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RESEARCH

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Biodynamic, organic and integrated agriculture effects on *cv. Italia* table grapes juice, over a 3-year period experiment: an ^{1}H NMR spectroscopy-based metabolomics study

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Abstract

Background The new trend demanding for "natural" agri-food products has encouraged the application of more sustainable and eco-friendly farming methods, which limit or avoid the use of synthetic chemicals. This approach is increasing in viticulture, one of the sectors with the highest commercial value since grapes and derived products are largely consumed foodstuffs, with appreciated nutritional and sensory features. In this work, ^{1}H Nuclear Magnetic Resonance spectroscopy (^{1}H NMR) was applied for the metabolic profiling of *cv. Italia* table grapes samples, from the same origin area, cultivated with different treatments (biodynamic, organic and integrated) and collected in three subsequent vintages. Multivariate statistical analysis was performed on NMR-data with the aim of comprehensively researching the possible influences on metabolites due to the use of diverse agricultural practices.

Results Both inter-annual variability (2020, 2021 and 2022 vintages) and different vineyard treatments (biodynamic, organic and integrated) resulted as significant drivers for samples differentiation in the preliminary unsupervised analysis of the (^{1}H NMR spectra derived) metabolic profile data. Nevertheless, supervised data analyses showed that inter-vineyards variability, due to application of diverse farming methods, had a comparable discriminating effect with respect to harvesting years. Ethanol, sugars (as α-/β-glucose), organic acids (as malate) and amino acids (as arginine, leucine, glutamine) resulted the most viticultural practices-dependent metabolites. Interestingly, results from pairwise comparisons between treatments indicated the biodynamic samples with respect to the organic ones as the best-observed differentiation. This was followed by the biodynamic vs integrated and organic vs integrated samples comparisons, in decreasing discrimination order, as confirmed by the descriptiveness and predictive ability parameters of the corresponding pairwise OPLS-DA models.

Conclusions Results highlighted that metabolites' composition in *cv. Italia* table grapes juice is significantly affected by the use of different kinds of vineyard managements (biodynamic, organic and integrated, here investigated). Metabolomics study, here employing ^{1}H NMR spectroscopy combined with multivariate statistical analysis, offers powerful tools to elucidate the metabolic differences among classes of samples.

Keywords Metabolomics, ^{1}H NMR, Multivariate statistical analysis, Grapes, Juice composition, Agricultural practices, Biodynamic, Organic, Integrated, Vintages

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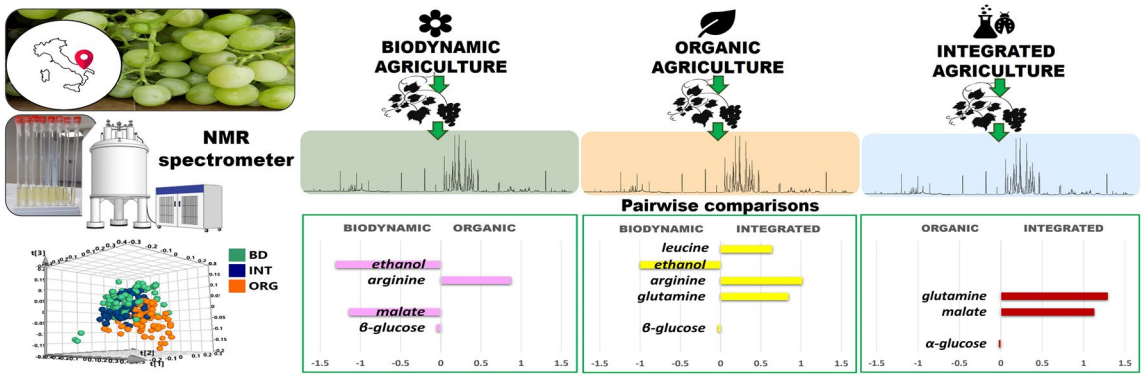
Graphical Abstract



Background

Grapes are considerably appreciated from their consumption as table fresh fruit to the production of wines, juices and jams. Thanks to its interesting versatility, this agri-food product is one of the most widely cultivated crops in the world. Its success is also due to vine's ability to adapt to very different pedoclimatic conditions, allowing it to conquer large areas of cultivation [1]. In 2022, the Italian land intended just for the production of table grapes amounted to 47 583 hectares and the total harvest was of 9 662 593 quintals [2]. Grapes have a great nutritional value thanks to their content in soluble sugars, minerals, nutraceutical and bioactive compounds [1, 3]. Moreover, the specific chemical composition can be influenced by various factors, including the cultivar, the farming method, the local climate [4], the microclimate in the vineyard [5, 6] and the soil features [7–10].

Regarding to farming method, a renewed awareness in consuming quality foods, in preserving both agro-ecosystems health and a good harvest yield, as well as in promoting a sustainable use of soils and resources, encourages study and adoption of environmentally friendly agronomic strategies [11, 12]. This is also a key issue to support the needs of a fast-growing human population [13]. Some of the contemporary regenerative farming methods are biodynamic and organic, which offer more sustainable food production systems in comparison to the industrial model. Industrialized production may obtain high yields over the short term through, for example, soil maximization and the help of chemical compounds. Nevertheless, over the long term, this may occur with high costs associated with losses of soil fertility, biodiversity and crop nutritional quality [14, 15]. The diffusion of more sustainable agricultural practices is a remarkable trend in viticulture [16–18]. These are usually accompanied by other management techniques in the vineyard aimed at improving production (e.g., trunk girdling or thinning of leaves, bunches and berries). Apart from the considered eco-friendlier agricultural methods, including biodynamic and organic, where the use of chemical compounds is strictly limited or banned, other methods seek to use strategies that are as sustainable as possible. One of them is the integrated treatment, which employs a low-pesticide-input pest management [19]. Large differences among vine cultivated with diverse treatments, resulting in a most vigorous growth for integrated protocol, were found by Meissner et al. [20]. Similarly, Döring et al. [21] assessed an increase of growth and yield for the integrated viticulture in comparison to the organic and biodynamic grapevines. These latter appeared characterized by higher disease incidence and severity of downy mildew, which partially accounted for the observed results [21]. By contrast, grapes from biodynamic and organic vineyards seemed to be more resistant to deterioration when compared with those ones from integrated treatment [22]. Furthermore, biodiversity at different trophic levels was improved in organic and biodynamic viticulture compared to integrated cultivation, including an increase in earthworm abundance [20, 23]. However, stimulation of soil nutrients cycling requires years to produce an important effect on N levels and microbial activity in the soil [23]. This is usually accomplished by application of compost, use of varied cover crop mixtures and denial of chemical compounds, as promoted in organic and biodynamic viticulture.

In particular, if biodynamically and organically managed vineyards are compared, Reeve et al. [24] observed no differences in yield, in soil quality and in microbial efficiency. On the other hand, in the biodynamic grapes, a great quality was also specifically found, in terms of

significantly higher levels of soluble solids (i.e., °Brix), total phenols and anthocyanins [24]. Regarding to the physiological responses of grapevines, lower stomatal conductance and lower leaf water potential were found in biodynamic with respect to organic *Vitis vinifera L. cv. Sangiovese*. Moreover, an increase in leaf enzymatic activities of endochitinase, exochitinase (β-N-acetylhexosaminidase and chitin 1,4-β-chitobiosidase) and β-1,3-glucanase, which are typically associated with plant biotic and abiotic stresses and with induced plant resistance, was also observed in biodynamic plants in comparison to those ones organically managed [25].

Influence of different agricultural management systems on the metabolites fingerprint of grapes was confirmed by some studies [24, 26, 27], although other authors did not observe significant variations in the metabolome of samples coming from different viticultural methods [28, 29]. NMR-based metabolomics studies on grapes from diverse agricultural practices are few in literature, mainly concerning winegrapes and wines [30–32]. By ^{1}H NMR, Picone et al. [32] evidenced various winegrape berries (*cv. Sangiovese*) metabolic profiles that could be correlated with the different physiological response of plants to the biodynamic and organic treatments. About table grapes, Gallo et al. [33], using ^{1}H NMR, evaluated the effects of conventional local practices (CONV) and organic management on the chemical composition for *cv. Superior Seedless*, as well as the inter-vineyards variability for *cv. Red Globe* grown with different farming techniques established by each producer. They observed that sugar concentrations were appreciably affected by the application of different cultivation practices, which, instead, resulted to have a less pronounced effect on organic acids content.

High-resolution Nuclear Magnetic Resonance (NMR) spectroscopy is normally combined with multivariate statistical analysis (MVA) in metabolomics studies. NMR spectroscopy is a very powerful tool, generally characterized by rapid sample preparation procedures and extremely reproducible results [34]. Nowadays, high field NMR instruments, gradient assisted and equipped with cryo-probes, also allowed to overcome the original low sensitivity problems with respect to other analytical techniques. Well-suited for metabolomics studies, capturing a "snapshot" of the metabolic profile of the analyzed sample at a given time, NMR spectroscopy provides detailed information on metabolites in complex mixtures. In the food science, NMR offers a wide range of applications: from studies of traceability, authenticity and safety of food [35, 36] to the monitoring of wine alcohol fermentation and aging [34], including also the analysis of a large variety of agri-food matrices [37–44]. The present work proposes a comparative study of metabolic profiles for *cv. Italia* table grapes juice from biodynamic, organic and integrated products, supplied by certified producers. Thus, this study may represent a useful completion of the investigations already carried out, based on the NMR-based profiling of grapes juice [32, 33], including for the first time a direct comparison of biodynamic (BD), organic (ORG) and integrated (INT) agriculture. The general biodynamic definition reported in the present work, specifically refers, for the investigated case, to the organic vineyards certified Reg. CE 848/18 [45] DEMETER production rules [46].

Methods

Origin of grape berries samples and sampling methods

A total of 210 grapes samples was obtained from bunches of *Vitis vinifera cv. Italia* table grapes, harvested at maturation according to the usual commercial practices. Grapes were collected from 2020 to 2022. They came from different specialized Italian farms placed in the same limited geographical area "Masseria Gaudella" included in the countryside of Castellaneta (Taranto province), in the south of Apulia Region (Italy) (Fig. 1a). Their geographic coordinates are: 40°34′46.13″N 16°52′6.20″E (BD farm), 40°34′26.43″N 16°53′0.61″E (ORG farm) and 40°34′52.23″N 16°53′10.57″E (INT farm). The vineyards selected for the study are located within a radius of approximately 1km (Fig. 1b), to homogenize as much as possible the pedoclimatic characteristics of the grapes growing area, as well as to minimize the possible variability effects of both occurring climatic conditions and soil properties on grape metabolism. According to the regions and provinces of Italian soil map [47], the selected vineyards are characterized by similar features with specific homologous pedological classification, as indicated in a detailed portion of the local map [48], more recently confirmed [49] (Additional file 1: Figure S1) and available from the regional database [50]. On the other hand, the soil characteristics could be influenced by the different land use in relation to the agronomic practices adopted, in particular for long-lasting management protocols, as recently demonstrated for the specific area of investigation [51]. The vine training system was a *covered tendone* with a layout measuring 3x3 m. The average of *cv. Italia* yield was, respectively, ca. 20–25 tons ha^{-1} year^{-1} for BD vineyard, ca. 20–30 tons ha^{-1} year^{-1} for ORG vineyard and ca. 35–50 tons ha^{-1} year^{-1} for INT vineyard. The field sampling was carried out during the ripening period of the *cv. Italia* grapes corresponding to the first half of September, collecting two bunches per plant. Selection of the plants to be sampled was randomized (as represented in the scheme, Fig. 1c) to guarantee for each vineyard a representative set of samples in the identified plots. The vineyards were managed according to the biodynamic,

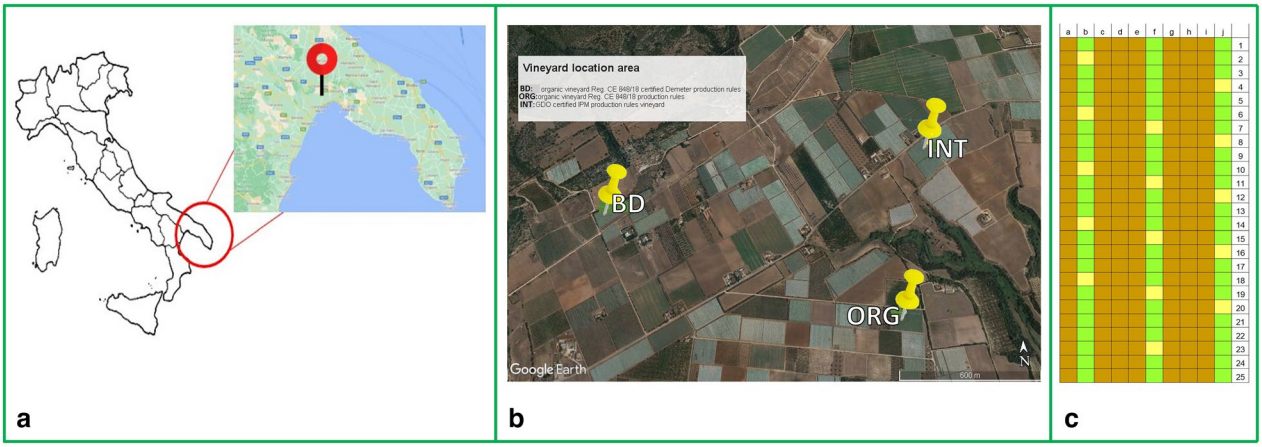


Fig. 1 Geographical area of samples origin, vineyard sites and sampling methods. **a** Map of Italy with place of origin of analyzed table grapes samples; **b** vineyards location area; **c** scheme of field sampling, green = rows of vine plants, yellow squares = sampled plants, brown = inter-row space

organic and integrated farming methods. Supplier farms are certified producers, in particular the biodynamic (BD) farm has organic vineyards certified Reg. CE 848/18 [45] DEMETER production rules [46], organic (ORG) farm has organic vineyards certified Reg. CE 848/18 production rules and integrated (INT) farm has integrated pest management (IPM) vineyards certified GDO production rules [52]. Questionnaires, based on bibliographic research [20–22, 53] and consisting in open/close-ended questions (Additional file 1: Figure S2), were supplied to farms to know detailed information about their viticultural practices (reported in Table 1). Immediately after collecting, the bunches of grapes were transported to the laboratory in three separated lots, respectively, of BD, ORG and INT grapes. In the same day of field sampling, fresh berries were selected for the preparation of samples (consisting in 10–15 berries), which were rapidly stored in freezer at − 20 ℃. For each farming method, berries were randomly selected from the bunches, to be representative of the total studied population and to minimize bias. During the experiment, every harvesting year, homogeneous sample replicates were derived from each provided lot (10 samples for 2020 which were increased to 30 samples for 2021 and 30 samples for 2022), reaching

Table 1 Summary of treatments indicated by supplier farms on questionnaires

Management practices	Biodynamic agriculture	Organic agriculture	Integrated agriculture
Perennial cover crop	• Soil grassed by spontaneous plants • Diverse cover crop mixture (Wolff mixture)	None, field treated with ploughing	None, field treated with herbicides and ploughing
Annual cover crop	• Soil grassed by spontaneous plants • Diverse cover crop mixture	None, field treated with ploughing	None, field treated with ploughing
Under-vine management	Mowing grass	Ploughing	• Herbicides • Ploughing
Fertilization method	• Compost with biodynamic preparations • Compost from manure (approximately 15 m^3/ha every 3 years)	• Ploughing up cover crop • Compost from manure • Commercial compost	• Mineral fertilizers • Synthetic fertilizers • Commercial compost
Plant protection	• Sulphur • Plant strengtheners • Bacillus thuringiensis	• Copper • Wettable sulphur • Plant strengtheners • Spinosad • Bacillus thuringiensis	• Systemic fungicides • Wettable sulphur • Rufast • Tracer • Topas
*Biodynamic preparations**	• n. 500 • n. 500P • n. 501 • n. 502–507 • Compost	None	None

* Biodynamic preparations listed in questionnaires referred to Santoni et al. [53]

a total amount of 30 samples for 2020, 90 samples for 2021 and 90 samples for 2022, as reported in Table 2.

Samples preparation for NMR analysis

From each sample, few grape berries were defrosted and squeezed to obtain the grapes juice. Then, 100 µL of phosphate buffer (1M KH_2PO_4, 0.05 % TSP, trimethylsilylpropanoic acid, as internal standard) and 2mM NaN_3 to prevent microbial contamination, at pH 3.3, according to Girelli et al. [54], were added to 900 µL of juice and subsequent centrifuged (at 10.000 g at room temperature for 5 min) to separate the solid phase from the liquid one. For ^{1}H-NMR direct analysis on juice, 700 µL of the supernatant were used to fill a 5 mm NMR tube.

NMR experiments and data processing

NMR spectra were acquired using a Bruker Avance III 600 Ascend NMR spectrometer (Bruker, Ettlingen, Germany), operating at 600.13 MHz for ^{1}H observation, using a TCI cryo-probe, equipped with a z axis gradient coil and automatic tuning-matching (ATM). After loading individual samples on a Bruker Automatic Sample Changer interfaced with the software IconNMR (Bruker), all the experiments were carried out at a temperature of 300 K in automation mode. An automated procedure consisting of locking, tuning, matching and shimming with a zgcppr Bruker standard pulse sequence was used for the spectra acquisition, alongside NOESY experiment to suppress the residual water signal. A total of 64 transients (with 16 dummy scans) were collected into 64 k data points with relaxation delay set to 5.0 s. A spectral width of 20.0276 ppm (12019.230 Hz) and an acquisition time of 2.7262144 s were used. Free induction decays (FIDs) were Fourier transformed, after being multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz. For every spectrum, the phase was manually adjusted, the baseline was corrected and the calibration was done by aligning TSP singlet to 0 ppm. All acquired ^{1}H NMR spectra were processed by using Topspin 3.5 (Bruker), which also allows for simultaneous visual inspection. The metabolites were assigned to the spectral peaks on the basis of 2D NMR spectra analysis (2D ^{1}H Jres, ^{1}H COSY, ^{1}H–^{13}C HSQC and HMBC) (Additional file 1: Figures S3, S4, S5) and by comparison using Chenomx software (Chenomx Inc., Edmonton, Canada) and literature data [8, 32, 33, 54–58]. The acquisition and processing of 2D spectra were performed as follows: a cosygpprqf Bruker standard pulse sequence with presaturation relaxation delay using gradient pulses for coherence selection allowed to acquire a bi-dimensional ^{1}H COSY spectrum. A spectral width in both dimensions 12,019 Hz (20.028 ppm), 2048 data points in f2, 1024 increments in f1, processed with an unshifted sine-bell squared (QSINE) window function in both dimensions before Fourier transform. An ^{1}H homonuclear J-resolved spectrum was obtained with a spectral width of 12 kHz for f2 dimension and 80 Hz for f1, 8096 data points in f2, 256 increments in f1, processed with zero filling in f1 to 4096 real data points, unshifted sine-bell squared window functions in both dimensions before Fourier transform. The ^{1}H–^{13}C gradient-selected HSQC spectrum was acquired with ^{1}H–^{13}C decoupling, 9 and 37 kHz spectral widths in the ^{1}H and ^{13}C dimensions, respectively, 2048 data points in f2, 256 increments in f1, forward linear prediction with 32 coefficients, zero filling to 4096 data points for the f1 dimension. Unshifted sine-bell squared (SINE) window functions were also applied in both dimensions before Fourier transform. The ^{1}H–^{13}C HMBC spectrum was obtained with 9 and 45 kHz spectral widths in the ^{1}H and ^{13}C dimensions, respectively, 2048 data points in f2, 512 increments in f1, forward linear prediction with 32 coefficients, zero filling to 4096 data points in f1. Unshifted sine-bell squared window functions were also applied in both dimensions before Fourier transform.

Table 2 Summary of analyzed samples divided per year and farming methods

Year	Biodynamic agriculture	Organic agriculture	Integrated agriculture	Total samples
2020	10	10	10	30
2021	30	30	30	90
2022	30	30	30	90

Multivariate statistical analysis on NMR spectroscopy data

The ^{1}H NMR spectra were divided into segments, called buckets or bins, of fixed 0.04 ppm width. The bucketing was performed by Amix 3.9.13 (Analysis of Mixture, Bruker, Biospin, Italy), as the subsequent step in NMR data processing. The spectral region between 4.78 and 5.00 ppm was excluded due to the presence of residual water signal and the remaining 233 buckets in the range 10.00–0.48 ppm were normalized to total area, mean-centered and integrated by Amix software. The total sum normalization was applied to minimize small differences due to sample concentration and/or little changes in experimental conditions among samples. After the alignment of buckets rows reduced spectra, the resulting data table was used in the MVA, employing the tools offered by Simca-P version 14 (Sartorius Stedim Biotech, Umetrics, Umeå, Sweden). In this work, a particularly careful alignment process was required to overcome shifts of some organic acid signals, despite the use of the buffer. Nevertheless, essentially for check purposes, a further data table was produced after manual adjustment of

some buckets by merging, into wider buckets, bins containing signals of tartaric (4.5; 4.46 ppm), malic (2.62; 2.66; 2.70 ppm) and citric (2.74; 2.78 ppm) acids, without any overlapping neighboring resonances [59]. The results from analogous MVA performed on the latter data table did not show significant variations with respect to the conclusions from the statistical analyses on NMR data with regular bucketing (fixed buckets of 0.04 ppm width), here reported.

A data table consists of rows (the observations or samples) and columns (the variables or buckets). Buckets represent the variables used as descriptors for each sample in chemometric analysis. The Pareto scaling method was applied to the variables, which is performed by dividing the mean-centered data by the square root of the standard deviation [60]. Then, the data matrix was analyzed according to unsupervised and supervised pattern recognition methods useful to have an overview of the intrinsic data variation. The first multivariate analysis is the Principal Component Analysis (PCA), an unsupervised method, which provides a summary of all observations, founding groupings, trends, outliers [61]. Starting from the data table, PCA can extract and show the systematic variation in a data matrix X, without using information on group identity to construct the model. Supervised methods, such as the Partial Least Squares Discriminant Analysis (PLS-DA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) are, then, used to discriminate samples grouped in classes with different characteristics. The PLS-DA allows to stress the maximum separation between groups of observations and to obtain information about the variables responsible for the observed separation. It is performed by rotating the main components, that are the axes expressing the variance of the data [62]. A modified PLS-DA method, that is the OPLS-DA, is able to powerfully reveal the most discriminating variables, filtering out variation that is not directly related to the focused discriminating response. For this purpose, the portion of the variance useful for predictive comparisons is separated from the not-predictive, which is, therefore, considered as orthogonal. As a result, the model interpretability is considerably improved [63]. The statistical models were validated using the internal cross-validation default method (7-fold) and the permutation test (100 permutations), available on the SIMCA-P software [64]. In particular, the permutation tests were used to validate the PLS-DA and OPLS-DA models, checking the degree of overfit and the possibilities of false-positive findings. Models were considered valid if: the Q^2 value on the actual data set is higher than all Q^2 values from the permuted data set, as well as the Q^2 regression line has a negative value of intercept on the *y*-axis [65]. There are some parameters useful to evaluate the quality of the MVA models: R^2, Q^2 and p [CV-ANOVA]. The first (R^2) represents the part of the original data that is explained by the model. Therefore, it is a measure of the overall fit of the model. The second (Q^2) is the part of the original data which the cross-validated model explains. It is a measure of the predictive power of the model, describing how well it can predict correct classification for a test sample. In supervised methods, the cross-validated analysis of variance (CV-ANOVA) provides a *p* value, a tool to understand the level of significance of the observed group separation analysis. An overview on these parameters allows to evaluate the model robustness. An ideal model should have closest to unity and similar R^2 and Q^2 values. In a real MVA, the model shows Q^2 value lower than R^2, but if it is particularly lower the robustness is compromised [66]. *Score scatter plot, loading scatter plot* and *S-line plot* are tools available on SIMCA-P software to display the results and they were performed for the developed models. The *score scatter plot* displays the scores, which summarize the relationship among the observations of a model, in a 2D (or 3D) scatter plot revealing the possible presence of outliers, groups, and other patterns in the data. The *loading scatter plot* shows in a 2D (or 3D) scatter plot the contribution of the original variables, describing each sample (in our case the buckets obtained from its NMR spectrum), in determining the sample position in the score scatter plot. The *S-line plot* for an OPLS-DA model with two classes visualizes the centred loading vector p(ctr) colored according to the absolute value of the correlation loading, p(corr). Changes in the metabolites content between two groups of observations can be evaluated as relative quantification. For this purpose, the *Fold Change (FC)* ratios of the normalized median intensity of selected bucket-reduced NMR unbiased signals of discriminant metabolites in the pairwise comparisons of treatment were calculated and reported as Log_2FC [54]. The statistical significance of the differences between the means, for each variable of the two groups, was assessed using the Student *t* test. A *p* value <0.05 (confidence level 95%) was considered statistically significant [54]. In this work, only metabolites with variable importance in projection (VIP) computed from all extracted components and correlation coefficient p(corr) absolute values higher than 1.0 and 0.5, respectively, were considered as potential significant discriminant compounds [67, 68] and taken into account for relative quantification.

Results

Cv. Italia table grapes composition

A representative spectroscopic fingerprinting of *cv. Italia* table grapes juice extracts can be observed in Fig. 2, where one of the acquired ^{1}H NMR spectra is reported. A

summary of the assigned peaks is reported on Table 3. To better visualize the peaks and the metabolites assigned to their relative chemical shifts, three main regions can be identified in the ^{1}H NMR spectra of table grapes juice: the aliphatic, the carbohydrate and the aromatic region [32]. The *aliphatic region* (range 0.9–3.2 ppm) is characterized by resonances mainly ascribable to amino acids and organic acids, which remarkably influence the organoleptic properties of grapes (Fig. 2a). Here, the most intense signals belong to malate, but a great variety of peaks of lower intensity corresponding to valine, leucine, isoleucine, lactate, alanine, arginine, proline, GABA, glutamine and citrate are also present. Moreover, the methyl signal of ethanol is detected, which gives important information about the active metabolic changes in grapes, such as some fermentation pathways of sugars occurring during fruit ripening and responsible for the production of organic acids [3]. The most intense peaks are recorded in the middle field region of the spectrum (range 3.2–5.6 ppm) (Fig. 2b), that is, the *carbohydrate region*. The dominant resonances of the whole spectrum, indeed, belong to sugar compounds that are present in high concentration in the grapes juice extract. Then, the *aromatic region*, ranging from 5.6 to 10 ppm (Fig. 2c), shows signals assigned mainly to aromatic compounds: syringate, pyridine alkaloids as trigonelline, amino acids as phenylalanine and tyrosine. Very important are the peaks associated with polyphenols as the hydroxycinnamic acids that have well-known antioxidant properties [69]. Moreover, the signals ascribable-to-not-aromatic compounds, such as formate and fumarate, are also present.

Statistical analysis

To reveal the possible data grouping of the grapes samples, an unsupervised PCA was applied on the whole *cv. Italia* NMR data set, where all the vintages (2020, 2021 and 2022) were considered. In the present PCA analysis [R^2X (cum) = 0.829, Q^2(cum) = 0.728], five components explained 82.9% of the total variance (38.9%, 23.7%, 9.9%, 5.6% and 4.7% for t[1], t[2], t[3], t[4] and t[5], respectively), describing the samples distribution in the space. The same t[1]/t[2] score scatter plot of the model was labeled according to both vintage (Fig. 3a) and farming method (Fig. 3b). Two main clusters are visible in Fig. 3a: the group of the vintages 2021–2022 shifted at more positive values of t[1] with respect to the group of the vintage 2020 mainly at negative values. Moreover, a further separation of samples can be observed along t[2]: the group of the vintages 2020–2021 at positive values of t[2] and the group of the vintage 2022 at negative values. A less evident clustering according to the different agricultural practices is observed in Fig. 3b. Nevertheless, an indication of differentiation along t[2], between the ORG and INT samples, could be observed in the t[1]/t[2] PCA score scatter plot (Fig. 3b). To visualize the separation of the BD samples with respect to the others, the analysis of the 3D PCA score scatter plot also including t[3] was required (Fig. 3c). Interestingly, at first glance, the observation of the 3D PCA score scatter plot (Fig. 3c) suggests the possible existence of specific characteristics in the metabolic profiles, differentiating essentially BD from ORG samples. Loading scatter plot for the PCA model was also performed, aiming to reveal the metabolites responsible for the observed separation of the samples groups (Additional file 1: Figure S6).

The observed trends (Fig. 3) in the PCA analysis indicated that the inter-annual variability due to the occurring climatic conditions might influence the metabolism of grapes, besides those produced by other factors, such as the application of different cultivation methods. However, to clearly identify and possibly quantify all the differences resulting from the ^{1}H NMR metabolic profiling of grapes juice samples, supervised MVA methods were, therefore, used.

The separation among samples classes could be improved by PLS-DA analysis. Two supervised (PLS-DA) analyses were performed and compared, based on the PCA clustering indications (Fig. 4). One PLS-DA analysis was set on the class "harvesting year" (score scatter plot in Fig. 4a) and the other one was set on the class "viticultural treatment" (score scatter plot in Fig. 4c). The two models, built using five components, were both reasonably good: the first one with R^2X (cum) = 0.789, R^2Y(cum) = 0.742, Q^2(cum) = 0.689; the second one with R^2X (cum) = 0.802, R^2Y(cum) = 0.723, Q^2(cum) = 0.705. Interestingly, the model parameters (descriptiveness/predictiveability) for the differentiation according to "viticultural treatment" were comparable to those characterizing the differentiation according to "harvesting year". Therefore, this result fully justified the further investigations on farming methods effects. Differences in the samples number per class, in the two PLS-DA models, should be also taken into account. Nevertheless, the results clearly indicate that both occurring climatic conditions specific

(See figure on next page.)

Fig. 2 Typical ^{1}H NMR spectrum of cv. Italia table grapes juice sample. Main resonances are labelled with the assigned metabolite. **a** Aliphatic region, mainly characterized by signals of amino acids and organic acids; **b** carbohydrate region, collecting mainly signals belonging to sugars and **c** Aromatic region (approximate zoom factor × 10), where signals principally of phenolic and other aromatic compounds are present

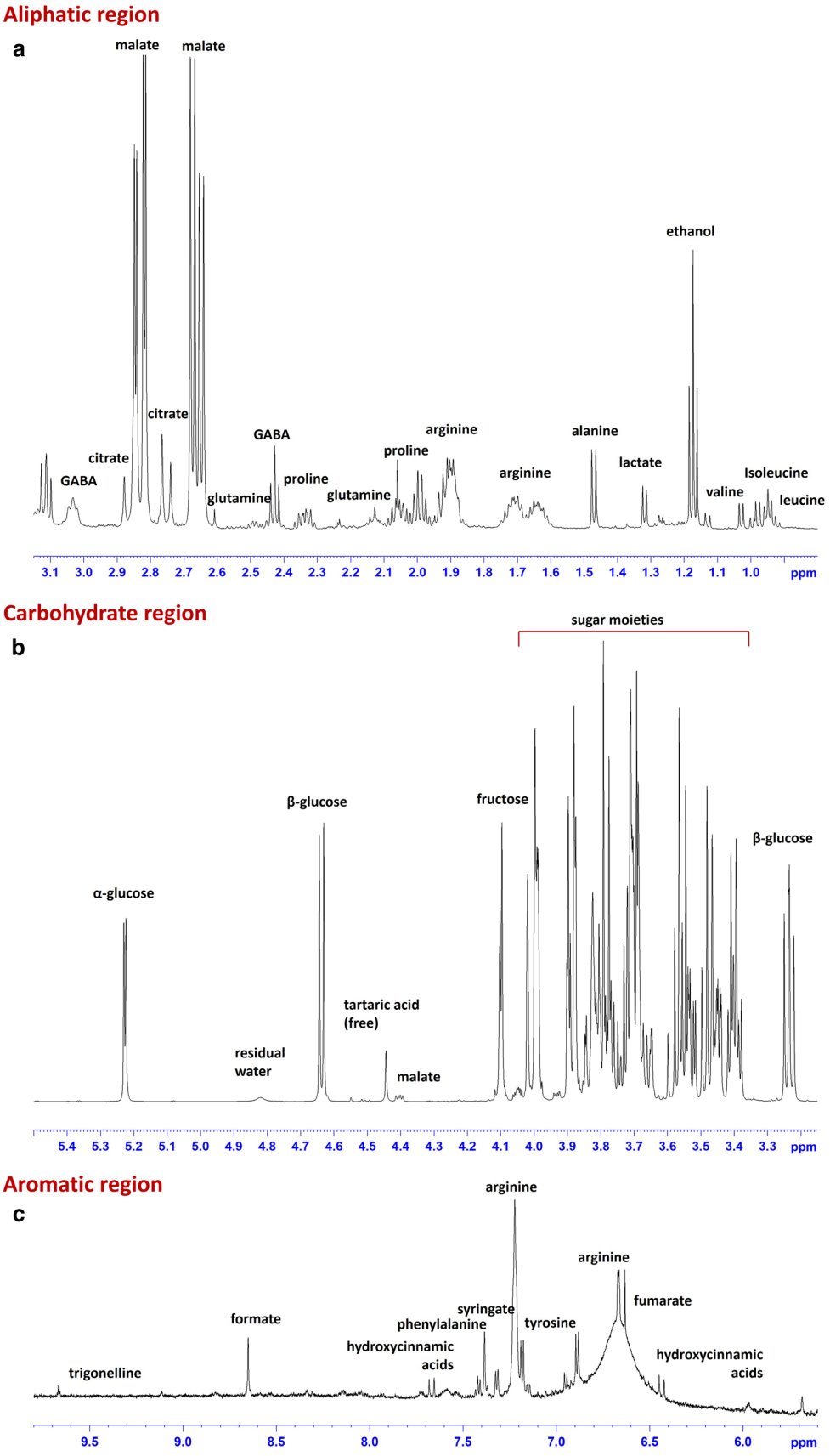


Fig. 2 (See legend on previous page.)

Table 3 [1]H-NMR chemical shift (δ) of metabolite resonances of cv. Italia table grapes juice

Metabolites	δ (ppm)
Leucine	0.94 (d[a]), 0.96 (d)
Isoleucine	1.00 (d)
Valine	1.03 (d)
Ethanol	1.18 (t)
Lactate	1.32 (d)
Alanine	1.46 (d), 3.79 (q)
Arginine	1.61–1.79 (m), 1.88–1.97 (m), 3.24 (dd), 6.52–6.80* (m), 7.21–7.29* (m)
γ-aminobutyrate (GABA)	1.90 (qu), 2.35 (t), 3.02 (t)
Proline	1.97–2.04 (m), 2.05–2.10 (m), 2.32–2.39 (m)
Glutamine	2.11–2.17 (m), 2.44–2.50 (m)
Malate	2.66 (dd), 2.82 (dd), 4.42 (dd)
Citrate	2.78 (d), 2.88 (d)
α-D-glucose	3.43 (dd), 3.50 (dd), 5.22 (d)
β-D-glucose	3.26 (dd), 3.40 (dd), 3.48 (t), 4.66 (d)
Fructose	4.01 (dd), 4.10 (d)
Tartaric acid (free)	4.46 (s)
Hydroxicinnamic acids	6.43 (d), 6.46 (d), 7.60 (d), 7.63 (d)
Fumarate	6.68 (s)
Tyrosine	6.91 (d), 7.19 (d)
Syringate	7.26 (s)
Phenylalanine	7.30 (d), 7.40–7.44 (m)
Trigonelline	8.08 (t), 8.80–8.86 (m), 9.13 (s)
Formate	8.66 (s)

[a] Letters in parentheses indicate the peak multiplicities; s = singlet; d = doublet; t = triplet; q = quartet; qu = quintet; dd = doublet of doublet; m = multiplet

*Multiplets at δ 6.52–6.80 and δ 7.21–7.29 could be assignable to the side chain amide protons of arginine at acidic pH. However, as broad signals they were not considered [58]

for each vintage and viticultural treatments influence the characteristics of the analyzed grapes juice samples, and the observed effects resulted at least comparable in importance. Permutation tests (n=100) were used to assess the validity and the degree of overfit of the two PLS-DA models (Additional file 1: Figure S7a).

Both PLS-DA analyses score scatter plots (Fig. 4a, c) showed a great separation among samples classes. In Fig. 4a, the t[1]/t[2] plot displayed the grapes samples belonging to the vintages 2020–2021 both at positive values of t[1], while the 2022 samples placed at negative values. This means that 2020–2021 grapes samples were more similar to each other with respect to those from 2022. The corresponding loading scatter plot (Fig. 4b) clearly indicates the metabolites responsible for vintages differentiation. In particular, table grapes samples from the 2020 to 2021 vintages appeared richer in α-/β-glucose, malate and citrate than those from 2022, while instead the 2022 samples contained more fructose and alanine.

In Fig. 4c, an important degree of separation among cultivation methods was observed in the t[1]/t[2] score scatter plot, with BD and INT table grapes samples occurring mainly at positive values of t[1] and well-differentiated from ORG samples placed, instead, at negative values. This result confirmed the PCA grouping indication (Fig. 3c). However, there are clearly other intrinsic aspects which determine the mutual differentiation between BD and INT-ORG samples along the t[2].

The PLS-DA loading scatter plot for the model set according to "viticultural treatment" classification shows the discriminating metabolites responsible of the observed separation within the farming methods

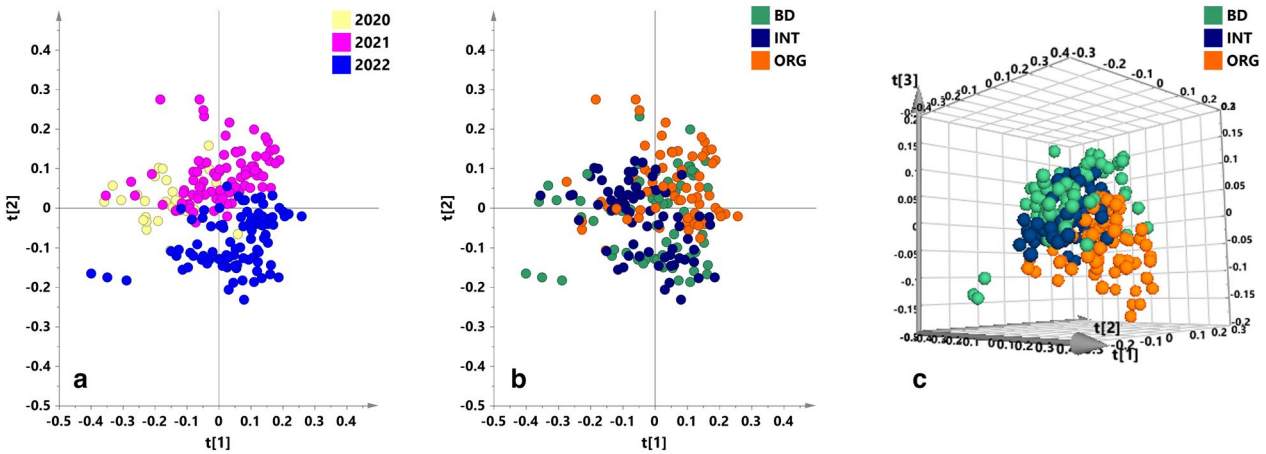


Fig. 3 PCA score scatter plot for all grapes juice samples. Model parameters: five components give R^2X (cum) = 0.829, Q^2(cum) = 0.728; PCA score scatter plot **a** colored by vintage and **b** colored by farming method; **c** 3D PCA score scatter plot; BD = biodynamic, INT = integrated, ORG = organic agriculture

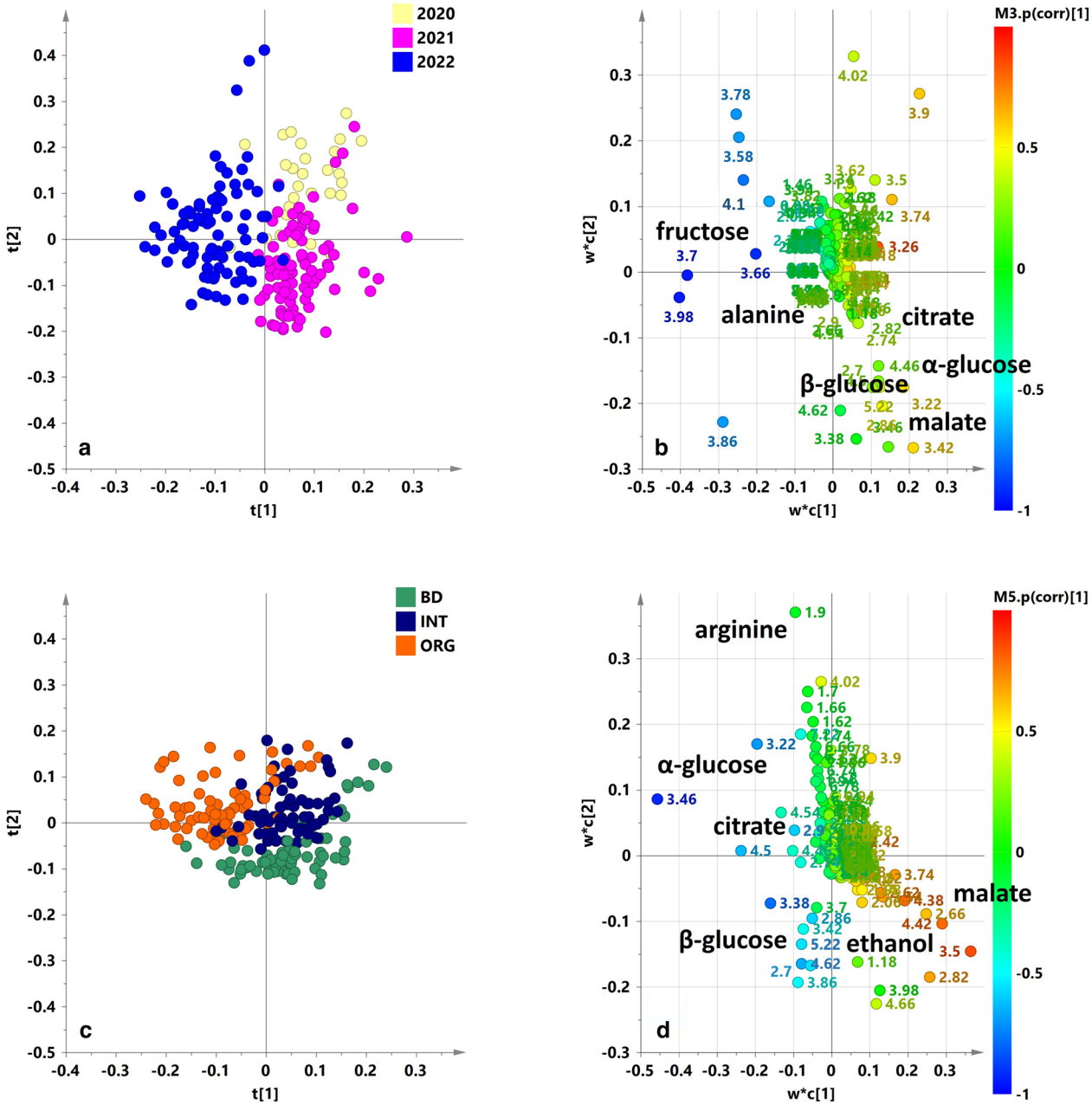


Fig. 4 PLS-DA analyses for all grapes juice samples. **a** PLS-DA score scatter plot from model set on class "harvesting year" (five components give R^2X (cum) = 0.789, R^2Y(cum) = 0.742, Q^2(cum) = 0.689), **b** PLS-DA loading scatter plot from model set on class "harvesting year", **c** PLS-DA score scatter plot from model set on class "viticultural treatment" (five components give R^2X (cum) = 0.802, R^2Y(cum) = 0.723, Q^2(cum) = 0.705) and **d** PLS-DA loading scatter plot from model set on class "viticultural treatment". Loading scatter plots for the models are coloured according to the absolute value of the correlation loading, p(corr). The colour bar associated with the plot indicates the correlation of the metabolites that discriminate the classes. w*c[1] and w*c[2] axes represented the weighted correlation vectors

clusters (Fig. 4d). In particular, a higher relative content of malate and ethanol characterized BD and INT grapes; while citrate, monosaccharides as α-/β-glucose and amino acids as arginine were more abundant in ORG samples. Noteworthy, from the comparative analysis of Fig. 4 loading scatter plots clearly results that the metabolites responsible for "viticultural treatment" discrimination do not correspond to those ascribable to "harvesting year" differentiation. These results further

buttress the need to thoroughly study the specific effects of different agricultural managements.

To better investigate differences of metabolites, which discriminate BD, ORG and INT products, separate pairwise OPLS-DA analyses were performed, setting "viticultural treatment" as classification category (Fig. 5). The score scatter plots of the three obtained models (Fig. 5a, c, e) displayed a good separation degree in each pairwise comparison (BD vs ORG, BD vs INT and ORG vs INT), confirmed by reasonably sound model parameters (Table 4). Remarkably, the BD vs ORG and BD vs INT OPLS-DA pairwise models showed higher descriptiveness and predictive ability parameters with respect to the ORG vs INT model. The present result evidently testifies a better differentiation of BD from both ORG and INT samples, when compared with the ORG vs INT discrimination. Indeed, a greater discrimination of samples usually relates to a higher predictive ability of the

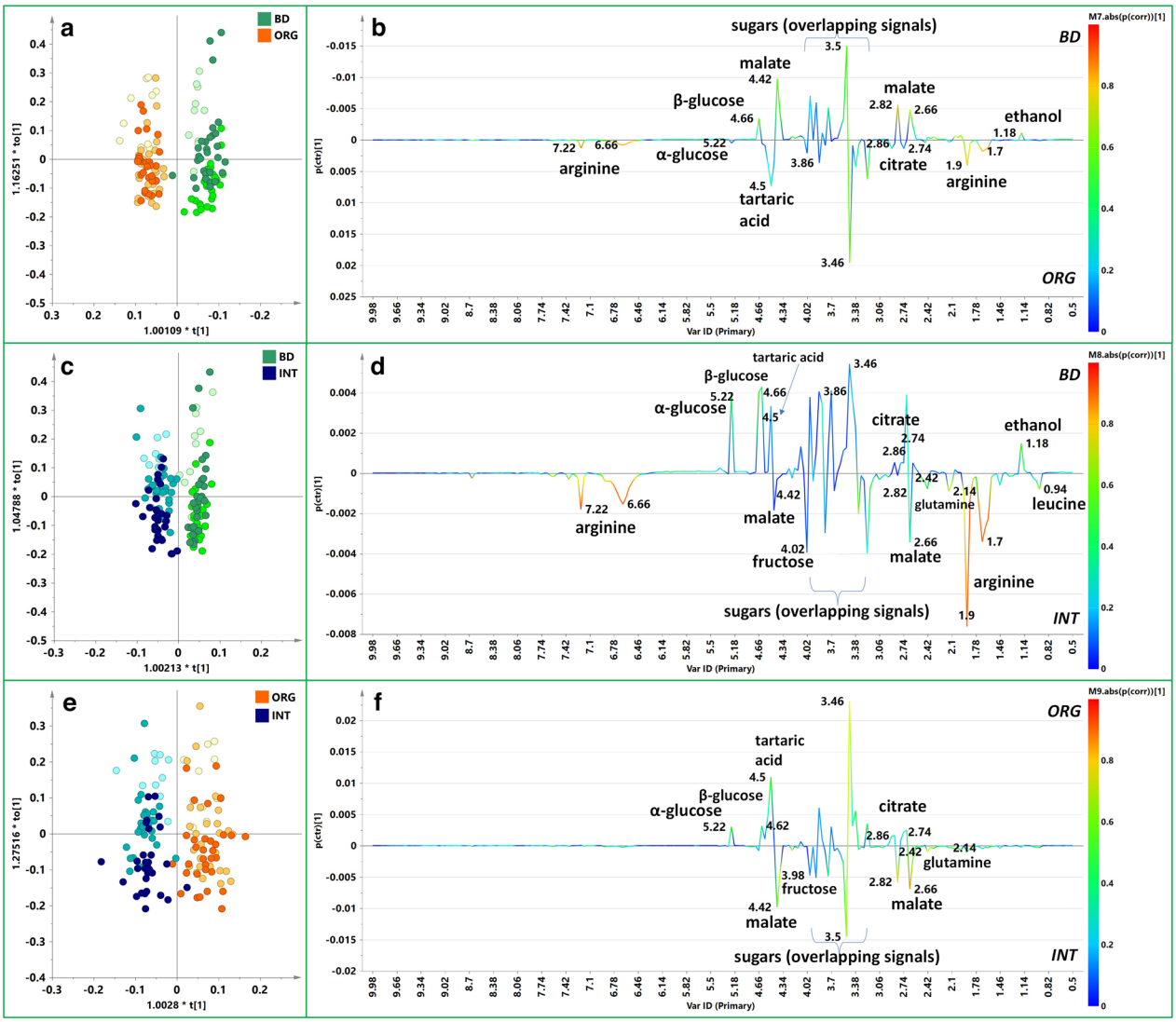


Fig. 5 OPLS-DA analyses, pairwise comparisons. **a** pairwise OPLS-DA score scatter plot (one predictive and four orthogonal components, $R^2X(cum) = 0.83$, $R^2Y(cum) = 0.912$, $Q^2(cum) = 0.889$) for biodynamic (BD) and organic (ORG) treatments, **b** S-line for the model; **c** pairwise OPLS-DA score scatter plot (one predictive and four orthogonal components, $R^2X(cum) = 0.84$, $R^2Y(cum) = 0.874$, $Q^2(cum) = 0.835$) for biodynamic (BD) and integrated (INT) treatments, **d** S-line for the model; **e** pairwise OPLS-DA score scatter plot (one predictive and four orthogonal components, $R^2X(cum) = 0.794$, $R^2Y(cum) = 0.824$, $Q^2(cum) = 0.779$) for organic (ORG) and integrated (INT) treatments, **f** S-line for the model. In the score scatter plots, the colour intensity of the dots increases progressively according to the vintage (light = 2020, medium = 2021, dark = 2022). The S-line plots for the OPLS-DA models visualize the centred loading vector (p(ctr)), colored according to the absolute value of the correlation loading, p(corr).The colour bar associated with the plot indicates the correlation of the metabolites that discriminate the classes

Table 4 Descriptiveness and predictive ability parameters of pairwise OPLS-DA analyses models

Pairwise comparison	R^2X(cum)	R^2Y(cum)	Q^2(cum)
BD vs *ORG*	0.83	0.912	0.889
BD vs *INT*	0.84	0.874	0.835
ORG vs *INT*	0.794	0.824	0.779

One predictive and four orthogonal components were considered for each model

corresponding models in the pairwise OPLS-DA evaluation [70]. Permutation tests (n=100) were used to check the validity and the degree of overfit of the three OPLS-DA models (Additional file 1: Figure S7b).

Interestingly, in all the comparisons (Fig.5), a very low differentiation according to the harvesting years was observed along the orthogonal component of the OPLS-DA score scatter plots. In addition, this result reinforces the already discussed comparable importance of vintages effects and viticultural treatments influences. For the pairwise OPLS-DA models, the corresponding S line plots (Fig. 5b, d, f) clearly indicate the metabolites responsible for BD vs ORG, BD vs INT and ORG vs INT differentiations. In the BD vs ORG comparison, the S-line plot for the OPLS-DA model (Fig. 5b) showed a higher relative content of ethanol, malate and β-glucose for BD table grapes samples with respect to ORG ones. On the contrary, ORG samples contained more arginine, citrate, tartaric acid and α-glucose than BD ones. In the pairwise comparison BD vs INT, the S-line plot for the model (Fig. 5d) indicated a greater abundance of ethanol, citrate, tartaric acid and carbohydrates such as α-/β-glucose in BD samples with respect to INT ones. On the other hand, INT samples were characterized by higher relative content of malate, fructose and amino acids such as arginine, glutamine and leucine. Finally, in the ORG vs INT comparison, the S-line plot for the model (Fig. 5f) pointed out the presence of more citrate, tartaric acid and α-/β-glucose for ORG samples than INT ones. These latter contained, instead, higher quantities of malate, fructose and glutamine.

A complete comparative view of occurring discriminating metabolites' trends in samples, relatively to all the investigated agricultural managements, is summarized in Fig. 6. Potential significant discriminating metabolites were identified using VIP and p(corr) values (Additional file 1: Tables S1, S2, S3). Only assigned metabolites showing a major contribution to the OPLS-DA models, with high statistical reliability $|p(corr)| \geq 0.5$ and strong discrimination power (VIP≥1), were selected. The relative quantification of these significant discriminant metabolites, for BD vs ORG, BD vs INT and ORG vs INT samples classes, was performed by considering the Fold Change (FC) ratio. FCs were calculated from the buckets' integral values of the relevant NMR signals for leucine, ethanol, arginine, glutamine, malate, α- and β-glucose. Significant (p value < 0.05) $\log_2$(FC) values are reported in the graph, as shown in Fig. 6a, b, c.

Discussion

The present study aimed at analyzing the effects of different viticultural treatments on metabolic profile of *cv. Italia* table grapes juice. We report, for the first time in this cultivar, a comparison among samples from biodynamic, organic and integrated agriculture, over a 3-year period, by using an ^{1}H NMR-based metabolomics analysis combined with MVA. Besides the sampling method and the analytical protocol, also other experimental aspects were kept constant over the three considered vintages (2020, 2021 and 2022): geographical area of samples origin, supplier farms and agricultural managements of vineyards. About the management, within each type of cultivation (biodynamic, organic and integrated) the used practices in the vineyard were kept

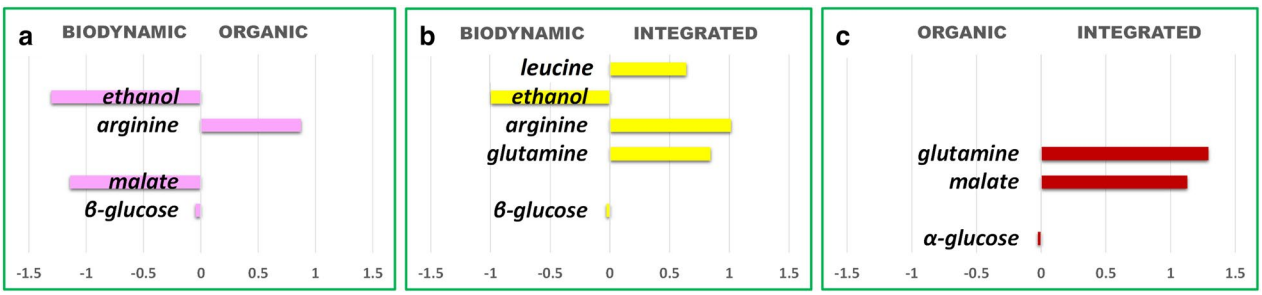


Fig. 6 Graphical representation of discriminant metabolites in each pairwise comparison for the investigated agricultural managements (only metabolites with $|p(corr)| \geq 0.5$ and VIP ≥ 1 were taken into account). Quantitative comparisons are, respectively, between **a** BD/ORG, **b** BD/INT and **c** ORG/INT samples classes. The *x*-axis reports significant (p value < 0.05) $\log_2$(FC) values. (FC = Fold Change, BD = biodynamic, ORG = organic, INT = integrated treatment)

the same and replicated year by year. The preliminary unsupervised PCA analysis, performed on the whole NMR data set, showed that both inter-annual variability (Fig. 3a) and different farming methods (Fig. 3c) resulted as significant drivers for samples differentiation. The two supervised PLS-DA analyses (Fig. 4a and c), respectively, set on "harvesting year" and "viticultural treatment" as classification categories, revealed that BD, ORG and INT cultivations determined discrimination among samples at least to a same extent as the vintages. This evidence emerged from the comparison of models' statistical parameters. The relative loading scatter plots (Fig. 4b, d) highlighted that discriminating metabolites for the observed classes separation were diverse between the two PLS-DA analyses. Thus, vintages and agricultural managements of vineyards seemed to differently affect the *cv. Italia* grape berries metabolic profiles. This aspect, combined with reasonably sound PLS-DA models parameters, buttressed the need to deepen the specific influences of farming methods. Thus, OPLS-DA pairwise comparisons (BD vs ORG, BD vs INT and ORG vs INT) were performed (Fig. 5a, c, e), along with the S-line for each model (Fig. 5b, d, f). As showed by the descriptiveness (R^2) and predictive ability (Q^2) values parameters, the highest discrimination was observed between BD vs ORG samples groups, followed by BD vs INT and, then, ORG vs INT comparisons (Table 4) in decreasing discrimination order. These results suggest that BD agriculture could produce clearly different foodstuffs from those obtained by ORG and INT treatments, which appear more similar to each other. Then, the relative quantification of discriminant metabolites ($|p(corr)| \geq 0.5$ and $VIP \geq 1$) was carried out by considering the Fold Change (FC) ratio for each pairwise comparison (Fig. 6a, b, c).

In the case of BD vs ORG comparison (Fig. 6a), significant relative content changes between samples groups from the two considered agricultural practices resulted for β-glucose, ethanol, malate and arginine. In particular, β-glucose, ethanol and malate relative contents were significantly higher in BD samples than ORG ones with 0.05-, 1.30- and 1.14-fold increases, respectively. With the exception of the sugars, similar findings were reported by Picone et al. [32] in *cv. Sangiovese* winegrapes regarding trends in ethanol and malate contents. The concomitant relative increase of the latter would suggest the activation of glycolysis toward fermentative metabolic pathways mostly in BD products [32]. Furthermore, the resulting greater abundance of arginine (0.87-fold increase) in ORG samples compared to BD ones is an outcome dissimilar to that described by other authors [32], who also observed an increased concentration of some amino acids in BD grapes with respect to ORG ones.

In the BD vs INT comparison (Fig. 6b), similarly as observed in the previous comparison (BD vs ORG), BD samples showed a statistically significant higher relative content of β-glucose and ethanol (0.03- and 1-fold increases, respectively) with respect to INT samples. In the case of β-glucose similar results was obtained, although not statistically meaningful, in the roots of red beet *Beta vulgaris* from BD agriculture with respect to both INT and ORG [71]. On the other hand, the large relative content of ethanol in BD samples, with one-fold increase respect to INT ones, is noteworthy and may suggest that the abundance of ethanol could be a specific feature of studied BD grapes. By opposite, in samples from INT practices a higher relative content of amino acids arginine, leucine and glutamine resulted. The application of fertilizers (mineral and synthetic, as declared by supplier farmers) in INT vineyards could account for the observed greater relative concentration of amino acids in INT samples [72] rather than in BD ones, which were grown up without these kinds of fertilization treatments.

Finally, in the ORG vs INT comparison (Fig. 6c), the resulting most discriminant metabolites were α-glucose, malate and glutamine. In particular, ORG samples showed a limited—although statistically significant—higher relative content of α-glucose (0.02-fold increase) with respect to INT samples. In accordance with some literature data on the total sugars content from apples produced by ORG and INT farming methods [73], the α-glucose content was found to be slightly higher in ORG samples compared to INT ones. Other authors found, instead, no substantial differences between foodstuffs from ORG and INT cultivations, in terms of sugars quantity (red beet: [71], apples: [74]), and this could justify the only α-glucose increase. On the other hand, malate and glutamine showed higher statistically significant content (1.13- and 1.29-fold increases, respectively) in INT samples than ORG ones. In the case of malate, the obtained result appears consistent with previous findings related to the highest malate and citrate total content in apple fruit from INT vs ORG production treatment [75].

Therefore, a general overview of the obtained data suggests that, also in the here studied systems, sugars content was clearly affected by the agricultural practices in accordance to some literature results [32, 33]. However, although significant, the here reported relative differences in α-/β-glucose content were not particularly pronounced in all the treatments' pairwise comparisons (Fig. 6). Moreover, apart from glucose, none of the other sugar metabolites appeared as significant discriminating factor in the present study. Hence, the total sum of soluble sugars in the grapes juice could remain approximately

unchanged, among the applied different agricultural practices, according to other literature data. A specific review proposed a meta-regression analysis showing that the juice sugar concentration of grapes from ORG and BD managed vineyards was almost the same as grapes from CONV or INT viticulture [23]. Further insights on the issue are required, since other authors found similar outcomes, detecting no statistically significant differences on sugars concentration also in other food products cultivated with different farming methods (red beet: [71], apples: [74]).

Focusing on organic acids, the most discriminant metabolite in agricultural practices pairwise comparisons was malate (Fig. 6). In particular, grapes juice samples from both BD and INT agriculture showed higher content of malate, with similar values of FC increase (respectively, 1.14 and 1.13, Fig. 6a and c), with respect to samples from ORG treatment. This result is in agreement with literature data related to grapes [32] and other food matrices as reed beet [71] and apple fruits [75]. Since malate is involved in diverse aspects of cellular metabolism in grapes, its quantity can be influenced by many factors. In general, the accumulation of malate in grapevines is known to be due to *de novo* synthesis during the pre-veraison. This occurs through metabolism of assimilates translocated from leaf tissues to berries, as well as photosynthetic activity within the fruit itself [76]. Furthermore, loss of malate is associated with post-veraison period, when it is involved in various catabolic pathways. The switch from net accumulation to degradation of malate occurs just before veraison. Since its content in fruits is extremely variable [76], many reasons could account for the abundance of malate in the studied BD and INT grapes juice samples, with respect to ORG ones, buttressing the need for further insights.

In the case of ethanol, significantly discriminant for BD grapes in both BD vs ORG and BD vs INT comparisons (Fig. 6a, b), it is known to be naturally present in grape berries as metabolism product and it was found to be one of the most farming methods-dependent metabolites [33]. However, as already proposed by Gallo et al. [33], it can be hypothesized that the occurring ethanol could be the sum of the ethanol naturally contained before harvest and that further formed by enzymatic degradation during storage and sample manipulation. The latter portion could be either negligible with respect to the starting amount or variable due to other specific factors (e.g., yeast population) depending on cultivation method [33].

Finally, pairwise comparisons (Fig. 6) highlighted remarkably that INT samples would appear the richest of some amino acid compounds, compared to both BD and ORG samples (Fig. 6b, c). It can be assumed that metabolism in INT vine would invest appreciably in anabolic pathways, as the amino acids' biosynthesis, boosted by the combined use of organic and mineral/synthetic fertilizers. These latter provide plants with important nutrients as nitrogen (N), which seem to increase the concentration of some amino acids in many crops [72]. The higher abundance of glutamine in INT samples could be an index of the general better nutritional status of INT vines, since glutamine is known to have an important anabolic role as N atoms source for biosynthesis of other amino acids and also biological molecules containing N [77, 78]. In our case, as declared in the questionnaires by the supplier farms, the dissimilar features in the fertilization method between INT and BD viticulture along other treatments (Table 1) may explain different plant's nutritional conditions and, consequently, the statistically significant differences in the relative quantity of some amino acids shown in the BD vs INT comparison (Fig. 6b). In the case of arginine, its content appears to be strictly dependent on farming methods, in accordance to previous work [33]. Arginine is important for N storage in plants and many mechanisms are involved in the regulation of its levels in plant tissues. Arginine synthesis and catabolism, both are linked to the overall nutritional status of the plant cell and its consumption seems to be coordinated with the sugar starvation status of plants. Thus, arginine catabolism allows mobilization of stored nitrogen and relates to other metabolic pathways, including fine-tuning of polyamines production (essential for development and stress responses of plants) [79]. Although the regulation of the different catabolic pathways concerning arginine requires further research, several regulatory mechanisms of its biosynthesis were identified [79]. Arginine accumulates in grape berries from shortly before veraison until the fruit ripens, when its biosynthesis stops [80]. Therefore, the observed lower arginine content in ripe BD table grapes juice compared to both ORG and INT samples (Fig. 6a, b), besides testifying a major intake of nutrients characterizing ORG and particularly INT agriculture, may also suggest a specific metabolic response by the BD plants. Indeed, BD agriculture could stimulate the natural resistance of the grapevines activating the synthesis of polyamines, which are important in plant defense processes and determine the arginine use, so explaining our findings.

Conclusions

The present metabolomics study demonstrated that *cv. Italia* table grapes juice composition is significantly affected by the use of different kinds of vineyard managements. The comparison here carried out was among three farming methods: biodynamic (BD), organic (ORG) and integrated (INT). The metabolic profiling of *cv. Italia* table grapes—from the same geographical

area and three different vintages 2020, 2021 and 2022—was performed with the ^{1}H Nuclear Magnetic Resonance spectroscopy as analytical platform and multivariate statistical analysis of the NMR spectra-derived data set. The preliminary unsupervised PCA analysis of the data revealed both inter-annual variability (vintages 2020, 2021, 2022) and different farming methods (BD, ORG, INT) as significant drivers for samples differentiation. In the following supervised PLS-DA data analyses, the inter-vineyards variability due to the diverse managements turned out to discriminate samples at least to a same extent as the harvesting year. Moreover, the observed discriminating metabolites for samples separation were different considering the viticultural practices rather than the harvesting year, as assigned class in the PLS-DA analyses. Noteworthy, OPLS-DA pairwise comparisons between field treatments indicated the biodynamic vs organic samples as the best differentiation, followed by the biodynamic vs integrated and organic vs integrated samples comparisons, in decreasing discrimination order. These results somehow quantified the relations of the overall changes for metabolites composition with different agronomic management. Although variation of product characteristics, according to the different agricultural practices, is an expected outcome, an estimation of its specific effect in a multi-year experiment, for the three different vineyard conductions here focused, is novel. Ethanol, sugars as α-/β-glucose, organic acids as malate and some amino acids as arginine, leucine, glutamine, resulted the chemical species mostly influenced by the viticultural treatments. In particular, besides ethanol, arginine content appears to be strictly dependent on farming methods, in accordance to previous work. The here observed lower arginine content in ripe BD table grapes juice with respect to both ORG and INT samples, besides testifying a major intake of nutrients characterizing ORG and particularly INT agriculture, also suggests a BD specific metabolic response possibly related to plant defense processes.

Abbreviations

NMR	Nuclear Magnetic Resonance
MVA	Multivariate Statistical Analysis
BD	Biodynamic
ORG	Organic
INT	Integrated
CONV	Conventional
ATM	Automatic tuning-matching
FID	Free induction decay
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
VIP	Variable importance for the projection
FC	Fold Change

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00553-5.

Additional file 1: Figure S1. local pedological map; **Figure S2.** questionnaires supplied to farms; **Figure S3.** [^{1}H,^{1}H]-cosy spectrum; Figure S4. [^{1}H,^{13}C]-HSQC spectrum; **Figure S5.** [^{1}H,^{13}C]-HMBC spectrum; **Figure S6.** PCA loading scatter plot; **Figure S7.** examples of permutation tests; **Table S1.** list of discriminating chemical descriptors with corresponding p(corr) and VIP, for BD vs ORG samples comparison in the related OPLS-DA model; **Table S2.** list of discriminating chemical descriptors with corresponding p(corr) and VIP, for BD vs INT samples comparison in the related OPLS-DA model; **Table S3.** list of discriminating chemical descriptors with corresponding p(corr) and VIP, for ORG vs INT samples comparison in the related OPLS-DA model.

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Author contributions
FPF and GC designed the research and choose the methodology; GC collected samples in the field experiment; CSC and MH performed the experiments; CSC and CRG analyzed the data; CSC, CRG and FPF interpreted the results; CSC wrote the manuscript and created the figures; CRG and FPF reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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