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^{1}H-NMR-based metabolomic profiles of different sweet melon (Cucumis melo L.) Salento varieties: Analysis and comparison

Chiara Roberta Girelli, Rita Accogli, Laura Del Coco, Federica Angilè, Luigi De Bellis, Francesco Paolo Fanizzi*

Di.S.Te.B.A., Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, prov.le Lecce Monteroni, Lecce, Italy

Abstract

Melon (Cucumis melo L.) is a significant source of substances able to provide human health benefits. From the 18th century in the Salento area (Apulia region), the cultivation of melon varieties (C. melo L.) has always been intense. Over the years, the production of this fruit has involved a large number of selected and preserved varieties in the different local districts. Unfortunately, most of the characteristics of locally grown vegetable varieties do not match the food industry requirements. Moreover, the agricultural land abandon leads these varieties to quickly disappear, thus affecting the intraspecific biodiversity. In order to characterize the inter-variety diversity of sweet melon (C. melo L. ssp. melo group inodorus) and the potential differences in the nutritional quality of fruits, a first investigation on the juice of five sweet melon varieties (locally known as “allungato”, “scurzune”, “egiziano”, “minna de monaca”, “pinto”), cultivated exclusively in the Salento area, was performed by 1H-NMR spectroscopy and Multivariate Analysis (MVA). The analysis grouped the samples into clusters according to the different variety. Interestingly, a different sugar (mono and disaccharides) content was observed among the grouped varieties, being sweetness the main characteristic of sweet melon quality and taste. A relative higher accumulation of monosaccharides (α-d and β-d glucose and α/β-d fructose) was found, in particular for the “minna de monaca” with respect to “allungato”, “egiziano” and “pinto” varieties. Moreover, a marked high content of polyphenols and aromatic aminoacids as phenylalanine and tyrosine characterize the “allungato”, “minna de monaca” and “pinto” varieties. An NMR-based metabolomic approach was used for the first time to describe these local landraces. This method may integrate other actions in order to achieving a reduction in the current rate of erosion of the biodiversity of Apulian horticultural species.

Keywords

Cucumis melo L. 1H-NMR spectroscopy Metabolomics Salento area Sugars Multivariate analysis

Abbreviations

NMR Nuclear Magnetic Resonance; MVA Multivariate Analysis; PCA Principal Component Analysis; PLS-DA Partial Least-Squares Discriminant Analysis; p[CV-ANOVA]Cross-validated analysis of variance; GABA γ-aminobutyrate ;FC Fold Change; HSD Tukey's honestly significant difference; ssp subspecies

1. Introduction

Due to the synergistic combination of antioxidants, phytochemicals, and dietary fibers and their benefits to human health, research on plants is increasing together with the demand for fresh-cut fruits and vegetables (Rodriguez-Pérez, Quirantes-Piné, Fernàndez-Gutièrrez, & Segura-Carretero, 2013; Tarachiwin, Masako, & Fukusaki, 2008). All fruit varieties have a specific chemical profile characterized by primary metabolites (such as amino acids, sugars and organic acids), involved in basic cell functions and the so-called secondary

metabolites, that are usually specific to the type of fruit. These components determine fruits nutritional value, aroma, taste and beneficial effects on health.

Melon (Cucumis melo L., 1753), a member of the Cucurbitaceae family, is one of the most cultivated and consumed fruit across wide areas of the world (FAO, 2016). This fleshy, sweet fruit exhibits great morphological variation on size, shape, colour and texture, taste and composition, so that it can be considered the most diverse species of the genus Cucumis (Liu, Kakihara, & Kato, 2004; Stepansky, Kowalski, & Perl-Treves, 1999). The species comprises feral, wild and cultivated varieties, the latter including sweet "dessert" melons, as well as non-sweet forms that are consumed raw, pickled or cooked (Stepansky et al., 1999). The intraspecific classification of C. melo includes two subspecies, melo and agrestis, and 15 groups or varietas, among these inodorous, chate, cantalupensis, reticulatus (ssp. melo) (Pavan et al., 2017). Moreover, naturally low in fat and sodium, with no cholesterol, melon is a significant source of phytochemicals, mainly polyphenols and other antioxidants, which provide potential health benefits, especially aiding the cardiovascular system (Lester, 1997; Maietti et al., 2012). Its chemical composition depends on cultivar, environmental conditions, and on the stage of fruit maturity (Villanueva, Tenorio, Esteban, & Mendoza, 2004). To date, in Italy, sweet melon production is mainly located in Sicily (27% of the total National production), followed by Lombardy, Campania, Apulia and Veneto (ISTAT, 2016). The Apulia region in Southern Italy is an important secondary centre of diversity for melons (Pavan et al., 2017). Thanks to the optimal pedoclimatic conditions of the Salento area (Apulia region, Southern Italy), the cultivation of melon (C. melo L.) varieties has always been intense, involving a large number of selected and preserved typical varieties in the different local districts from the 18th century (Accogli, Nicolì, & De Bellis, 2014; Laghetti, Accogli, & Hammer, 2008; Mannarini, 1914). Among these, the cucumber melon 'Meloncella' (C. melo L. group chate) traditionally cultivated for its unripe fruits, and several landraces of winter sweet melon (C. melo group inodorus) with long shelf-life (Laghetti et al., 2008; Pavan et al., 2017). Unfortunately, most of the characteristics of locally grown vegetable varieties do not match the food industry requirements and new cultivars with greater commercial interest as cantalupensis and reticulatus progressively replace its traditional landraces (Laghetti et al., 2008; Pavan et al., 2017). Moreover, the intraspecific biodiversity of vegetable crops in the Apulia region has been eroded, due to several factors such as agricultural land abandon, ageing of the farming population, failure to pass information down the generations (leading to loss of knowledge and historical memory) (Accogli et al., 2014; Laghetti et al., 2008). The loss of family vegetable gardens near settlements, has led to a considerable loss of genetic diversity especially among folk varieties grown for family consumption. It is therefore important to recover the use of local varieties, also to have available a supply of genes useful for genetic improvement to resist diseases and hostile climate. The recovery and enhancement of local varieties are necessary to restore the concentrations of healthy phytochemicals in plants and vegetables since the genetic improvement makes the edible plants bigger, less indigestible, less lignified but often impoverished (Renna, Serio, Signore, & Santamaria, 2014). In order to characterize the inter-variety variability of C. melo and the potential differences in the nutritional quality of fruits, a first investigation on the juice of these sweet melon varieties cultivated in the Salento area, was performed by 1H-NMR spectroscopy and Multivariate Analysis (MVA). We focused our study on the rarest sweet melon (C. melo L. ssp. melo group inodorus) local varieties, seriously exposed to genetic erosion risk, being cultivated only by few farmers (Accogli, Conversa, Ricciardi, Sonnante, & Santamaria, 2018), locally known with the folk names: "allungato", "scurzune", "egiziano", "minna de monaca" and "pinto". The metabolomic analysis of 1H-NMR data from fruit extracts provides useful information on the relationship between the major metabolites and the sensory characteristics of the fruits. Nevertheless, the effects of pedoclimatic conditions and agricultural practices on metabolic profiles of fruits are well known (Sobolev et al., 2015). It was observed that juices from the same cultivar but different geographical origin result in a different metabolic profile (Tomita et al., 2015). In this scenario, it could be possible to assess a geographic specificity (traceability) of these local products,

and in particular, with respect to other sweet melon varieties, cultivated in other geographical or specific areas. As the chemical composition of each plant is strictly related to the genotype (Maietti et al., 2012), the specific metabolic profile could define varieties and geographical origin of fruits. As known, (Kim, Choi, & Verpoorte, 2010; Sobolev et al., 2015), metabolomics uses an untargeted approach by which a "snapshot" of the metabolic profile of the analyzed sample at a given time can be obtained. In the case of fruits analysis, the largest number of metabolites are extracted by choosing a suitable solvent or a solvent mixture. Among the analytical techniques used to analyze the fruits, Nuclear Magnetic Resonance Spectroscopy (NMR) is a major tool in a wide range of metabolomic studies and it has rapidly expanded into the field of mixture analysis and multiple screening applications, during the past years. NMR spectroscopy is a non-invasive, robust, and reliable quantitative technique that provides detailed information on molecular structures of the state of a solution based on atomic nuclear interactions and their properties. It combines truly quantitative and structural information with high throughput (a 1D spectrum can be measured in a few minutes) and excellent reproducibility, mostly relying on minimum sample preparation without derivatization needs (typically just buffer addition) (Spraul et al., 2009). Thanks to its potential applications in plant functional genomics, food science, and human nutrition, plant metabolomics is a growing science and has been successfully applied also to fleshy fruits (Biais et al., 2009). Thus, the aim of this work is to provide a better characterization of these local horticultural varieties, contributing to their valorization. This should help to conserve genetic resources in agriculture achieving a reduction in the current rate of erosion of these specific local sweet melon varieties.

2. Materials and methods

2.1. Melon sample growth, collection and handling

A total of 45 sweet melon juices fruit samples were obtained from five sweet melon local Salento varieties, "allungato", "scurzune", "minna de monaca", "pinto", "egiziano" (Fig. 1). Morphological and physiological characterization for each variety is summarized in Table 1. Melon plants were supplied by local farms in the Salento peninsula (province of Lecce) with their prior informed consent, within the framework of the project "Biodiversity of Apulian vegetable species" (Rural Development Programme, European Agricultural Fund for Rural Development, Reg. EC. No. 1698/2005), aimed at the safeguard and characterization of Apulian rural biodiversity (Ministero delle politiche agricole e forestali, 2013). Melon plants were growth according to local traditional agronomic practices. Sowing is carried out in open field between the first and second decades of April, in rows distanced 1.5–2 m, with 3–5 seeds placed for each hole. The dry culture practiced in this area until the 1970s is actually replaced by daily water dripping irrigation system. Three melons for each variety were harvested at maturity according to size, skin colour change and consistency between July and August 2017. Juice was obtained from fruits by removing skin and squeezing 35–40 g of flesh. Each juice sample consisted of three homogeneous replicates Technical and analytical reproducibility was assessed by the superimposition of technical replicates in the MVA (PCA and PLS-DA) score plots (Allwood et al., 2014).

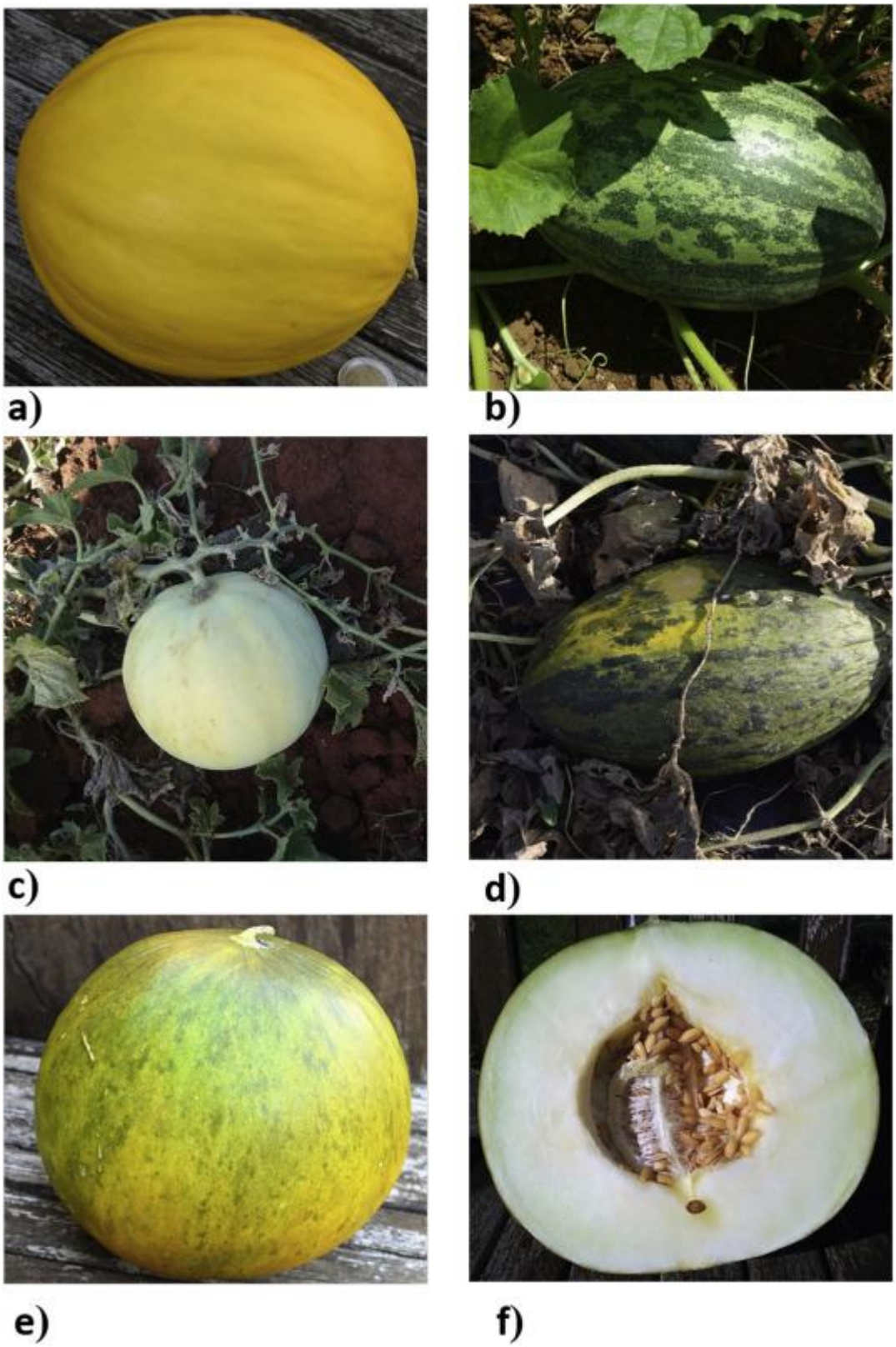

Fig. 1. Fruits of sweet melon varieties described in this study. a) “allungato”; b) “scurzune”; c) “egiziano”; d) “pinto”; e) and f) “minna de monaca”. (Accogli et al., 2018).

Table 1. Summary of morphological and physiological characters scored on 15 mature melon fruits. Measurements were averaged on three fruits per variety (Accogli et al., 2018; Ministero delle politiche agricole e forestali, 2013).

Melon variety	Origin	Fruit shape and size	Skin colour and texture	Flesh colour and juiciness	Shelf life (days)
Allungato	Tricase (LE)-39°56 N 18°22′ E	Oval-elliptical 20×15 cm	Strong yellow; smooth;	White; compact; few juicy;	100–150
Scurzune	Muro Leccese (LE)-40°06′ N 18°20′ E	Wide elliptical 22×25 cm	Green with dark spots; wrinkled;	White/green; tender; juicy;	100–150
Egiziano	Muro Leccese (LE)-40°06′ N 18°20′ E	Globular 20×25 cm	Ivory; smooth;	White; compact; juicy;	90–100
Minna de monaca	Collepasso (LE)-40°04′ N 18°10′ E	Oblate 23×27 cm	Green with small dark spots; wrinkled;	White/green, tender; juicy;	150–200
Pinto	Muro Leccese (LE)-40°06′ N 18°20′ E	Oval-elliptical 20×15 cm	Green with brown irregular spots; wrinkled;	Cream; compact; few juicy;	100–150

2.2. NMR spectroscopy

For 1H-NMR direct analysis on juice, 100 µL of phosphate buffer (1 M KH2PO4, 0.1% TSP, trimethylsilylpropanoic acid, as internal standard, D2O and 2 mM NaN3 to prevent microbial contamination, pH 2.9, according to Godelmann et al., 2013; Girelli, De Pascali, Del Coco, & Fanizzi, 2016; Longobardi et al., 2013, Spraul, Schütz, Humpfer, et al., 2009; Spraul et al., 2009) were added to 900 µL of juice and subsequent centrifuged (at 10.000g at room temperature for 5 min) in order to remove solids. Then, 700 µL of the supernatant were filled into a 5 mm NMR tube. All measurements were performed on a Bruker Avance III 600 Ascend NMR spectrometer (Bruker, Germany) operating at 600.13 MHz for 1H observation, equipped with a z axis gradient coil and automatic tuning-matching (ATM). Experiments were run at 300 K in automation mode after loading individual samples on a Bruker Automatic Sample Changer, interfaced with the software IconNMR (Bruker). For each sample, a zgcppr Bruker standard pulse sequence was applied to suppress the residual water signal. A total of 32 transients (with 4 dummy scans) were collected into 64 k data points with relaxation delay set to 5.0 s. A spectral width of 12,019.230 Hz (20.0276 ppm) and an acquisition time of 2.7262144 s were used. 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 3.5 pl 7 (Bruker). The metabolites were assigned on the basis of 2D NMR spectra analysis (2D 1H Jres, 1H COSY, 1H-13C HSQC and HMBC) and by comparison with literature data (Biais et al., 2009; Girelli et al., 2016; Sobolev, Segre, & Lamanna, 2003). A cosygpprqf Bruker standard pulse sequence with presaturation during relaxation delay using gradient pulses for selection was used to acquire a bi-dimensonal 1H-1H COSY spectrum. A spectral width of 12,019.230 Hz (20.0276 ppm) in both dimensions, 2 K data points in f2, 256 increments in f1 and an unshifted sine-bell squared (QSINE) window function in both dimensions. The 1H homonuclear J-resolved spectrum was acquired with the following parameters: 2 s relaxation delay, 10 µs 90° 1H pulse, spectral width 12 kHz in the proton dimension and 120 Hz in the J dimension, 4 K data points in f2 and 128 increments in f1. Zero filling in f1 to 4096 real data points and unshifted sinusoidal (SINE) window functions in both dimensions were used before Fourier

transformation. The 1H-13C gradient-selected HSQC spectrum was registered in the Echo –Antiecho - TPPI gradient selection mode with decoupling during acquisition, with the following parameters: 12 µs 90° 13C hard pulse and 80 µs for GARP 13C decoupling, 9 and 45 kHz spectral widths in the proton and carbon dimensions respectively, 2 K data points in f2 and 256 increments in f1. A number of 32 coefficients for forward Linear Prediction, followed by zero filling to 4 K real data points was applied in the f1 dimension before Fourier transformation. A 90°-shifted sinusoidal (SINE) window functions were also applied in both dimensions. The 1H-13C HMBC spectrum was obtained with a 1.5 s relaxation delay, 90° pulse of 10 µs for 1H and 12 µs for13C, 9 and 45 kHz spectral widths in the proton and carbon dimensions respectively, 2 K data points in f2, 512 increments in f1, a number of 32 coefficients for forward Linear Prediction, followed by zero filling to 4 K real data points was applied in the f1 dimension, processed with the use of unshifted sine-bell squared (QSINE) window functions in both dimensions.

2.3. NMR data processing and statistical analysis

1H NMR spectra were segmented in rectangular buckets (0.04 ppm width) and integrated using the Bruker Amix 3.9.13 (Analysis of Mixture, Bruker, Biospin) software. The spectral region between 5.02 and 4.7 ppm was discarded and the remaining 231 buckets in the range 10.00–0.50 ppm were then normalized to total intensity and subsequently mean centered. The data table generated by all aligned buckets row reduced spectra was used for multivariate data analysis. Each bucket row represents the entire NMR spectrum, and all the molecules present in the sample. Each bucket in a buckets row reduced spectrum is labeled with the value of the central chemical shift for its specific 0.04 ppm width. The variables used as descriptors for each sample in chemometric analyses are the buckets. The description of statistical analyses refers to Pareto scaled data obtained dividing the mean-centered bucket values by the square root of the standard deviation (van den Berg, Hoefsloot, Westerhuis, Smilde, & van der Werf, 2006). Multivariate statistical analysis (MVA) was performed by using Simca-P version 14 – (Sartorius Stedim Biotech, Umeå, Sweden). In particular, unsupervised (principal component analysis, PCA) and supervised (partial least squares discriminant analysis, PLS-DA) pattern recognition methods were performed to examine the intrinsic data variation. PCA is at the basis of the multivariate analysis (Jackson, 2005 and is one of the most common ways to reduce collinearity (Eriksson, Byrne, Johansson, Trygg & Vikström, 2013). A PCA model provides a summary, or overview, of all observations or samples in the data table. In addition, groupings, trends, and outliers can also be found. 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). To further maximize the separation between sample classes (already observed by PCA), Partial Least-Squares Discriminant Analysis (PLS-DA) was then applied. The PLS-DA is the regression extension of PCA, which gives the maximum covariance between the measured data (X variable, matrix of buckets related to metabolites in NMR spectra) and the response variable (Y variable, matrix of data related to the class membership). The PCA and PLS-DA models were validated using internal cross-validation default method (7-fold) and further evaluated with permutation test (400 permutations) all available on the SIMCA-P software (Eriksson, Byrne, Johansson, Trygg, & Vikstrom, 2013). The quality of the models was described by R2, Q2 and p[CV-ANOVA] parameters. The first (R2) is a cross validation parameter defined as the portion of data variance explained by the models and indicates goodness of fit. The second (Q2) represents the portion of variance in the data predictable by the model. In our MVA usually 3–5 components gave optimal R2(cum) and Q2(cum) values. The minimal number of components required can be easily defined since R2(cum) and Q2(cum) parameters display completely diverging behavior as the model complexity increases (Wheelock & Wheelock, 2013). Cross-validated analysis of variance (p[CV-ANOVA]) provides a p-value indicating the level of significance of group separation in PLS-DA analyses (Trygg & Wold, 2002; Wheelock & Wheelock, 2013). Moreover, the change in discriminating metabolite content among the observed groups 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) (Multi Integrate routine from the AMIX-Tools - Bruker Amix 3.9.14, software, Bruker, Biospin). TSP was used for chemical shift calibration and quantification (Cazor, Deborde, Moing, Rolin, & This, 2006). Signals corresponding to alanine (1.46 ppm), γ-aminobutyrate (GABA, 3.02 pmm), α-d-glucose (5.23 ppm), β-d-glucose (4.64 ppm), α/β-d-fructose (4.10 ppm) and sucrose (5.40 ppm) were integrated. In particular, the changes in metabolite levels between two groups were calculated as Log2 fold change (FC) ratio of the normalized median intensity of the corresponding signals in the spectra of the groups (Ghini et al., 2015, Girelli et al., 2016). Analysis of variance (One Way-ANOVA) with Tukey's honestly significant difference (HSD) post hoc test were used to examine mean differences among discriminating metabolite content for the sweet melon varieties. Statistical significance was set at least at an adjusted p-values < 0.05 (R Development Core Team, 2011).

3. Results and discussion

3.1. Visual inspection of 1H-NMR spectra and assignment of the peaks

A spectroscopic fingerprinting of sweet melon fruit extracts can be observed in the 1H NMR spectrum. Its relative expansions of significant spectral regions, with some peak assignments, are reported in Fig. 2a, b, c, d. The most intense peaks (assigned to sucrose, α-β glucose, fructose) were observed in the middle field region of the spectrum (range 3.2–4.2 ppm and 4.3–5.7 ppm) (Fig. 2b and c). Minor signals assigned to inositol, β-galactose, trehalose were also detected. Resonances from aliphatic groups of aminoacids and organic acids were also identified in the high field region of the spectrum, at range 1–3.2 ppm (Fig. 2a). Moreover, ethanol, an important indicator of metabolic changes (Biais et al., 2009), as fermentation of sugars during fruit ripening, was detected. In particular, peaks corresponding to isoleucine, valine, citrulline, GABA, acetate, glutamine and glutamate, citrate, asparagine and aspartate were observed in the 1–3.2 ppm range. In this region, the most intense signal was assigned to the alanine doublet (1.46 ppm). The low field aromatic region (Fig. 2d), in the spectral range from 6 to 10 ppm, showed peaks assigned to phenolic compounds (aromatic aminoacids as phenylalanine and tyrosine, alkaloids as trigonelline and salts of dicarboxylic acid as fumarate). As already observed in literature (Clausen, Pedersen, Bertram, & Kidmose, 2011; Girelli et al., 2016; Goulas et al., 2015; Sobolev et al., 2003), the two broad signals observed at 6.90 and 7.60 ppm were assigned to molecules, such as polyphenols. It should be noted that, due to the weakness of their intensities, the aromatic spectral region was reported with a high peak intensities enhancement. A summary of the peak assignments obtained is reported in Table 2.

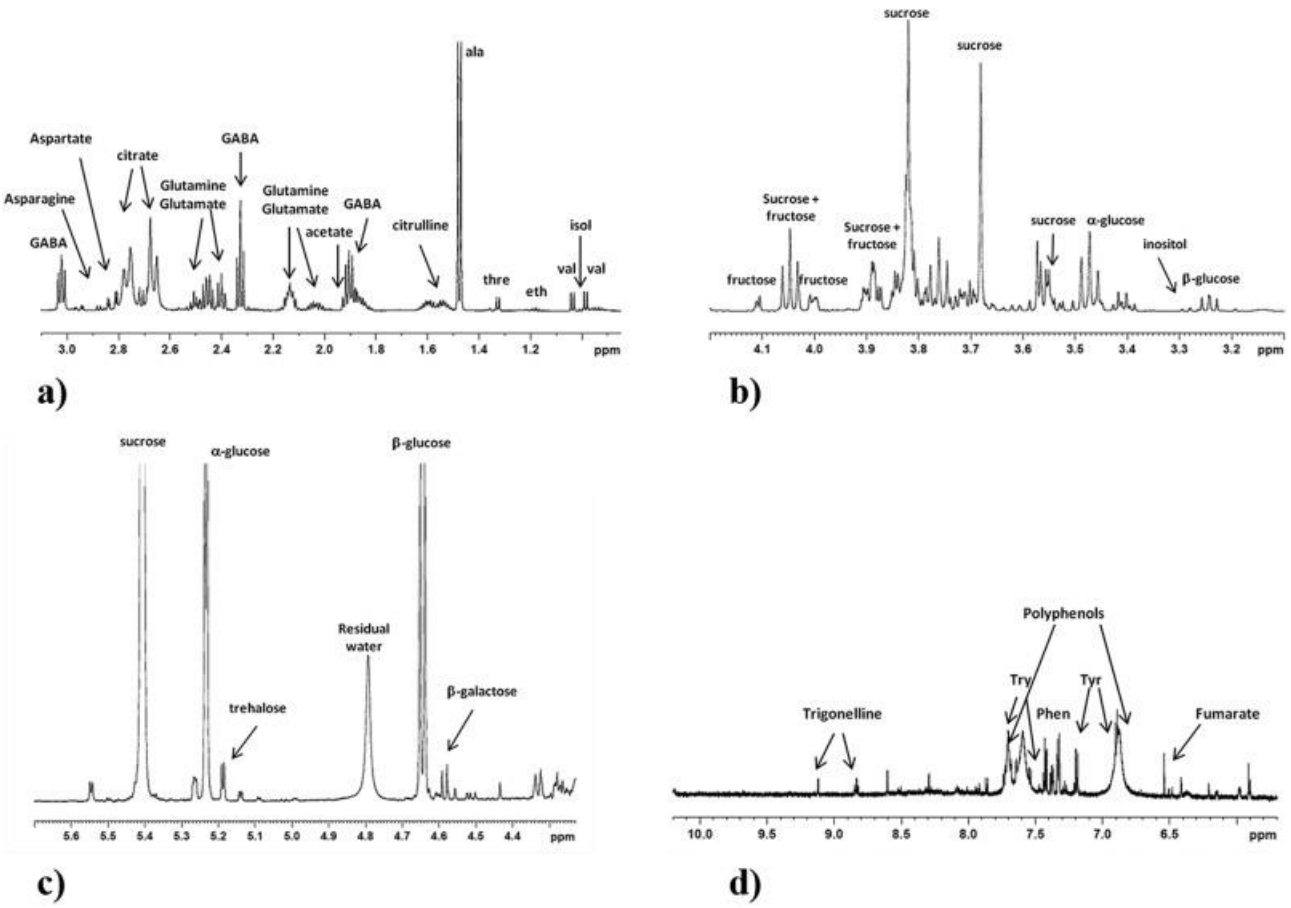

Fig. 2. 1H NMR spectrum (600 MHz, D2O) of sweet melon fruit juice samples in a) high, b–c) middle, and d) low frequency regions.

Table 2. Chemical shift (δ) and assignment of metabolite resonances in the 1H NMR spectrum of sweet melons juice.

Metabolites	δ (ppm)
Isoleucine	1.00 (d, β-CH3)
Valine	0.98 (d, CH3), 1.03 (d, CH3), 2.26 (m, β-CH)
Ethanol	1.17 (t, CH3)
Threonine	1.32 (d, γ-CH3), 4.26 (α-CH)
Alanine	1.46 (d, CH3), 3.79 (m, α-CH)
Citrulline	1.57 (m, γ-CH2), 1.87 (m, β-CH2), 3.15 (t,γ-CH2)
γ-aminobutyrate (GABA)	1.90 (m, β-CH2), 2.35 (t, α-CH2), 3.02 (t, γ-CH2)
Acetate	1.93 (s, CH3)
Glutamine	2.13 (m, β-CH2), 2.44 (m, γ-CH2), 3.76 (m, α-CH)
Glutamate	2.36 (m, γ-CH2)
Aspartic acid	2.71 (dd, half β-CH2), 2.85 dd (half β-CH2)
Citrate	2.69 (d, half CH2), 2.78 (d, half CH2)

Asparagine	2.95 (dd, half β-CH2)
Choline	3.19 (s, N(CH3)3)
β-d-glucose	3.26 (dd, H2), 3.48 (t, H3),4.64 (d, H1)
Inositol	3.28 (t, H4), 3.60 (T, H3 H6)
β-galactose	3.47 (t, H2), 4.58 (d, H1)
α-d-glucose	3.5 (dd, H2), 5.23 (d, H1)
Sucrose	3.55 (dd, H2), 3.67 (s, H2'), 3.81 (m, CH2–6), 4.20 (d, H3'), 5.40 (d, H1)
Fructose	4.01 (H5), 4.1 (d, H3,H4)
Trehalose	5. 18 (d, H1)
Polyphenols	6.90, 7.60 (broad signals)
Tyrosine	6.91 (m, H3, H5 ring), 7.19 (m, H2, H6 ring)
Tryptophane	7.20 (t, H5), 7.27 (t, H6), 7.55 (d, H7), 7.72 (d, H4)
Phenylalanine	7.43 (m, H3, H5), 7.37 (m, C4H), 7.30 (m, H2, H6)
Trigonelline	8.08 (t, H4), 8.84 (t, H3, H5), 9.13 (s, H2)
Adenosine-like	8.52 (s,CH2 ring)

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

3.2. Statistical analysis

In order to reveal a general data grouping of the classes and identify discriminating metabolites, an unsupervised PCA analysis was applied, as first attempt, to the bucket reduced NMR spectra dataset. In the PCA analysis the total variance of 97.1% was explained by three components (t[1], t[2], t[3] for 62%, 22.8% and 5.63%, respectively), describing the samples distribution in the PCA model space. The t[1]/t[2] scoreplot for the model showed a separation for the samples along the first two principal components (two coordinates, t[1]/t[2], for a two dimensions reduced model) (Fig. 3a). In particular, samples varieties were grouped in two macro-classes along the principal component t[1]: "scurzune" and "minna de monaca" varieties placed at negative values (between -1 to 0), while "allungato", "egiziano" and "pinto" varieties were found at positive (between 0 and 1) values of t[1]. By examining the loading scatter plot for the model (Fig. 3b), it was possible to define the variables (loadings) corresponding to the NMR signals of discriminating metabolites along t[1] and t[2] components. At positive values of t[1], samples are characterized by high relative content of sucrose (loadings at 3.82, 4.06, 4.22, 5.42, 4.06 ppm), aminoacids as alanine (1.46 ppm) and GABA (2.34 ppm). Samples grouped at negative values of t[1] are characterized by high level of monosaccharides, such as α and β- glucose (5.22 and 4.62 ppm, respectively) and fructose (4.02, 4.1 ppm). The variation in discriminating metabolite content among the varieties belonging to each observed group was calculated by integration (normalized median intensity) of specific signals, identified by NMR-based untargeted MVA and reported as Log2 fold change (FC) ratio. Signals corresponding to alanine (1.46 ppm), GABA (2.34 pmm), α-d-glucose (5.23 ppm), β-d-glucose (4.64 ppm), α/β-d-fructose (4.10 ppm) and sucrose (5.40 ppm) were integrated. A statistically significant higher level of monosaccharides (α-d-glucose, β-d-glucose, α/β-d-fructose) in "scurzune" "minna de monaca" varieties with respect to "allungato", "pinto" and "egiziano" varieties was observed (Fig. 4). "Minna de monaca" showed a

statistically significantly higher level of all monosaccharides with respect to “allungato” and “egiziano” varieties, and a significant higher level of fructose with respect to “pinto” variety.

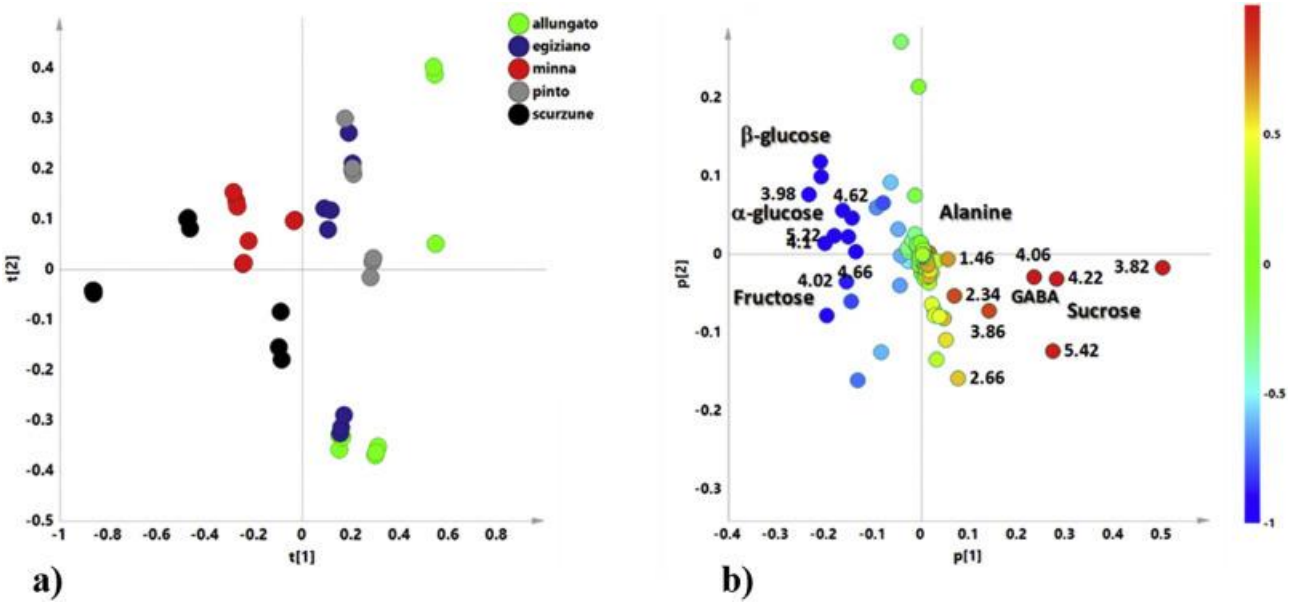

Fig. 3. a). PCA t[1]/t[2] scoreplot for sweet melons varieties (three components gives R2X(cum) = 0.904 and Q2(cum) 0.858; b) Loading scatter plot for the model colored according to the correlation scaled coefficient (* p(corr) ≥ |0.5|). The colour bar associated to the plot indicates the correlation of the metabolites in segregating among classes.

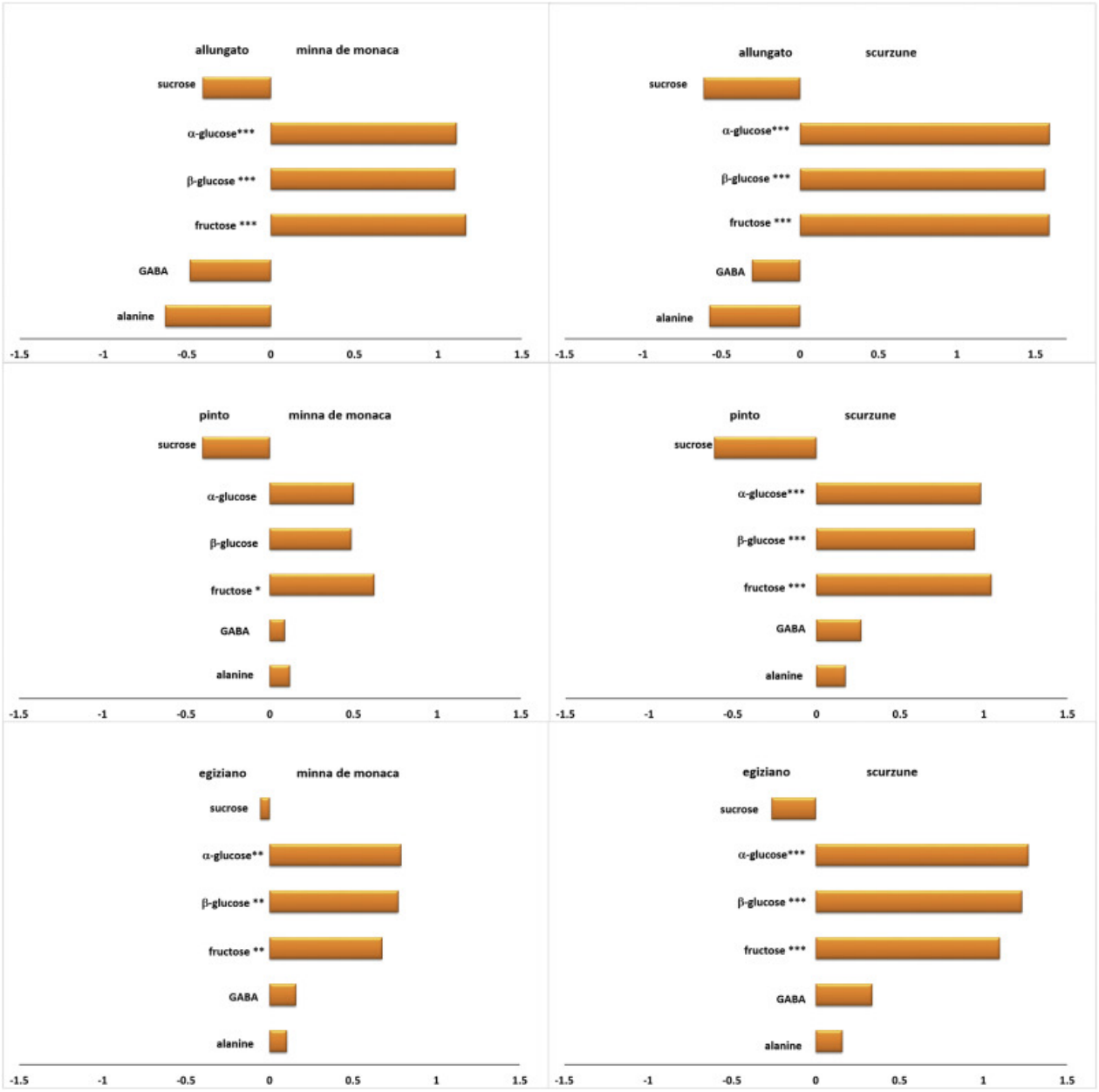

Fig. 4. Discriminant metabolite comparison between sweet melon varieties. The values of − Log2 (FC) (x axis) are reported. Statistical significance (Multiple Comparisons of Means, Tukey's honestly significant difference, HSD post hoc test) was set at least at an adjusted p-values < 0.05 and indicated with 0 '***' 0.001 '**' 0.01 '*' 0.05'. Metabolites with − Log2 (FC) negative values have higher concentration in "allungato", "pinto" and "egiziano" varieties, while − Log2 (FC) positive values indicated metabolites with higher concentration in "scurzune" and "minna de monaca" varieties.

In order to characterize also the metabolites with distinctive signals in the aromatic region, unsupervised (PCA) and supervised (PLS-DA) analyses were performed, for the sweet melon samples, discarding the aliphatic region of the spectra (middle and low frequencies, from 6 to 0 ppm) and reasonably good models were obtained (PCA five components, R2X (cum) = 0.929, Q2(cum) = 0.847; PLS-DA five components, R2X (cum) = 0.916, R2Y(cum) = 0.664, Q2 (cum) = 0.556). A certain degree of separation of the varieties was observed in the t[2]/t[3] PCA score plot, with "egiziano" and "scurzune" occurring at negative values of t[3] component and well differentiated from "pinto", "minna de monaca" and "allungato" varieties (data not shown). The separation among the samples classes could be improved by PLS-DA analysis maximizing covariance between the measured data (X) and the response variable (Y). The t[2]/t[3] components in the

PLS-DA scoreplot clearly showed a samples clustering in two main groups along the t[2] component (Fig. 5a). In this case, “scurzune” and “egiziano” varieties grouped together at negative values of t[2] component, while “allungato”, “pinto” and “minna de monaca” varieties placed between – 0.006 and 0.028 values of t[2] component. The loading scatter plot obtained for the model explained the aromatic molecular component characteristic for the observed groups. In particular, a marked content of polyphenols and aromatic aminoacids as phenylalanine and tyrosine was observed in “allungato”, “minna de monaca” and “pinto” varieties (Fig. 5b). These biologically active components have positive influence on health such as antioxidant activity, so protecting cells from damage by reactive oxygen species (ROS) (Dai & Mumper, 2010). The higher level of these compounds observed in “allungato”, “minna de monaca” and “pinto” varieties resulted in good nutrition properties. Moreover, in the case of “allungato” and “pinto” varieties, we observed a relative higher content of sucrose, the most important variable in determining melon fruit quality (Katzir et al., 2008; Yamaguchi, Hughes, Yabumoto, & Jennings, 1977). This particular taste profile, together with nutritive properties, may boost consumer demand leading to a future exploitation of such biodiversity. It should be noted that, polyphenol signals integration, useful for quantitative analyses, was not possible, appearing as two broad signals. Therefore, the Log2 fold change (FC) ratio for the relative content of tyrosine and phenylalanine (calculated for intensities of the corresponding signals at 7.19 and 7.43 ppm, respectively was also calculated for the studied varieties. A significant higher relative content of tyrosine and phenylalanine in “minna de monaca” variety with respect to “egiziano” and higher level of tyrosine “minna de monaca” with respect to “scurzune” variety was observed. Both “allungato” and “pinto” showed a significant higher level of phenylalanine with respect to “egiziano” variety (Fig. 6).

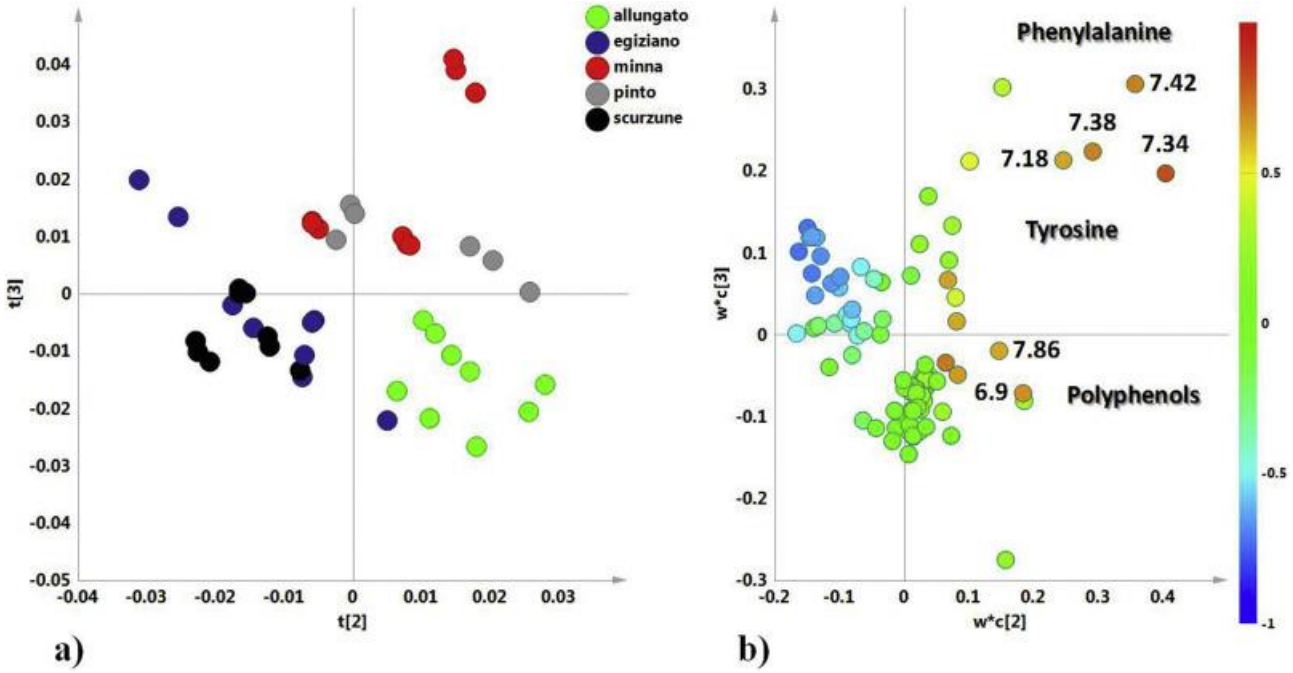

Fig. 5. a) PLS-DA t[2]/t[3] scoreplot for sweet melons varieties focusing aromatic spectral region (five components, R2X (cum) = 0.916, R2Y (cum) = 0.664, Q2 (cum) = 0.556, p[CV]-anova = 2.20*10−6. b) loading scatter plot for the PLS-DA model, colored according to the correlation scaled coefficient (* p(corr) ≥ |0.5|). The colour bar associated to the plot indicates the correlation of the metabolites in segregating among classes.

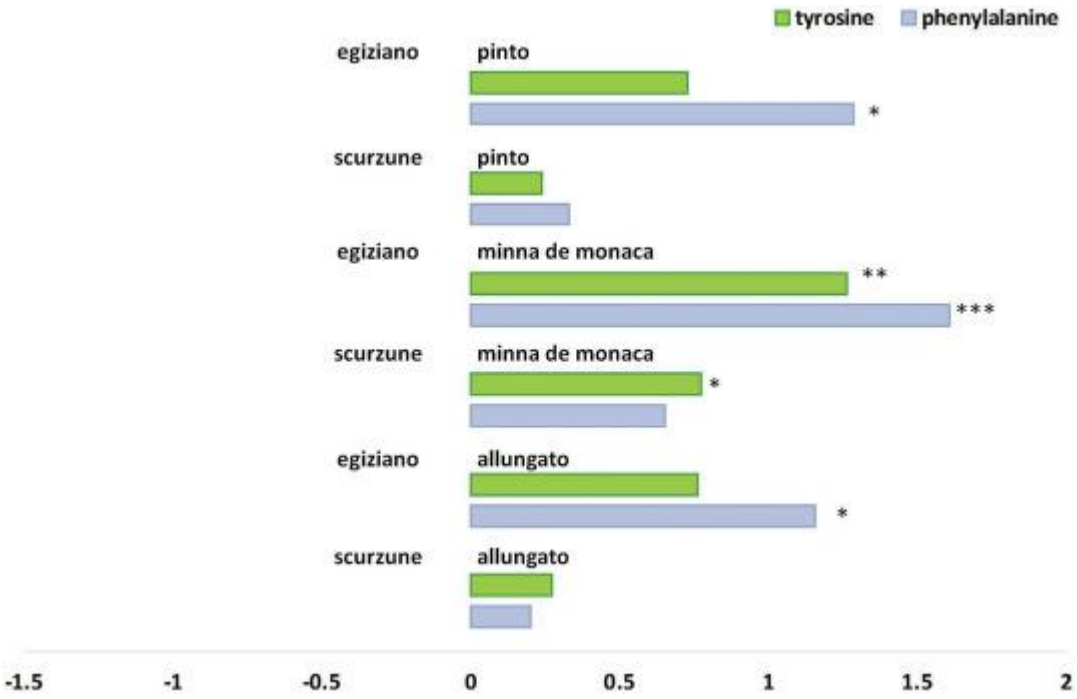

Fig. 6. Tyrosine and Phenylalanine (represented as green and blue histograms respectively) comparison between sweet melon grouping varieties in the 1H-spectrum aromatic region. (A: “allungato”; P: “pinto”, E: “egiziano”, M: “minna de monaca”, S: “scurzune”). The values of − Log2 (FC) are provided (Multiple Comparisons of Means test Tukey's honestly significant difference (HSD) post hoc test). Statistical significance was set at least at an adjusted p-values < 0.05 and indicated with 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05’. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

In this work a 1H NMR metabolic profile analysis in combination with MVA was performed for the first time in order to compare five sweet melons varieties, all exclusively cultivated in the Salento peninsula (South-East Italy), with well known important organoleptic differences and particularly appreciated for their long shelf-life. The benefits of cultivating these rare melon folk varieties could reside in the evaluation of the main metabolites which characterize their nutritional qualities. Interestingly, the multivariate discrimination results grouped samples into clusters according to the five different varieties. A further inspection of the statistical models, based on the NMR data, revealed a two-class differentiation, mainly due to types and amounts of different sugars content (monosaccharides, α and β- glucose and fructose and the disaccharide sucrose). In particular, these results clearly demonstrated that the specific sugar content was strongly dependent on the fruit variety, although the studied sweet melons were all cultivated over a modest area of the Salento peninsula, with the same pedoclimatic conditions and harvested in the same period. Our results allow, to clearly quantify the differences for the local varieties of sweet melons through the characterization and quantification of their molecular components. Although further investigations are needed to support the differentiation among the varieties studied, the 1H-NMR spectroscopy analytical methodology, could provide clear information on the relationship between the principal metabolites and the sensory characteristics for this specific studied foodstuff. Such a correlation is extremely important nowadays, in food chemistry, not only for promoting and establishing local and traditional products valorization actions, but also for supporting with instrumental data commercial values based on organoleptic analyses.

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Authors' contribution

CRG: prepared the samples for NMR analysis, performed the NMR experiments, analyzed and interpreted the NMR and statistical data, wrote and reviewed drafts of the manuscript, prepared the final writing. R.A.: selected and collected the samples, wrote and reviewed drafts of the paper, and contributed to the final writing. L.D.C: analyzed and interpreted the NMR and statistical data, reviewed drafts of the manuscript, prepared the final writing. F.A. contributed to prepare the samples for NMR analysis and to perform NMR experiments. L.D.B.: reviewed drafts of the paper and contributed to the final writing. FPF supervised NMR experiments and statistical analysis, reviewed drafts of the paper and contributed to the final writing. All authors read and approved the final manuscript.

Additional information

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References

Accogli, R., Conversa, G., Ricciardi, L., Sonnante, G., & Santamaria, P. (2018). Nuovo Almanacco BiodiverSO. Biodiversità delle Specie Orticole del Salento: Università degli Studi di Bari “Aldo Moro”.

Accogli, R., Nicolì, F., & De Bellis, L. (2014). Tradizioni e saperi attorno ad alcune varietà locali di Cucumis melo L. in Salento (Puglia). Thalassia Salentina, 36, 43–52.

Allwood, J. W., Cheung, W., Xu, Y., Mumm, R., De Vos, R. C., & Deborde, C. (2014). Metabolomics in melon: A new opportunity for aroma analysis. Phytochemistry, 99, 61–72.

Biais, B., Allwood, J. W., Deborde, C., Xu, Y., Maucourt, M., & Beauvoit, B. (2009). 1H NMR, GC−EI-TOFMS, and data set correlation for fruit metabolomics: Application to spatial metabolite analysis in melon. Analytical Chemistry, 81(8), 2884–2894.

Cazor, A., Deborde, C., Moing, A., Rolin, D., & This, H. (2006). Sucrose, glucose, and fructose extraction in aqueous carrot root extracts prepared at different temperatures by mean of direct NMR measurements. Journal of Agricultural and Food Chemistry, 54(13), 4686–6481.

Clausen, M. R., Pedersen, B. H., Bertram, H. C., & Kidmose, U. (2011). Quality of sourcherry juice of different clones and cultivars (Prunus cerasus L.) determined by a combined sensory and NMR spectroscopic approach. Journal of Agricultural and Food Chemistry, 59(22), 12124–12130.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10), 7313–7352.

van den Berg, R., Hoefsloot, H. J., Westerhuis, J., Smilde, A., & van der Werf, M. J. (2006). Centering, scaling, and transformations: Improving the biological information content of metabolomics data. BMC Genomics, 7, 142.

Development Core Team, R. (2011). R: A language and environment for statistical computing the R foundation for statistical computing, Vienna, Austria (2011). Retrieved 03/07/2018, from http://www.R-project.org/.

Eriksson, L., Byrne, T., Johansson, E., Trygg, J., & Vikström, C. (2013). Multi-and megavariate data analysis basic principles and applications. Vol. 1. Umetrics Academy.

FAO (Food and Agriculture Organization of the United Nations) (2016). Retrieved 03/07/ 2018, from http://www.fao.org/faostat/en/#data/QCGhini.

Girelli, C. R., De Pascali, S. A., Del Coco, L., & Fanizzi, F. P. (2016). Metabolic profile comparison of fruit juice from certified sweet cherry trees (Prunus avium L.) of Ferrovia and Giorgia cultivars: A preliminary study. Food Research International, 90, 281–287.

Godelmann, R., Fang, F., Humpfer, E., Schütz, B., Bansbach, M., & Schäfer, H. (2013). Targeted and nontargeted wine analysis by 1H NMR spectroscopy combined with multivariate statistical analysis. Differentiation of important parameters: Grape variety, geographical origin, year of vintage. Journal of Agricultural and Food Chemistry, 61(23), 5610–5619.

Goulas, V., Minas, I. S., Kourdoulas, P., Lazaridou, A., Molassiotis, A. N., & Gerothanassis, I. P. (2015). 1H NMR metabolic fingerprinting to probe temporal postharvest changes on qualitative attributes and phytochemical profile of sweet cherry fruit. Frontiers in Plant Science, 6, 1–11.

ISTAT. (2016) Retrieved 03/07/2018, from http://www.italiafruit.net/? AspxAutoDetectCookieSupport=1

Jackson, J. E. (2005). A user's guide to principal components. Vol. 587. John Wiley & Sons.

Katzir, N., Harel-Bega, R., Protnoy, V., Tzuri, G., Koren, E., & Lev, S. (2008). Melon fruit quality: A genomic approach.

Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. Nature Protocols, 5(3), 536.

Laghetti, G., Accogli, R., & Hammer, K. (2008). Different cucumber melon. (Cucumis melo L.) races cultivated in Salento (Italy). Genetic Resources and Crop Evolution, 55(4), 619–623.

Lester, G. (1997). Melon (Cucumis melo L.) fruit nutritional quality and health functionality. HortTechnology, 7(3), 222–227.

Liu, L., Kakihara, F., & Kato, M. (2004). Characterization of six varieties of Cucumis melo L. based on morphological and physiological characters, including shelf-life of fruit. Euphytica, 135(3), 305–313.

Longobardi, F., Ventrella, A., Bianco, A., Catucci, L., Cafagna, I., & Gallo, V. (2013). Nontargeted 1H NMR fingerprinting and multivariate statistical analyses for the characterisation of the geographical origin of Italian sweet cherries. Food Chemistry, 141(3), 3028–3033.

Maietti, A., Tedeschi, P., Stagno, C., Bordiga, M., Travaglia, F., & Locatelli, M. (2012). Analytical traceability of melon (Cucumis melo var reticulatus): Proximate composition,bioactive compounds, and antioxidant

capacity in relation to cultivar, plant physiology state, and seasonal variability. Journal of Food Scien ce, 77(6), C646–C652.

Mannarini, A. (1914). Orticoltura Salentina: monografia: Ambiente, Importanza economica, Pratiche colturali, Studio botanico-agrario delle razze ortive locali.

Ministero delle politiche agricole e forestali (2013). Linee guida per la conservazione e caratterizzazione della biodiversità vegetale, animale e microbica di interesse per l'agricoltura. Piano Nazionale sulla Biodiversità di Interesse Agricolo, INEA. Retrieved 03/07/2018, from http://dspace.crea.gov.it/bitstream/inea/556/1/Sintesi_PNBA_INEA.pdf.

Pavan, S., Marcotrigiano, A. R., Ciani, E., Mazzeo, R., Zonno, V., & Ruggieri, V. (2017). Genotyping-by-sequencing of a melon (Cucumis melo L.) germplasm collection from a secondary center of diversity highlights patterns of genetic variation and genomic features of different gene pools. BMC Genomics, 18(1), 59.

Renna, M., Serio, F., Signore, A., & Santamaria, P. (2014). The yellow–purple Polignano carrot (Daucus carota L.): A multicoloured landrace from the Puglia region (southern Italy) at risk of genetic erosion. Genetic Resources and Crop Evolution, 61(8), 1611–1619.

Rodriguez-Pérez, C., Quirantes-Piné, R., Fernàndez-Gutièrrez, A., & Segura-Carretero, A. (2013). Comparative characterization of phenolic and other polar compounds in Spanish melon cultivars by using high-performance liquid chromatography coupled to electrospray ionization quadrupole-time of flight mass spectrometry. Food Research International, 54(2), 1519–1527.

Sobolev, A. P., Mannina, L., Proietti, N., Carradori, S., Daglia, M., & Giusti, A. M. (2015). Untargeted NMR-based methodology in the study of fruit metabolites. Molecules, 20, 4088–4108.

Sobolev, A. P., Segre, A., & Lamanna, R. (2003). Proton high-field NMR study of tomato juice. Magnetic Resonance in Chemistry, 41(4), 237–245.

Spraul, M., Schütz, B., Humpfer, E., Mörtter, M., Schäfer, H., & Koswig, S. (2009). Mixture analysis by NMR as applied to fruit juice quality control. Magnetic Resonance in Chemistry, 47, S130–S137.

Spraul, M., Schütz, B., Rinke, P., Koswig, S., Humpfer, E., & Schäfer, H. (2009). NMR based multi parametric quality control of fruit juices: SGF profiling. Nutrients, 1(2), 148–155.

Stepansky, A., Kowalski, I., & Perl-Treves, R. (1999). Intraspecific classification of melons (Cucumis melo L.) in view of their phenotypic and molecular variation. Plant Systematics and Evolution, 217, 313–332.

Tarachiwin, L., Masako, O., & Fukusaki, E. (2008). Quality evaluation and prediction of Citrullus lanatus by 1H NMR-based metabolomics and multivariate analysis. Journal of Agricultural and Food Chemistry, 56(14), 5827–5835.

Tomita, S., Nemoto, T., Matsuo, Y., Shoji, T., Tanaka, F., & Nakagawa, H. (2015). A NMR based,non-targeted multistep metabolic profiling revealed L-rhamnitol as a metabolite that characterised apples from different geographic origins. Food Chemistry, 174, 163–172.

Trygg, J., & Wold, S. (2002). Orthogonal projections to latent structures (O-PLS). Journal of Chemometrics, 16, 119–128.

Ghini, V., Unger, F. T., Tenori, L., Turano, P., Juhl, H., & David, K. A. (2015). Metabolomics profiling of pre- and post-anesthesia plasma samples of colorectal patients obtained via Ficoll separation. Metabolomics, 11(6), 1769–1778.

Villanueva, M. J., Tenorio, M. D., Esteban, M. A., & Mendoza, M. C. (2004). Compositional changes during ripening of two cultivars of muskmelon fruits. Food Chemistry, 87(2), 179–185.

Wheelock, Å. M., & Wheelock, C. E. (2013). Trials and tribulations of 'omics data analysis: Assessing quality of SIMCA-based multivariate models using examples from pulmonary medicine. Molecular BioSystem, 9(11), 2589–2596.

Yamaguchi, M., Hughes, D., Yabumoto, K., & Jennings, W. (1977). Quality of cantaloupe muskmelons: Variability and attributes. Scientia Horticulturae, 6(1), 59–70.