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^{1}H HR-MAS NMR-based metabolomics study of different persimmon cultivars (*Diospyros kaki*) during fruit development

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ABSTRACT

^{1}H HR-MAS NMR spectroscopy was used to track the metabolic changes throughout the whole development of astringent ('Giombo') and non-astringent ('Fuyu') cultivars of persimmon (*Diospyros kaki*). The NMR data revealed the low concentration of amino acids (threonine, alanine, citrulline and GABA) and organic acids (malic acid). In addition, the signals of carbohydrates (sucrose, glucose and fructose) seemed to play the most important role in the fruit development. In both cultivars, the growth was characterized by fluctuating sucrose concentration along with a constant increase in both glucose and fructose. In the initial growth stage, the polyphenol composition was quite different between the cultivars. Gallic acid was detected throughout the growth of 'Giombo', while for 'Fuyu', signals of polyphenols disappeared over time. Additional multivariate analysis suggested that these cultivars share many metabolic similarities during development. These findings might help the comprehension of fruit development, which in turn, impacts the quality of the fruits.

1. Introduction

Persimmon (*Diospyros kaki*) is recognized as the most important species for fruit production in the *Diospyros* genus (Ebenaceae) (Yonemori, Sugiura, & Yamada, 2010). In 2013, world production of persimmons reached 4.6 million tonnes, with China accounting for 43% of this total. Other major producers include the Republic of Korea, Japan, Brazil and Azerbaijan (FAOSTAT, 2013).

In addition to its economic value, some studies have shown that persimmon is one of the most bioactive fruits (Daood, Biacs, Czinkotai, & Hoschke, 1992; Gorinstein et al., 1998; Veberic, Jurhar, Mikulic-Petkovsek, Stampar, & Schmitzer, 2010). In this context, reports have been found regarding antioxidant, cytotoxic and antidiabetic activities, as well as the beneficial effect on coronary diseases (Katsube et al., 2004; Kawase et al., 2003; Lee, Cho, Tanaka, & Yokozawa, 2007; Santos-Buelga & Scalbert, 2000).

The chemical composition of persimmon is the key to understand not only the aforementioned biological activity, but also to ensure the nutritional and organoleptic properties which, in turn, affect consumer satisfaction. The quality and enjoyment of fruits are intimately tied to their chemical composition, in this context, compounds, such as carbohydrates, organic acids, polyphenols and carotenoids, play key roles (Colaric, Veberic, Stampar, & Hudina, 2005; Daood et al., 1992). For instance, the sugar/organic acid ratio is a common quality index for fruits (Bassi & Selli, 1990). Giordani, Doumett, Nin, and Del Bubba (2011) reported a comprehensive review of the primary and secondary metabolites in fresh persimmons, highlighting the analytical methods employed for the determination of sugars, vitamin C, carotenoids and polyphenols. In a critical manner, the authors evaluated the overall significance of literature results concluding that the literature data are affected by a number of sources of variability, such as ripeness stage and analytical methods that should be more controlled and standardized in order to obtain more reliable and comparable results. The situation is further complicated by the vast number of varieties, which adds additional variation in the chemical investigation of persimmons. In Japan alone, over 100 cultivars are grown (Yamagishi, Matsumoto, Nakatsuka, & Itamura, 2005; Zhou, Zhao, Sheng, Tao, & Yang, 2011).

In contrast, a versatile, non-selective, NMR based approach, termed high resolution magic angle spinning (HR-MAS) NMR spectroscopy, has allowed the characterization of foodstuff

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providing information on a wide variety of compounds (Santos, Fonseca, Lião, Alcantara, & Barison, 2015; Valentini et al., 2011). In this technique, with the purpose of eliminating the contribution of dipolar coupling and differences in magnetic susceptibility, samples are submitted to fast spinning about the so-called "magic angle" (54.74°) (Santos et al., 2015). As a result of such a procedure, the line broadening in the spectra is significantly reduced, which results in a boost of signal-to-noise ratio and resolution. In a single NMR experiment, it is possible to identify and quantify non-polar and polar substances; thus, both primary and secondary metabolites can be evaluated. In addition, NMR spectra are acquired directly from samples avoiding the loss of information provoked by the extraction procedures (Santos et al., 2015). These features make such a technique ideal for tracking chemical changes associated with complex processes, such as fruit development.

As such, there is a clear need for a better understanding of the chemical processes (including the metabolites involved) behind fruit growth and ripening. Such processes affect the whole characteristic of fruits, including taste, texture and bioactivity. Thus, knowledge about how the fruits grow can provide important molecular indicators to monitor the crop both during the cultivation or after the harvest (Buesa et al., 2013; Harima et al., 2003; Lee, Kim, Kim, & Park, 2005). The present work aims to evaluate the metabolic changes during the development of persimmon cultivars 'Fuyu' (non-astringent) and 'Giombo' (astringent) through ^{1}H HR-MAS NMR and chemometric analysis. In persimmons, the astringency sensation is produced by the presence of high-molecular-weight tannins in fruit flesh. Such compounds damage the normal lubrication of oral surfaces, at least in part, by the precipitation of salivary protein (Lyman & Green, 1990).

2. Materials and methods

2.1. Samples

Persimmon fruits of cultivars 'Fuyu' and 'Giombo' during the whole period of the development (from September 2012 to March 2013) were provided by Fruticultura Boutin Company (Porto Amazonas, Paraná, Brazil). The fruits were cultivated under the same field conditions. For each cultivar, ten fruits were collected at the medium height of the persimmon tree, covering the entire length of the plantation randomly. The samples were kept in a freezer (-80 °C) until analysis.

2.2. Sample preparation for NMR analysis

For the acquisition of the ^{1}H HR-MAS NMR spectra, the fruits were thawed and split in half, and a slice of one of the parts was pulverized after freezing with liquid nitrogen. Approximately, 12.0 ± 1.0 mg was packed into a 50 μl zirconium rotor followed by the addition of 40 μl of D_2O phosphate buffer (pH 6.4), with 0.5% TMSP-d_4, 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt. The buffer solution was used to prevent the pH-dependent variation of some NMR signals (Shintu, Caldarelli, & Franke, 2007). This is essential as fruit development is characterized by changes in its pH, which could in turn lead to variation in pH sensitive NMR resonances (Gil et al., 2000). In general, molecules with ionization state that change with changing pH also display varying NMR chemical shifts at different pHs (del Campo, Berregi, Caracena, & Santos, 2006).

2.3. NMR measurements

^{1}H HR-MAS NMR spectra were recorded at 293 K on a Bruker AVANCE spectrometer operating at 9.4 T, observing ^{1}H at 400.13 MHz, equipped with a 4 mm four channel (^{1}H, ^{13}C, ^{15}N and ^{2}H) HR-MAS probe. The samples were spun at the magic angle (θ = 54.74°) at 5 kHz. ^{1}H HRMAS-NMR spectra were acquired by using a water suppression pulse sequence, *noesypr1d* (Bruker library), using 64 K data points over a 5999 Hz spectral width averaged over 512 transients. A recycle delay of 1.0 s, power for presaturation of 43 dB and a mixing time of 100 ms were used, the later to help reduce the water signal through T_1 relaxation and chemical exchange. The saturation of the water residual signal was achieved by irradiation during recycle and the NOE mixing time at the H_2O/HOD NMR frequency. The spectra were apodized via an exponential multiplication corresponding to a 0.3 Hz line broadening in the transformed spectrum and zero filled by a factor of 2. The magic angle was adjusted daily using the ^{79}Br signal from a powdered KBr for reference. The samples were locked on the deuterium signal from D_2O, and the magnetic field homogeneity was optimized for each sample. The total experiment time was 60 min for each sample, including the time used for rotor preparation.

In order to support the NMR chemical shift assignments in the ^{1}H HR-MAS NMR spectra, liquid-state NMR experiments were performed on persimmon samples (representing the beginning, middle and end of growth) extracted directly in D_2O. One-bond and long-range ^{1}H−^{13}C correlation from HSQC and HMBC NMR experiments were optimized for an average coupling constant $^{1}J_{(C,H)}$ and $^{LR}J_{(C,H)}$ of 140 and 8 Hz, respectively. The experiments were performed on a Bruker AVANCE III 600 NMR spectrometer operating at 14.1 T, observing ^{1}H and ^{13}C at 600.13 and 150.90 MHz, respectively, equipped with a 5 mm inverse detection four channel (^{1}H, ^{13}C, ^{15}N and ^{31}P) probe with an actively shielded field. ^{1}H and ^{13}C NMR chemical shifts are given in ppm referenced to TMSP-d_4 signal at 0.00 ppm.

2.4. Normalized integral areas and statistical analysis

In order to monitor the metabolic changes revealed in the NMR data, the normalized integral areas of identified compounds were calculated using the multi-integration tool-kit of AMIX-viewer (Bruker BioSpin, Rheinstetten, Germany). The results were evaluated using the analysis of variance (ANOVA) single factor analysis with significance level of 0.05. The means were compared using Tukey's test.

2.5. Multivariate data analysis

All ^{1}H HR-MAS NMR spectra were manually phased, baseline corrected and aligned by TopSpin® software. The chemical range between 0.50 and 9.50 ppm represented all ^{1}H NMR resonances in the samples. Such range was segmented in continuous small buckets of 0.02 ppm wide. The areas between 4.5–5.2 and 2.20–2.26 were excluded in the ^{1}H NMR spectra to eliminate, respectively, the residual H_2O/HOD signals, as well as the signal of remaining acetone from the cleaning procedure of the rotor. The area under each bucket was integrated in AMIX® 3.9.12 (Bruker BioSpin, Rheinstetten, Germany) using the special integration mode. The spectra were scaled to total intensity, which in turn divides the measured signal intensity for each bucket by the integral of the complete spectrum. Thus, the spectral differences resulting from the variation of the amount of samples can be corrected. After this procedure, a matrix was created in which each row represented persimmon samples and each column contained the integrated area of the original spectroscopic intensities within each bucket region. The initial matrix was composed of 70 samples (lines) and 446 variables (columns). For the principal components analysis (PCA), the variables were scaled to unit variance (autoscale). This means that all columns have the same weight and all

variables have an equal opportunity to be considered in the PCA. This scaling is needed, for example, if the metabolites concentration are very different in the same samples, enabling all compounds (major or minority) to be taken into account in the multivariate analysis. To identify the metabolites that were contributing to the fruit development, PCA loading plots, which represent the relative weight for each bucket, were also acquired for each of the PCA scores plots.

3. Results and discussion

3.1. Global changes in metabolites during persimmon development

Before the acquisition of the data (comprised of 140 persimmons, 70 fruits for each of the two cultivars), the NMR methodology was optimized as described in Supplementary Material. The optimization stage was important as the heterogeneity and physical state of the fruit could impact the analysis. For example, the differences of hardness in fruits at the beginning and the end of development might influence the results of the chemometric analysis (Pérez, Iglesias, Ortiz, Pérez, & Galera, 2010).

The spectral profile of the persimmon cultivars during the complete stage of development are shown in Figs. S7 and S8. The spectra of both cultivars presented an overlapped signal at the 3.0–6.0 ppm region, which seemed to dominate the changes during the process. For a better visualization and discussion of the results, the spectra were divided into three regions: organic acid/amino acids (0.5–3.0 ppm), carbohydrates (3.0–6.0 ppm) and aromatic compounds (6.0–9.5 ppm) (Gil et al., 2000).

3.1.1. Organic and amino acid region (0.5–3.0 ppm)

The general weak intensity of signals in the 0.5–3.0 ppm region supported the low concentrations of amino acids and organic acids in persimmon (Figs. S7 and S8). These results are in accordance with Ryu et al., 2016, where they described the use of solution NMR to characterize Japanese persimmon aqueous extracts (Ryu et al., 2016). The analysis of this region allowed the identification of ethanol (1.19 ppm, t), threonine (1.34 ppm, d), alanine (1.48 ppm, d), citrulline (1.61 ppm, m), GABA (2.30 ppm, t) and malic acid (2.70, d) as the major compounds (Fig. 1). All the assigned signals are reported in Table S1. In order to monitor the behavior of these compounds, the normalized integral area of individual metabolites was calculated (Fig. 2).

Malic acid was the only organic acid identified in this region (Fig. 2), significant differences of its content were observed only for 'Fuyu', in the samples of September and March (Tukey's test, $p < 0.05$). Senter, Chapman, Forbus, and Payne (1991), when studying different persimmon cultivars found in Japan, also reported the increase of malic acid with maturity (Senter et al., 1991). Besides malic acid, the authors described the presence of succinic, citric and quinic acids.

Malic acid, along with citric acid, are considered the most dominant acids in fruits effectively contributing to their organoleptic characteristics. It has been hypothesized that the proportion of these two acids influences the perception of sweetness (Lobit, Genard, Soing, & Habib, 2006). As malate fluctuations have previously been correlated to mainly environmental rather than genetic variation, comparison of malate content between cultivars is of less interest (Lobit et al., 2006).

In terms of amino acids, citrulline, threonine, alanine, and GABA were identified; however, due to low signal and baseline variation, only the two latter amino acids provided reliable integral areas. The intensities of alanine and GABA signals were statistically different between September and March for the 'Giombo' cultivar, what also happened with 'Fuyu' only for alanine. Regarding the normalized area of GABA for the 'Fuyu' cultivar, differences between November and March were noticed. In addition, the visual inspection of the citrulline signal suggested a decrease of its content as the fruits developed. The decrease of amino acid content, within this context, has been related to protein synthesis and degradation during maturation, as well as their relative dilution as the fruits get larger (Ackermann, Fischer, & Amado, 1992).

Regarding ethanol content, no significant changes were observed for 'Giombo', while for 'Fuyu', variations were statistically representative during September and January (Fig. 2). Ethanol, together with acetaldehyde, leads to dramatic changes either

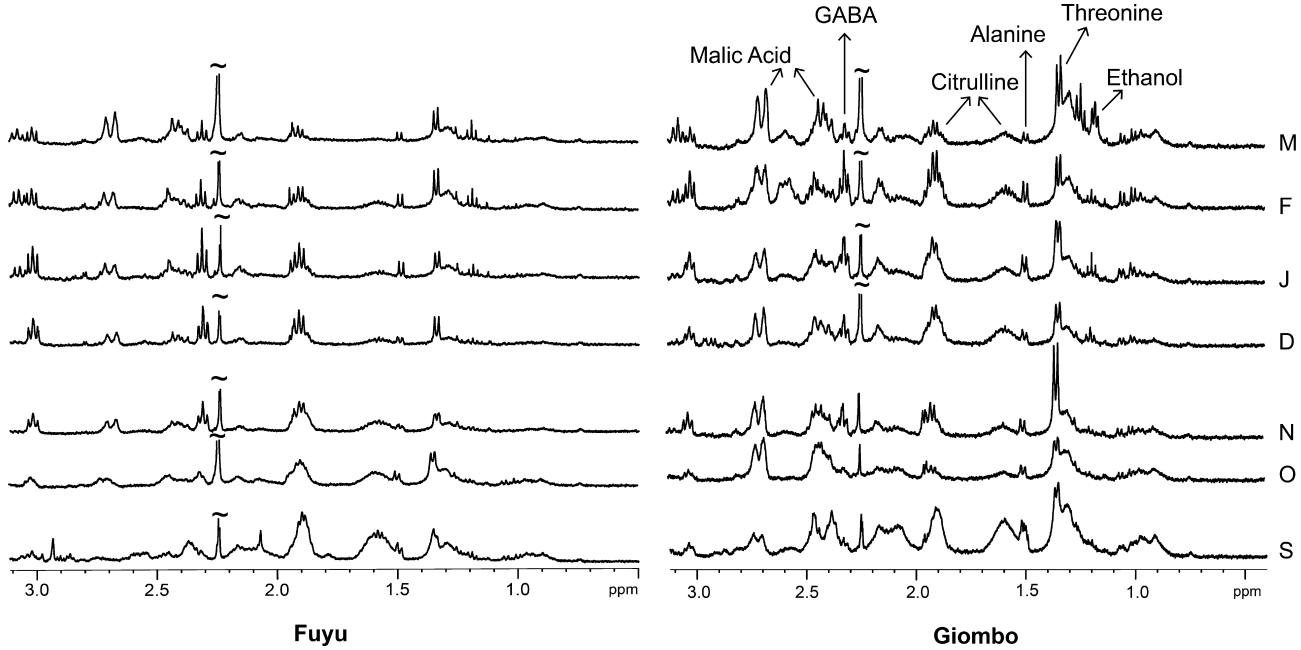

Fig. 1. Expansion of 0.5–3.0 ppm regions of the ^{1}H HR-MAS NMR spectra of persimmon during the process of development (from September (S) to March (M)).

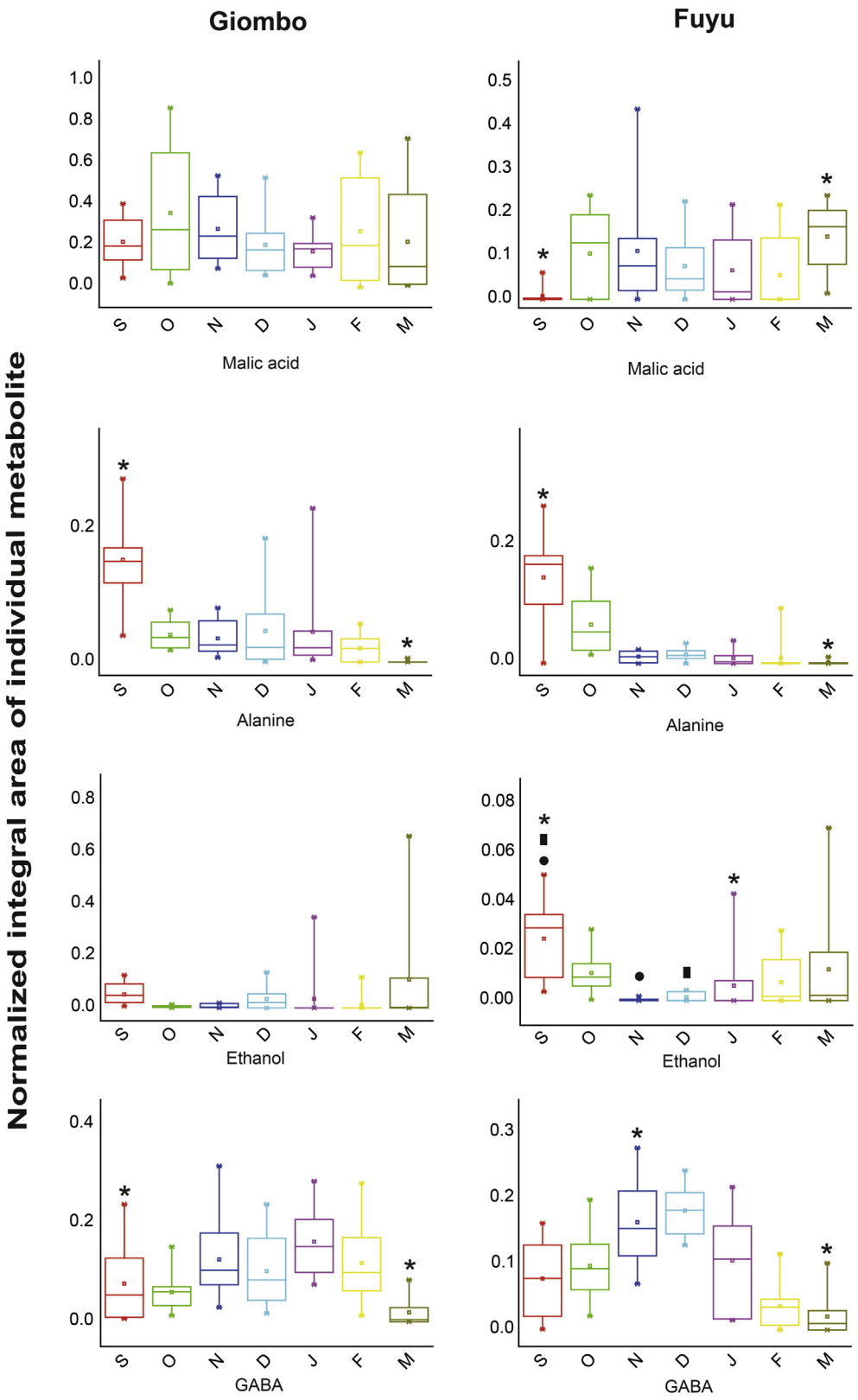

Fig. 2. Monthly evaluation (from September to March) of the normalized integral areas of metabolites present in 'Fuyu' and 'Giombo' cultivars (box plot). The symbols in the graphs reveal significant differences in the levels by Tukey's test at $p < 0.05$. Samples containing the same symbol mean that they are statistically different.

when the fruit is on the tree or in postharvest period (Pesis, 2005). Sugiura, Yonemori, Harada, and Tomama (1979) postulated the production of these two metabolites in the seeds and flesh, imparted the high degree of astringency found in the persimmon fruit. In addition, it has been suggested that ethanol and acetaldehyde levels at harvest could be criteria for indicating the optimal harvesting time (Zerbini, Giudetti, Rizzolo, & Grassi, 2001).

3.1.2. Carbohydrate region (3.0–6.0 ppm)

Persimmons are a rich source of carbohydrates. Fig. 3 demonstrates it is possible to monitor carbohydrate flux during all stages of development. Fructose, glucose and sucrose were the major sugars in persimmon flesh, which is in accordance with the findings in the literature (Del Bubba et al., 2009; Giordani et al., 2011; Ryu et al., 2016). In order to evaluate the sugar's role in fruit development, the normalized integral area of the major carbohydrates was calculated for both cultivars (Fig. 4). At the start and end of development, the magnitude of intensities of sucrose, glucose and fructose signals were virtually the same in both 'Fuyu' and 'Giombo', however, differences in the levels of sugars along the process were observed. The signal intensity of the anomeric hydrogen of sucrose started at values close to zero for the cultivars presenting a significant increase in January for 'Giombo', and for 'Fuyu' in November and December. At the end of fruit development, none of cultivars presented sucrose in their composition.

In general, a permanent increase of signal areas of glucose and fructose was clearly observed until the end of fruit growth (Fig. 4). The same trends were previously reported for several persimmon cultivars (Senter et al., 1991). The integrals of signals related to α e β-glucose increased continually during fruit development. For 'Fuyu', there was a statistically significant increase of their contents in January and February, from which the carbohydrate production seemed to reach the stability. For 'Giombo', January and February samples were statistically different regarding the other months.

Although carbohydrate composition of persimmon is qualitatively well-defined in literature, up to now, there is a lack of understanding regarding how the levels of sugars (sucrose, glucose, and fructose) change during the fruit development (Del Bubba et al., 2009). Nevertheless, the increment of total sugar content has been constantly described in the literature regardless of whether the cultivar is astringent or non-astringent (Giordani et al., 2011). That increment has been characterized in different manners: 1) the boost of sucrose and reducing sugar; 2) increasing and decreasing of sucrose followed by a constant increase of glucose and fructose, and 3) the relevant increase of sucrose and the virtually steady levels of glucose and fructose (Giordani et al., 2011).

The lack of a well-defined trend may be explained by the persimmons' genetic variability, as well as variations in experimental design of the employed analytical methods. Further complications are the absence of a protocol for determining the actual development stage of samples and also the lack of control for enzymatic activity (invertase) (Zheng & Sugiura, 1990), which is increased when fruit are pulped or extracted. As such, these variations make comparison among different studies involving the chemical composition of persimmons challenging.

Using the methodology described in the present work, the trend of carbohydrate during fruit development in both cultivars could be described by increasing and decreasing of sucrose followed by a steady increase of glucose and fructose. Such a trend is in line with one of those reported by Giordani et al., 2011. However, it is worth highlighting that the methodology employed in this work, allowed the spectra acquisition directly from the samples during all development stages of fruits cultivated under the same environmental conditions, avoiding the problems with misclassification of the fruit development stage, as well as the influence of invertase.

3.1.3. Aromatic region (6.0–9.5 ppm)

The investigation of the aromatic region revealed considerable differences between the chemical composition of the cultivars; with alterations being most noticeable in spectra for the September samples. The analysis of this region allowed the assignment of signals related to fumaric acid (6.52 ppm), gallic acid (7.06), trigonelline (9.11 ppm), epigallocatechin (6.58 ppm) and catechin (6.97 ppm), in 'Fuyu', and, only gallic acid in 'Giombo' (Fig. 5 and Table S1). Both epigallocatechin and catechin have been reported as one of the monomeric flavan-3-ol units of the condensed tannins found in persimmon (Akagi et al., 2010; Li et al., 2010; Nakatsubo et al., 2002).

These results support the significant synthesis of polyphenols occurring in the early stages of growth, which is in line with results reported by Del Bubba et al. (2009) and Tessmer et al. (2016). It is worth noting that an abrupt decline in aromatic content was observed for 'Fuyu' after the first month. From October on, only the signal of fumaric acid was detected. According to Yonemori and Matsushima (1985), early cessation of tannin cell development is the main cause of natural astringency loss in non-astringent fruits since it leads to the dilution of tannin cells in the flesh as fruits grow. On the other hand, the signal of gallic acid (7.06 ppm) remained until the final stage of development in 'Giombo', reaching a maximum in December. This result suggests the continuation of the synthase of tannins in late stages of fruit development (Ikegami, Yonemori, Kitajima, Sato, & Yamada, 2005). The biosynthetic pathway of condensed tannin production is not well understood, however, the enzyme gallic acid transferase, vacuolar membrane transporters for leucoanthocyanidins and proanthocyanidins, and condensing enzyme have been reported to be involved in this process (Park et al., 2004). From the nutritional view-point, the highest content of polyphenols, usually found in astringent cultivars, may be considered better sources of natural antioxidants (Suzuki, Someya, Hu, & Tanokura, 2005).

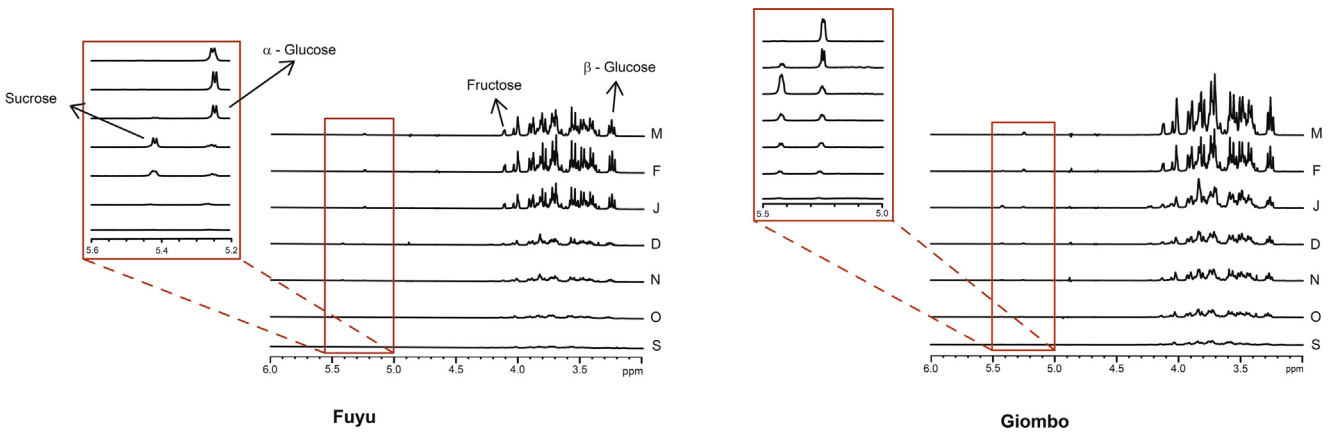

Fig. 3. Expansion of 3.0–6.0 ppm regions of the ^{1}H HR-MAS NMR spectra of persimmon during the process of development (from September to March).

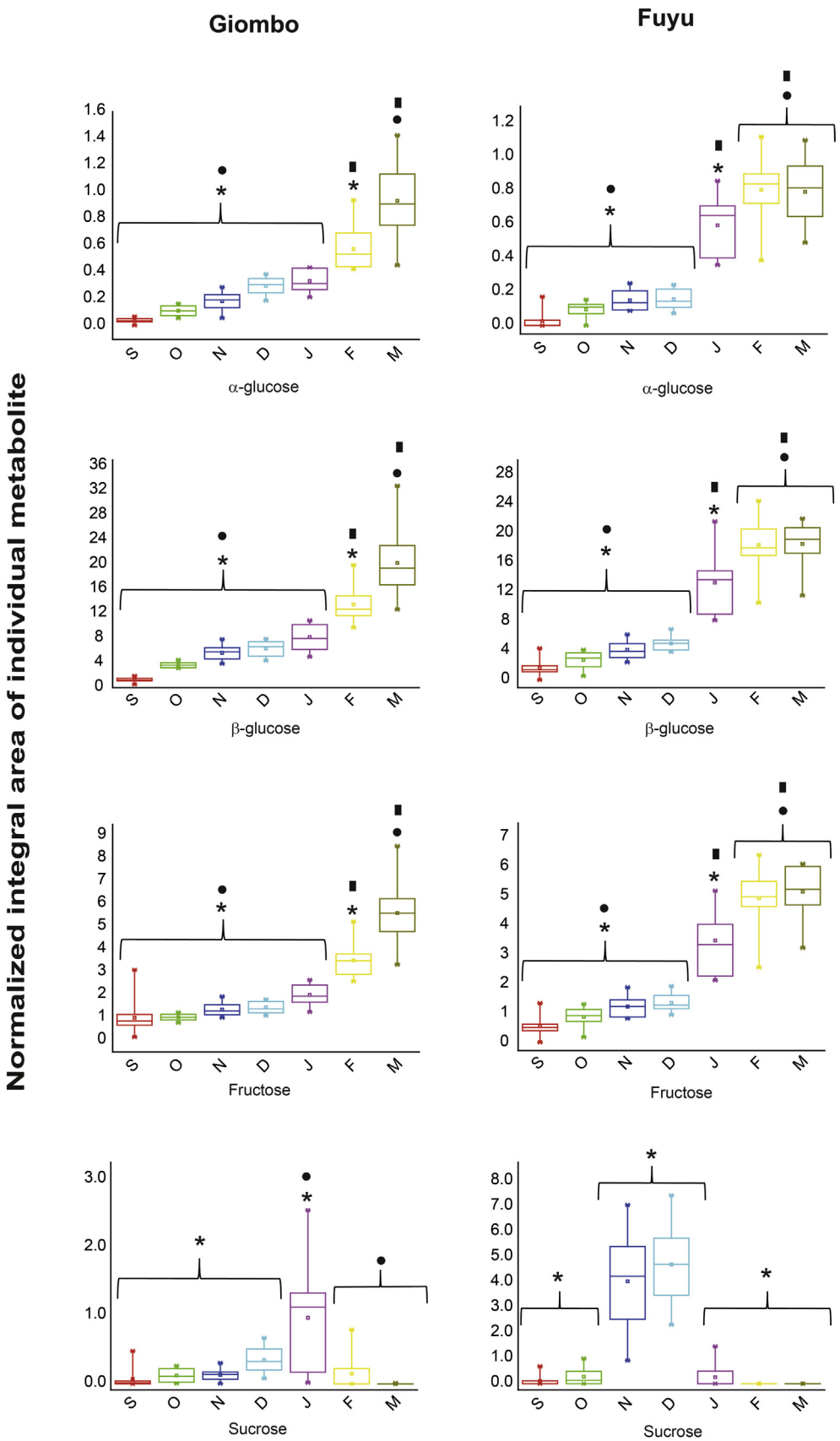

Fig. 4. Monthly evaluation (from September to March) of the normalized integral areas of major carbohydrates present in 'Fuyu' and 'Giombo' cultivars (box plot). The symbols in the graphs reveal significant differences in the levels by Tukey's test at $p < 0.05$. Samples containing the same symbol mean that they are statistically different.

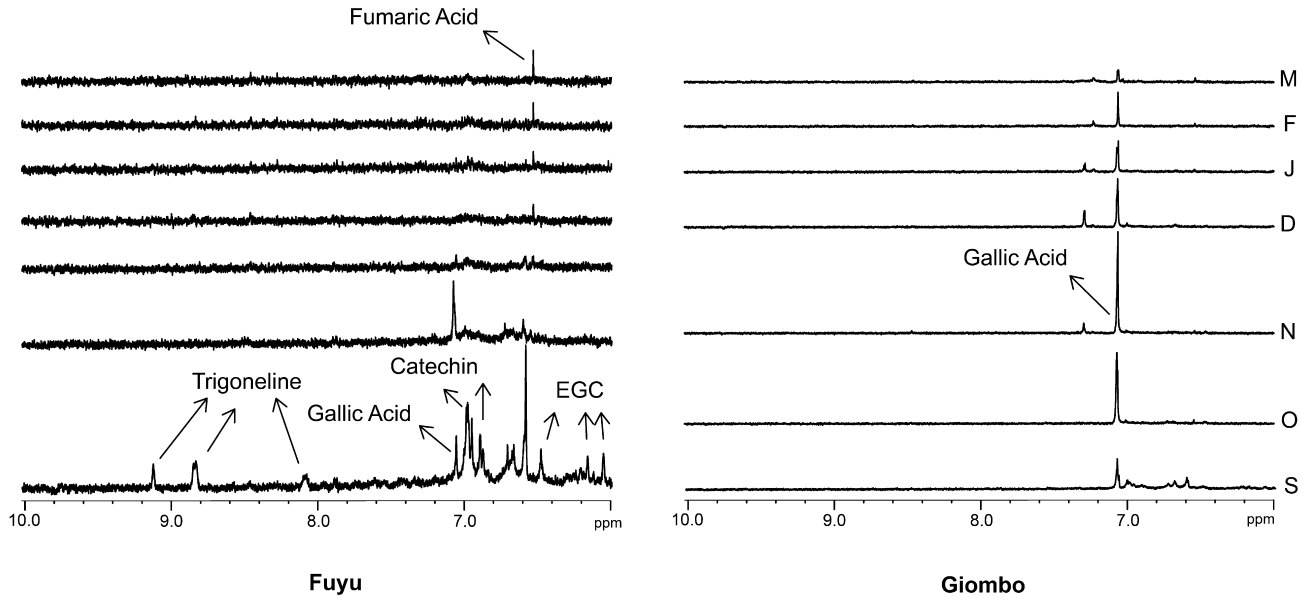

Fig. 5. Expansion of 6.0–10.0 ppm regions of the ^{1}H HR-MAS NMR spectra of persimmon during the process of development (from September to March). EGC: epigallocatechin.

3.2. Multivariate NMR data analysis

In order to discriminate the samples according to their developmental stage, as well as to compare the developmental process between the cultivars, a multivariate statistical analysis, PCA, was carried out.

3.2.1. 'Fuyu' and 'Giombo' PCA

Scores and loading plots obtained through the PCA analysis, applied to the bucketed spectra of 'Fuyu', are depicted in Fig. 6. The two first principal components explained 92.05% of total variance with PC1 representing 89.23% of the total variability and PC2 describing an additional 2.82%. According to the scores plotted with these two components, a well-defined discrimination of two groups has been observed. The first one was composed of September samples (FS) occupying the positive region of PC1 and PC2, whereas, the second group, constituted for samples of February (FF) and January (FJ), on negative side of PC1 and positive side of PC2. The evaluation of the PC1 and PC2 loadings allowed the determination of compounds with the highest impact on variance (Fig. 6). Organic acids, amino acids and aromatic compounds (all aforementioned and shown in Figs. 1 and 5) influenced the discrimination of FS samples. It is noteworthy that the bucket at 3.20–3.22 ppm, assigned to the methylic hydrogens of $N(CH_3)_3$ group of choline, also appeared as relevant for that discrimination (Pérez et al., 2010). For the group formed by FJ and FF samples, the bucket associated to the signal of α-glucose (bucket at 5.24–5.26 ppm) was responsible for the discrimination. The high dispersion of samples of October, November and December on different quadrants makes it difficult to correlate them with the PC's selected. However, based on NMR data, these samples were influenced by the presence of sucrose, as can be seen in Fig. 3. This is further supported by the bucket at 5.40–5.42 ppm on the positive side of PC1 and the negative side of PC2. Finally, samples from March did not form a concise group. For a clearer grouping of these samples, more data may be required to provide better statistical discrimination. However, among the samples analyzed, the partial discrimination could be attributed to buckets at 3.25–3.28 ppm and 4.12–4.14 ppm (assigned to β-glucose and fructose) on the negative side of PC1 and PC2.

For the data set obtained with the spectra of 'Giombo', a model using the two first components was built, and 90.34% of the total variance of data was explained (Fig. 6). The first principal component described 87.95% of the variance, whereas, the second component reported an additional amount of 2.39%. Samples of September were clustered on the positive region of PC1 and negative region of PC2 (Fig. 6). The analysis of these regions on loadings revealed the influence of the same compounds reported to FS. The grouping of the samples of February and March on the negative side of PC1 $\times$ PC2 plane was also observed. The buckets related to carbohydrate signals were responsible for that discrimination being possible to assign signals from the buckets at 5.24–5.26 ppm, 3.24–3.28 and 4.10–4.14 (α and β-glucose as well as fructose, respectively). The remaining samples (October, November, December and January) could not be discriminated since they were widely spread on the selected PC's. However, the bucket at 5.41–5.43 ppm suggested the influence of sucrose signal to those samples, which is in agreement with the increase of sucrose depicted in Fig. 4.

The multivariate analysis of NMR data allowed visualizing the influence of all metabolic changes at the same time, providing a comparative interpretation of samples from different months and cultivars. The two cultivars seem to develop in a similar manner: at the initial stage, there was an influence of organic acid, amino acid, polyphenols and choline, while for the rest of the months, the changes were related to the presence of carbohydrates. Sucrose levels varied during the middle of the growth process suggesting it has an important role, with the contribution of glucose and fructose becoming more pronounced towards the end of the growth. The grouping of samples from different months suggested that even though fruits are at different temporary stages they may present a similar metabolic profile. Such information might be useful for the development of postharvest strategies, for example, to ensure the most appropriate harvesting time or even to extend the shelf life and storage of fruits.

In addition, although, the persimmon skin colour has been used as the criterion for harvesting or even to assess the internal physicochemical changes that fruits undergo during maturity, our results suggested this correlation might not be always achieved (Besada & Salvador, 2011; Pérez et al., 2010; Salvador, Arnal, Carot, Carvalho,

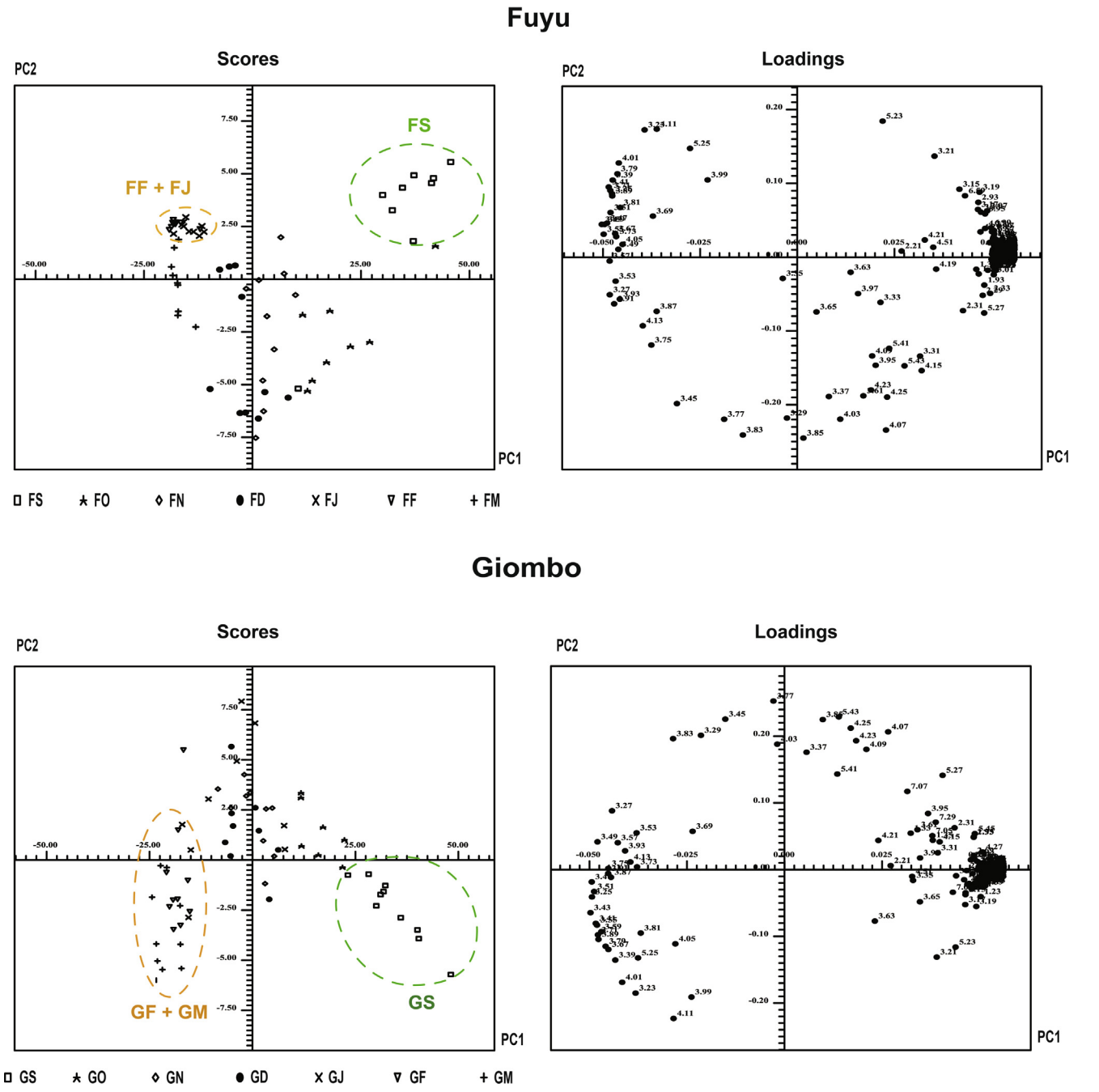

Fig. 6. Principal components analysis (PCA) of persimmon using 66 fruits for each cultivar. For 'Fuyu': scores plot of PC1 (89.23%) versus PC2 (2.82%), while for 'Giombo' scores plot of PC1 (87.95%) versus PC2 (2.39%). The respective loadings of plots of PC1 versus PC2 are also depicted, discriminating the compounds responsible for the separation of groups. The samples are mentioned by means of the initial letter of the cultivar (F for 'Fuyu' and G for 'Giombo') followed by the initial letter of months (from September to March).

& Jabaloyes, 2006; Salvador et al., 2007; Tessmer et al., 2016). For an actual definition of fruit stages, the chemical composition should be taken into consideration.

4. Conclusions

In summary, the HR-MAS NMR spectroscopy, combined with principal component analysis was shown to be an efficient approach to tracking the multiple chemical constituents of different persimmon cultivars ('Fuyu' and 'Giombo') throughout the fruit development. In both cultivars, the abundance of amino acids and organic acids was quite low. On the other hand, carbohydrates were more prominent and varied during the fruits growth cycle. The trend of these sugars could be described by an increase and decrease of sucrose, followed by a continued increase of glucose and fructose. In addition, at the initial stage of development, a noticeable difference was observed in the aromatic region of the two cultivars. For 'Giombo', the signal related to the gallic acid remained in the spectra until the end of the growth, while for 'Fuyu', signals of polyphenols were detected only during the first month of growth. Finally, the grouping of samples from different months in PCA, in the end of the fruit development, suggested that for an actual definition of fruit stages, the chemical composition should be taken into consideration. The findings obtained in this

work may lead to the improvement of the postharvest strategies applied to this crop. For a deeper understanding of the key mechanism involved in the persimmon fruit growth, a modeling approach should be performed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.06.133.

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