineland, NJ, USA) and subsequent centrifugation (15 min and 3000 rpm at room temperature). Thereafter, 100 µL of phosphate buffer (1 M KH2PO4, 0.1%, TSP as internal standard, D2O and NaN3), were added to 900 µL of juice for NMR sample preparation. In order to ensure the stability of the chemical shifts, the

pH of each sample was adjusted to the pH value (3.22 ± 0.01) of the juice sample reference, according to Godelmann et al. (2013). From the prepared mixture, 600 μL were filled into a 5 mm NMR tube.

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). All measurements were performed on a Bruker Avance III 600 Ascend NMR spectrometer (Bruker, Karlsruhe, Germany) operating at 600.13 MHz for 1H observation, equipped with a z axis gradient coil and automatic tuning-matching (ATM). A time delay of 5 min was set between sample injection and preacquisition calibrations to ensure complete temperature equilibration (300 K). Experiments were run at 300 K in automation mode after loading individual samples on a Bruker Automatic Sample Changer, interfaced with the software Icon NMR (Bruker). For each sample a one-dimensional NOESY experiment (referred to as 1D-NOESY), including solvent signal saturation during relaxation, mixing time and a spoil gradient, was performed. For each experiment 64 free induction decays (FIDs) were acquired, using a spectral width of 12,019 Hz (20.0276 ppm), an acquisition time of 2.7 s, a relaxation delay of 4 s, and a mixing time of 10 ms. The resulting FIDs were multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz before Fourier transformation phasing, and baseline correction. All spectra were referenced to the TSP signal (δ = 0.00 ppm). NMR data were processed using TopSpin 2.1 (Bruker). The metabolites were assigned on the basis of 2D NMR spectra analysis (2D 1H Jres, 1H COSY, 1Hsingle bond13C HSQC and HMBC) and by comparison with published data (Hou et al., 2008, Clausen et al., 2011, Longobardi et al., 2013, Goulas et al., 2015, Barclay et al., 2012, Gabriel et al., 2013).

2.3. NMR data processing and chemometric analysis

1H NMR spectra were segmented in rectangular buckets of fixed 0.04 ppm width and integrated using the Bruker Amix 3.9.13 (Bruker, Biospin) software (bucket tables reduced spectra are reported in Supplementary information as Tables S1 and S2). The spectral region between 4.72 and 5.1 ppm was discarded due to the presence of residual water signal and the remaining 223 buckets in the range 10.00–0.50 ppm were then normalized to total area and mean-centered. Then, the Pareto scaling method, which is performed by dividing the mean-centered data by the square root of the standard deviation, was applied to the variables (van den Berg, Hoefsloot, Westerhuis, Smilde, & van der Werf, 2006). The data table generated with all the spectra was processed by Multivariate Statistical Analysis, using Simca-P version 14 (Umetrics, Sweden). In particular, unsupervised Principal Component Analysis (PCA), supervised Partial Least Squares Discriminant Analysis (PLS-DA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) pattern recognition methods were performed. PCA is at the basis of the multivariate analysis (Jackson, 1991). This method can extract and display the systematic variation in a data matrix X formed by rows (the considered observations), and columns (the variables describing each sample i.e. in our case the buckets originating from each NMR spectrum). The PLS-DA method was performed in order to justify the number of t latent variables used in OPLS-DA model. OPLS-DA is a modification of the usual PLS-DA method which filters out variation that is not directly related to the focused discriminating response. This is accomplished by separating the portion of the variance useful for predictive purposes from the not predictive variance (which is made orthogonal). The result is a model with improved interpretability. Furthermore, OPLS-DA focuses the predictive information in one component, facilitating the interpretation of spectral data. The quality of the models was described by R2, Q2 values and p[CV-ANOVA]. The PLS-DA and OPLS-DA models were validated using internal cross-validation default method (7-fold) and further evaluated with permutation test (400 permutations) of SIMCA-P software (Loo et al., 2009, Holmes et al., 2008, Trygg and Wold, 2002).

Whenever possible, the change in discriminating metabolite content (identified by NMR based untargeted MVA) among the two cultivars was determined by analyzing the integrals of selected distinctive unbiased NMR signals after spectra normalization (to the total spectrum excluding the residual water region) (Ghini et al., 2015), using TSP for chemical shift calibration and quantification (Cazor, Deborde, Moing, Rolin, & This, 2006). This was the case of malic acid (4.46 ppm), α-d-glucose (5.23 ppm), β-d-glucose (4.65 ppm) and α/β-d-fructofuranose (4.10 ppm) (Goulas et al., 2015). The normalized median intensity of the selected signals in the spectra of the two groups were calculated after removal of very limited number (2 Ferrovia and 1 Giorgia samples) of occurring far outliers (Reimann, Filzmoser, & Garret, 2005). Metabolites differences were represented as Log2 fold change (FC) ratio of the calculated median intensities of the corresponding selected signals (Ghini et al., 2015). Results were validated by Kruskal-Wallis significance testing, using the R statistical environment, Version 3.2.4, on a 64bit Windows machine (R Development Core Team, 2011). The levels of statistical significance were at p-values < 0.05 with 95% confidence level. On the other hand specific metabolite assignment was not possible for the polyphenols in the juice samples, since selective extraction is usually required for this task (Goulas et al., 2015, Clausen et al., 2011).

3. Results and discussion

In order to investigate their possible different nutraceutical properties, the metabolic profile comparison of the Ferrovia and Giorgia fruit juices, obtained from cultivars certified trees, was performed by 1H NMR spectroscopy and MVA. A typical 600 MHz 1H 1D-NOESY NMR spectrum of sweet cherry juice is reported in Fig. 1 and the peaks of the main metabolites are labeled Sugars and organic acids characterize the aliphatic region (middle and low frequencies, from 5.5 to 0.5 ppm) whereas phenolic compounds are typical for the aromatic region (high frequencies, 9.0–6.0 ppm). Relevant 1H NMR data are reported in Table 1.
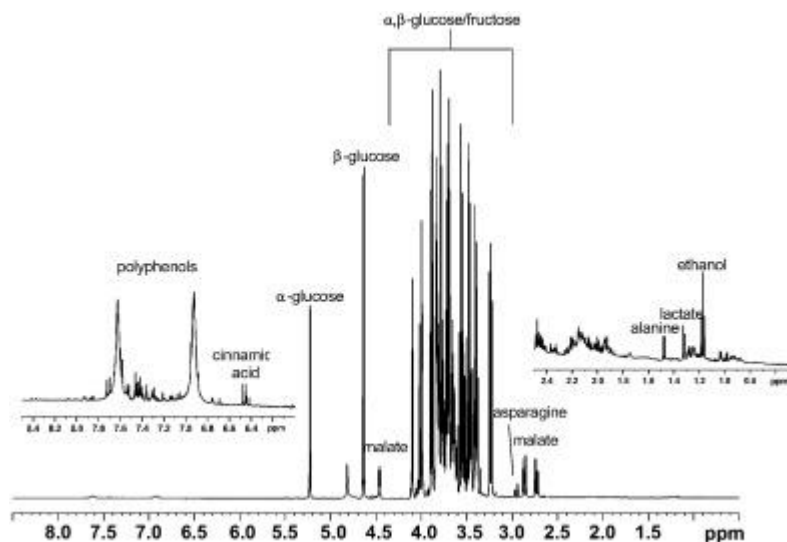

Fig. 1. 1H 1D-NOESY NMR spectrum of a sweet cherry juice sample. The peaks of the main metabolites are indicated.

Table 1. Chemical shift (δ) and assignment of metabolite resonances in the 1H NMR spectrum of sweet cherry juice.

Metabolites	δ (ppm)
Leucine	0.94 (da, CH3), 0.96 (d, CH3)
Isoleucine	0.93 (t, CH3), 1.00 (d, CH3)
Valine	0.98 (d, CH3), 1.03 (d, CH3)
Ethanol	1.17 (t, CH3), 3.65 (q, CH2)
Lactate	1.32 (d, CH3)
Alanine	1.48 (d, CH3), 3.79 (m, CH)
γ-Aminobutyrate	1.93 (m, CH2), 2.41 (t, CH2), 3.02 (t, CH2)
Glutamate	2.09 (m, CH2), 2.15 (m, CH2), 2.45 (m, CH2)
Glutamine	2.14 (m, CH2), 2.48 (m, CH2), 3.77 (m, CH)
Malate	2.74 (dd, CH2), 2.86 (dd, CH2), 4.46 (dd, CH)
Asparagine	2.86 (dd, CH2), 2.95 (dd, CH2), 4.03 (m, CH)
Choline	3.18 (s, N(CH3)3)
Methanol	3.35 (s, CH3)
β-d-Glucose	4.65 (d, CH), 3.48 (t, CH), 3.46 (ddd, CH), 3.40 (dd, CH), 3.26 (dd, CH)
α-d-Glucose	5.23 (d, CH), 3.53 (dd, CH), 3.43 (dd, CH)
α/β-d-Fructofuranose	4.10 (m, 2CH), 4.01 (dd, CH2), 3.70 (dd, CH2), 3.65 (dd, CH2), 3.59 (d, CH2), 3.55 (d, CH2)

Pyrimidine nucleotides   5.85–6.00

Hydroxycinnamic acids   6.43, 6.46, 7.63, 7.60

Fumarate   6.68 (s, CH)

Polyphenols   6.90, 6.95, 7.53, 7.60

Tyrosine 6.95 (m, C3,5H ring), 7.13 (m, C2,6H ring)

Phenylalanine   7.43 (m, C3,5H), 7.37 (m, C4H), 7.30 (m, C2,6H)

Anthocyanins   8.6 (s,H-4)

aLetters in parentheses indicate the peak multiplicities; s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet.

Metabolic profiles of Ferrovia and Giorgia juice samples, obtained by 1H NMR spectroscopy, were therefore studied with multivariate analyses (PCA, PLS-DA, OPLS-DA) performed on bucket reduced 1H NMR spectra. The original dataset (223 buckets from the spectral region 10.00–0.50 ppm) was rearranged in a new multivariate coordinate space in which the dimensions are ordered by decreasing explained variance of the considered data. On the first attempt, in order to reveal a general data grouping of the two sample classes (Ferrovia and Giorgia) an unsupervised PCA analysis was applied to the bucket reduced NMR spectra dataset.

In the PCA analysis three components explained 92.7% of total variance (80.00%, 9.34% and 3.25% for t[1], t[2] and t[3], respectively), describing the samples distribution in the space. The visual inspection of Fig. 2 score plot showed a weak but clear separation for most of the samples along t[2] component. Positive (between 0 and 0.3) and negative (between 0 and − 0.2) values were observed in the score plot for Ferrovia and Giorgia juice samples, respectively (Fig. 2). In order to improve the separation between the two classes, supervised analyses (PLS-DA and OPLS-DA) were performed.
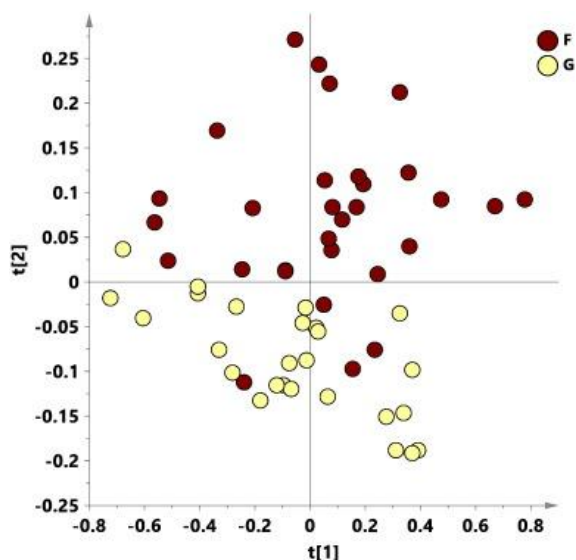

Fig. 2. PCA (t[1]/t[2]) score plot for Ferrovia and Giorgia varieties (three components give R2X = 0.927 and Q2(cum) = 0.891).

By supervised multivariate analytical methods (OPLS-DA), the identity of each group of samples is specified in the model such that the maximum variance of the groups can be attained in the hyperspace. Two performance indicators were used to assess both the supervised model complexity and the eventual overfit degree: the cross validation (CV) and the response permutation test (n = 400).

The OPLS-DA (1 predictive + 3 orthogonal components, R2X = 0.93, Q2 = 0.75, p[CV-ANOVA] = 9 ∗ 10− 12) gave a good model showing a separation among the two cultivars (Ferrovia and Giorgia) along the predictive component t[1] (Fig. 3a). The predictive component accounted for 9.72% of the total explained variance and the uncorrelated (orthogonal)

components to[1], to[2] and to[3] corresponded to 75.8%, 4.94% and 2.48% respectively of the explained variance. By examining the S-plot and the Volcano Plot (Fig. 3b and c) of the original variables it was possible to define the metabolic components distinctive for each cultivar. In particular samples from Giorgia variety were characterized by higher content of malic acid (δH 2.74, 2.84, 4.46), the main organic acid in sweet cherry (Serrano, Guillén, Martínez-Romero, Castillo, & Valero, 2005), whereas Ferrovia samples showed higher level of sugars (δH 3.62, 3.66, 3.74, 3.82). It should be noted that the strongly discriminating sugars signals highlighted by circles in Fig. 3b account for the overlapping multiplets of α- and β-glucose, and fructose, the major sugars in sweet cherry (Goulas et al., 2015). Moreover, the change in discriminating metabolite content (identified by NMR based untargeted MVA) among the two cultivars were determined by analyzing the integrals of selected distinctive unbiased NMR signals after spectra normalization (to the total spectrum excluding the residual water region) (Ghini et al., 2015), using TSP for chemical shift calibration and quantification (Cazor et al., 2006). This was possible for malic acid (4.46 ppm), α-d-glucose (5.23 ppm), β-d-glucose (4.65 ppm), α/β-d-fructofuranose (4.10 ppm). In particular, Log2 fold change (FC) ratio of the normalized median intensity of the corresponding signals in the spectra of the two groups were calculated. A statistically significant higher level of malic acid in Giorgia with respect to Ferrovia cultivar with a concomitant increase (although much less pronounced) of sugars content in Ferrovia with respect to Giorgia samples was found (Fig.4). The trend detected by Log2 fold change (FC) analysis could be also clearly observed by direct comparison of two representative Ferrovia and Giorgia juice samples spectra (Fig. 5). Interestingly, the observed differences are in accord with the only previous paper where the data for some metabolites for the two cultivars (Ferrovia and Giorgia), among several others, have been reported (Ballistreri et al., 2013). However it should be noted that a completely different technique (targeted HPLC analysis) was used to analyze them. It is known that sugars and organic acids are responsible of the main organoleptic properties of cherry fruits. The observed differences in sugars and malic acid content could be considered significant for evaluating the nutraceutical properties of these fruits. The content of organic acids determines the acidity of the fruit juice avoiding fermentation processes and keeping stable the organoleptic properties (Serrano et al., 2005, Usenik et al., 2008). These consist mainly of malic acid, well observed in the present study and accounting for more than 98% of the total content (Ballistreri et al., 2013), but also of citric, shikimic and fumaric detectabled only as very minor components in the NMR spectra of pure fruit juice without selective extraction (Goulas et al., 2015). In our study also ethanol and lactate presence (Longobardi et al., 2013) was observed according with previous findings (but these two metabolites were detected as very minor components. Malic acid is also naturally present in body's cells in which it stimulates metabolism and increases energy production (Xie et al., 2011). Sugars and organic acids ratio is also considered to be at the base of consumer acceptance or preference together with visual appearance (Crisosto et al., 2003).

a)

b)

c)

Fig. 3. a) OPLS-DA t[1]/to[1] score plot for sweet cherry juice samples from Ferrovia and Giorgia varieties. b) S-plot for the model displaying the predictive loading colored according to the correlation scaled loading (p(corr)). c). Volcano plot for the model displaying the predictive loading using a combination of Variables Influence in Projection (VIP) and the p(corr). The labels indicate the metabolite signals (ppm) in the buckets reduced 1H 1D-NOESY NMR spectra.
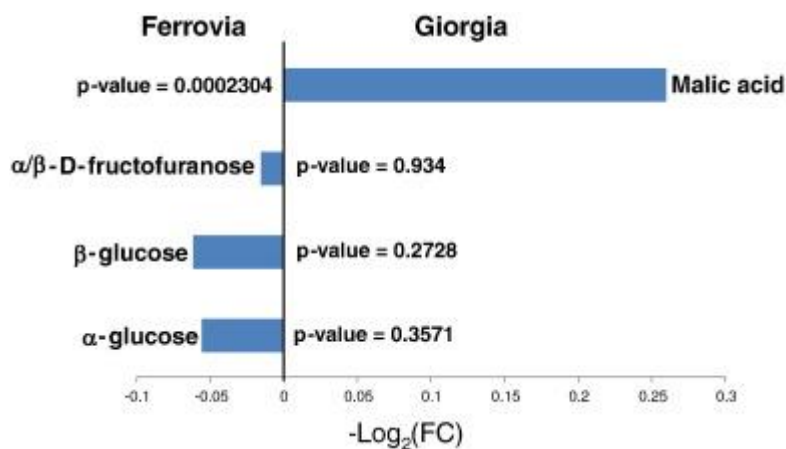

Fig. 4. Discriminant metabolite comparison. The values of $-$ Log2(FC) and the p-values are provided (Kruskal-Wallis test, p-value < 0.05). Metabolites with $-$ Log2(FC) negative values have higher concentration in Ferrovia samples, while malic acid having positive $-$ Log2(FC) value results significantly higher in Giorgia samples (Kruskal-Wallis test with p-value < 0.05).
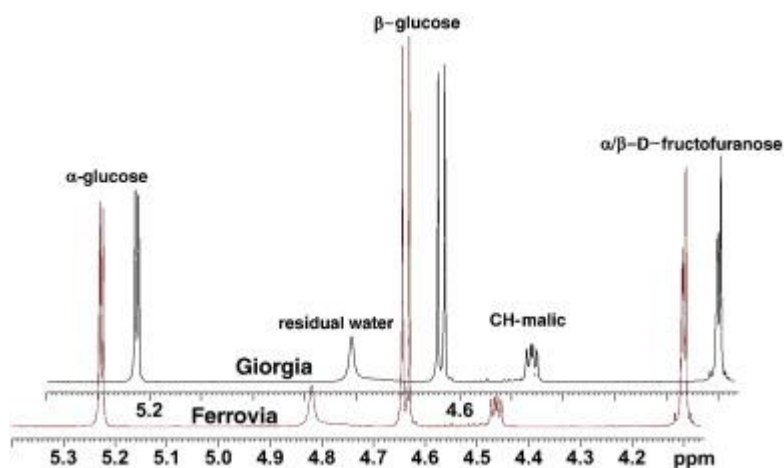

Fig. 5. Representative 1H NMR spectra of Giorgia and Ferrovia juice samples calibrated by matching the TSP intensity. The peaks of the selected discriminating metabolites used in fold change ratio calculation (FC) are indicated.

In order to deeply analyze the potential differences also in the polyphenols content, a new bucketing from 1H 1D-NOESY spectra was further performed considering only the aromatic region between 9.00 and 6.00 ppm. Unsupervised PCA analysis was carried out giving a model where the first three components explained the 99.3% of variance. The t[1]/t[3] scoreplot showed the better separation between the classes (Fig. 6).
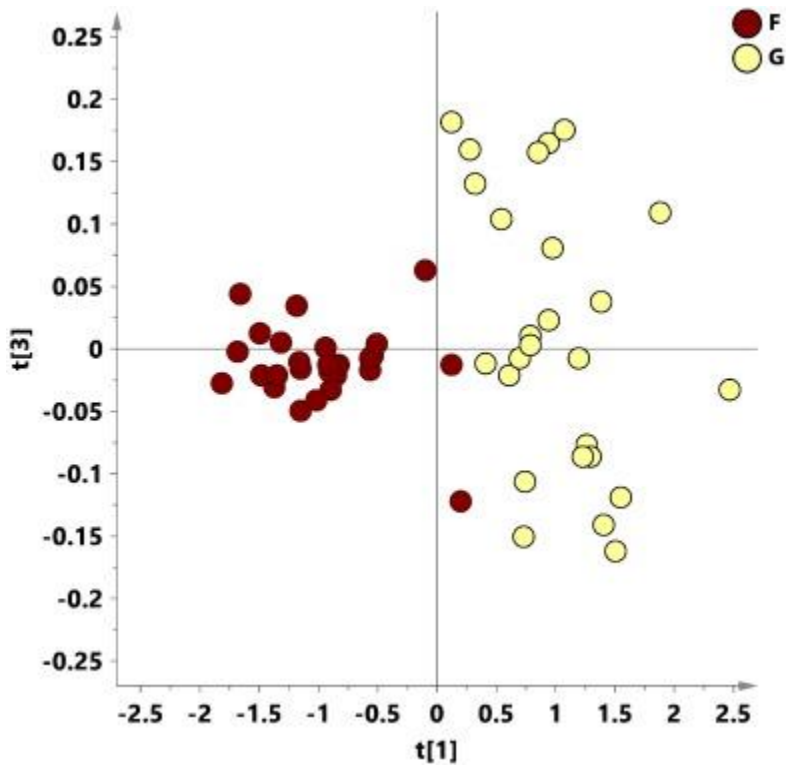
Fig. 6. PCA (t[1]/t[3]) scoreplot for Ferrovia and Giorgia varieties (three components give R2X = 0.993 and Q2(cum) = 0.989).

A supervised OPLS-DA analysis was also accomplished, to improve the partition of the two classes (Ferrovia and Giorgia). In this case, six components (1 predictive + 5 orthogonal components) gave a good model (R2X = 0.998, Q2 = 0.952, p[CV-ANOVA] = 1.99 ∗ 10− 22), showing a clear separation among the varieties (Fig. 7a). The predictive component explained 78.6% of the total variance and the five uncorrelated (orthogonal) components to[1], to[2], to[3], to[4] and to[5] corresponded to 8.51%, 6.01%, 3.52%, 2.64% and 0.57% respectively of the explained variance. When the number of orthogonal components was reduced, to four (R2X = 0.997, Q2 = 0.94, p[CV-ANOVA] = 2.49 ∗ 10− 22) and three (R2X = 0.995, Q2 = 0.899, p[CV-ANOVA] = 2.31 ∗ 10− 19), the decrease in the model quality parameters is negligible). Both the S-plot and the Volcano Plot revealed a higher content of polyphenols (δH 6.9, 6.94, 7.62, 7.66) in Giorgia juice samples (Fig. 7b and c).
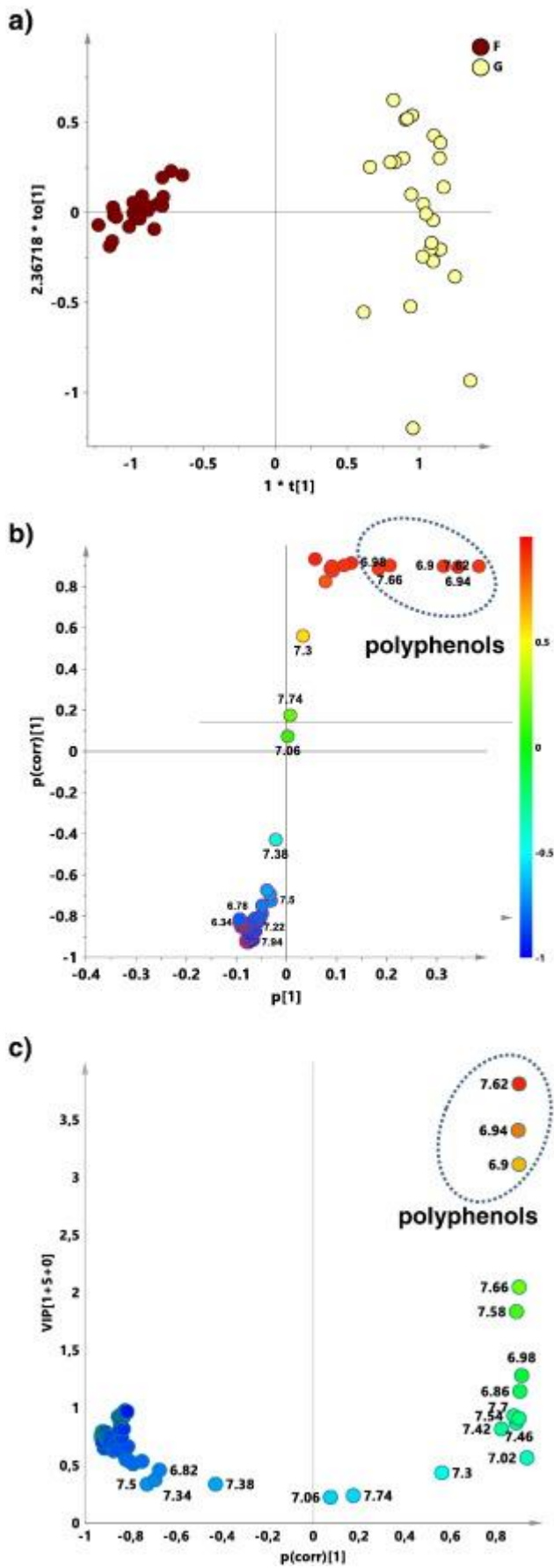
Fig. 7. a) OPLS-DA t[1]/to[1] scoreplot for cherry samples from Giorgia and Ferrovia varieties focusing aromatic spectral region. b) S-plot for the model displaying the predictive loading colored according to p(corr). c) Volcano plot

for the model displaying the predictive loading using a combination of Variables Influence in Projection (VIP) and the p(corr). The labels indicate the metabolite signals (ppm) in the buckets reduced 1H 1D-NOESY NMR spectra.

As previously described in the literature (Clausen et al., 2011, Goulas et al., 2015), polyphenols could be observed also in the Ferrovia and Giorgia juice samples as two broad signals (6.94 and 7.66 ppm) in a peaks overcrowded region of the 1H 1D-NOESY NMR spectra. However, these two peaks are distinctive of several different species generally ascribable to this class of compounds. On the other hand hydroxycinnamic acids (neoclorogenic, p-coumaroylquinic and clorogenic acid at 6.43, 6.46, 7.60, 7.63 ppm) NMR signals could be also detected in the 1H 1D NOESY NMR spectra of the studied samples. In particular, we could discriminate the signals of chlorogenic acid at 6.46 and 6.43 ppm (cross peaks with 7.71 and 7.65 ppm, respectively; J coupling of 16 Hz), on the basis of 2D COSY and J resolved spectrum NMR spectra and in accord with the literature data (INRA Bordeaux-Aquitaine Centre (France), 2015). Anyhow, use of distinctive specific unbiased signals, useful for polyphenols quantitative discrimination, in the cherries juice samples, was not possible, since preconcentration by selective extraction is usually required for this task (Goulas et al., 2015, Clausen et al., 2011). In general, polyphenols, also responsible of the fruit pigmentation, are known to determine the antioxidant activity of sweet cherries, which are therefore considered as a reservoir of bioactive compounds (Ballistreri et al., 2013, Usenik et al., 2008, Goulas et al., 2015). Finally, anthocyanins, important class of antioxidant phenolic compounds, could be also detected in the spectra of cherry juices as a broad small singlet at 8.6 ppm. The same specific signal has previously reported as distinctive of anthocyanins in the 1H NMR spectra in D2O of sweet cherries methanolic extracts (Goulas et al., 2015).

4. Conclusions

In this work we performed for the first time an untargeted 1H NMR based MVA comparison of two typical Apulian sweet cherry cultivars, Ferrovia and Giorgia by metabolic profiling the fruit juices. Despite the usually lower commercial value of Giorgia with respect to the Ferrovia cherries, a higher content of malic acid and phenolic compounds was found in Giorgia juice samples. The observed differences in sugars and malic acid content are in accord with the results of targeted HPLC analyses (Ballistreri et al., 2013), available in the only previous paper where the data for some metabolites for both the two cultivars (Ferrovia and Giorgia), among several others, have been reported. According to the used untargeted MVA procedures, the model validation was assessed by the satisfactory model quality parameters (R2, Q2 values and p[CV-ANOVA]). Nevertheless, it is noteworthy that, in the comparison of the two focused cultivars, the two approaches (1H NMR spectroscopy and Multivariate Analysis (MVA) and the previously reported targed HPLC quantification) gave similar results. Moreover, the use of an untargeted method such as 1H NMR based MVA, proved as a reliable and much faster tool to discriminate the metabolic content of different fruit cultivars. Indeed high-throughput NMR-based methods allow to obtain a simultaneous multiple metabolites snapshot of biological samples, without overlooking or underlooking important discriminating features (Dìaz et al., 2016). On the other hand, the use of 1H NMR data for quantitative comparison of discriminant metabolites is only possible when these latters can be identified by selective unbiased distinctive NMR signals in the mixture spectra. Nevertheless the results of this preliminary work could be also useful to encourage a more informed consumption of sweet cherry fruits based on their possible health beneficial properties beside their organoleptic and/or external characteristics (Crisosto et al., 2003, Chauvin et al., 2009).

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2016.11.014.

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