SUPPLEMENTARY MATERIAL

Monitoring of pistachio (Pistacia Vera) ripening by high field Nuclear Magnetic Resonance spectroscopy

Fabio Sciubba1*, Damiano Avanzato2, Angela Vaccaro2, Giorgio Capuani1, Mariangela Spagnoli1, Maria Enrica Di Cocco1, Irina Nikolova Tzareva3, Maurizio Delfini1

1Dipartimento di Chimica, Sapienza Università di Roma, Piazzale Aldo Moro, 5, 00185 Roma Italy.
2CRA-FRU- Centro di Ricerca per la Frutticoltura, Via Fioranello, 5, 00134 Roma, Italy
3Fruit Growing Institute, Ostromila, 12, 4004 Plovdiv, Bulgaria

*Corresponding Author: Dr. Fabio Sciubba, Dipartimento di Chimica, Sapienza Università di Roma, piazzale Aldo Moro 5, 00185 Roma, Italy. Tel. ++39 06 49913124 Fax: ++39 06 49913214; e-mail: fabio.sciubba@uniroma1.it
Abstract: The metabolic profiling of pistachio (*Pistacia vera*) aqueous extracts from two different cultivars, namely ‘Bianca’ and ‘Gloria’, was monitored over the months from May to September employing high field NMR spectroscopy. A large number of water-soluble metabolites were assigned by means of 1D and 2D NMR experiments. The change in the metabolic profiles monitored over time allowed the pistachio development to be investigated. Specific temporal trends of amino acids, sugars, organic acids and other metabolites were observed and analyzed by multivariate Partial Least Squares (PLS) analysis. Statistical analysis showed that while in the period from May to September there were few differences between the two cultivars, the ripening rate was different.

Keywords: NMR spectroscopy; pistachio; metabolic profiling; ripening

Figure S1 $^1$H NMR spectrum of Bianca pistachio hydroalcoholic extract at 298 K relatives to the months from May (upper) to September (lower).
Figure S2 Bidimensional NMR $^1$H–$^1$H TOCSY spectrum of Bianca pistachio hydroalcoholic extract at 298 K relatives to the month of May
Figure S3 Bidimensional NMR $^1$H-$^{13}$C HSQC spectrum of Bianca pistachio hydroalcoholic extract at 298 K relatives to the month of May
Figure S4 Bidimensional NMR $^1$H-$^{13}$C HMBC spectrum of Bianca pistachio hydroalcoholic extract at 298 K relatives to the month of May
Table S1 Table of the metabolites identified in the $^1$H NMR spectrum of the aqueous extracts of pistachios. Diagnostic resonances employed for quantification are evidenced in bold.

| COMPOUND                    | ASSIGNMENT                  | $\delta$ (ppm) | MULTIPLICITY |
|------------------------------|-----------------------------|----------------|--------------|
| **Organic acids**            |                             |                |              |
| Citric acid                 | $\alpha,\gamma$-CH         | 2.67           | d            |
|                             | $\alpha',\gamma'$-CH       | 2.71           | d            |
| Acetic acid                 | CH$_3$                      | 1.92           | s            |
| Shikimic acid               | CH$_2$-7                    | 2.20-2.78      | m            |
|                             | CH-6                        | 3.96           | dd           |
|                             | CH-5                        | 3.71           | m            |
|                             | CH-4                        | 4.40           | t            |
|                             | CH-3                        | 6.46           | m            |
| Shikimic acid 3-phosphate   | CH-3                        | 6.48           | m            |
| Fumaric acid                | $\alpha,\beta$-C=C         | 6.56           | s            |
| Gallic acid                 | CH-2,6                      | 7.05           | s            |
| Gallic acid ester           | CH-2,6                      | 7.06           | s            |
| Formic acid                 | HCOO                        | 8.46           | s            |
| Malic acid                  | $\alpha$-CH                | 4.31           | dd           |
|                             | $\beta,\beta'$-CH          | 2.38, 2.69     | dd           |
| Quinic Acid                 | CH$_2$-1                    | 1.89, 2.09     | dd           |
|                             | CH-2                        | 4.02           | m            |
|                             | CH-3                        | 3.55           | m            |
|                             | CH-4                        | 4.15           | m            |
|                             | CH$_2$-5                    | 2.06, 2.00     | dd           |
| **Amino acids**             |                             |                |              |
| Alanine                     | $\beta$-CH$_3$             | 1.49           | d            |
|                             | $\alpha$-CH                | 3.80           | q            |
| Threonine                   | $\gamma$-CH$_3$            | 1.33           | d            |
|                             | $\alpha$-CH                | 3.60           | m            |
|                             | $\beta$-CH                 | 4.27           | m            |
| Arginine                    | $\gamma$-CH$_2$            | 1.69           | m            |
|                             | $\beta$-CH$_2$             | 1.90           | m            |
|                             | $\delta$-CH$_2$            | 3.23           | t            |
|                             | $\alpha$-CH                | 3.77           | t            |
| Valine                      | $\gamma$-CH$_3$            | 0.99           | d            |
|                             | $\gamma'$-CH$_3$           | 1.05           | m            |
|                             | $\beta$-CH                 | 2.29           | m            |
|                             | $\alpha$-CH                | 3.62           | m            |
| Isoleucine                  | $\delta$-CH$_3$            | 0.95           | t            |
|                             | $\gamma'$-CH$_3$           | 1.02           | d            |
|                             | $\gamma$-CH                | 1.25           | m            |
|                             | $\gamma'$-CH$_3$           | 1.49           | m            |
|                             | $\beta$-CH                 | 1.99           | m            |
|                             | $\alpha$-CH                | 3.69           | m            |
| Leucine                     | $\delta,\delta'$-CH$_3$   | 0.97           | m            |
|                             | $\beta$-CH$_2$             | 1.73           | m            |
|                             | $\alpha$-CH                | 3.74           | m            |
| Aspartate                   | $\beta$-CH$_2$             | 2.70           | dd           |
|                             | $\beta'$-CH$_2$            | 2.79           | dd           |
|                             | $\alpha$-CH                | 3.91           | m            |
| Asparagine                  | $\beta$-CH$_3$             | 2.86           | dd           |
|                             | $\beta'$-CH$_3$            | 2.98           | dd           |
|                             | $\alpha$-CH                | 4.01           | m            |
| Tyrosine       | CH-5  | 6.88  | d  |
|----------------|-------|-------|----|
|                | CH-6  | 7.20  | d  |
| Phenylalanine  | CH-2,6| 7.32  | d  |
|                | CH-4  | 7.38  | d  |
|                | CH-3,5| 7.42  | d  |
| Glycine        | α-CH₂ | 3.57  | s  |
| Carbohydrates  |       |       |    |
| α-Glucose      | CH-1  | 5.25  | d  |
|                | CH-2  | 3.55  | n.d.|
|                | CH-3  | 3.72  | n.d.|
|                | CH-4  | 3.42  | n.d.|
|                | CH-5  | 3.84  | n.d.|
|                | CH₂-6,6’| 3.73, 3.90 | n.d.|
| β-Glucose      | CH-1  | 4.69  | d  |
|                | CH-2  | 3.26  | n.d.|
|                | CH-3  | 3.50  | n.d.|
|                | CH-4  | 3.42  | n.d.|
|                | CH-5  | 3.48  | n.d.|
|                | CH₂-6,6’| 3.74, 3.91 | n.d.|
| Sucrose        | GLC CH-1 | 5.42 | d  |
|                | CH-2  | 3.59  | n.d.|
|                | CH-3  | 3.79  | n.d.|
|                | CH-4  | 3.48  | n.d.|
|                | CH-5  | 3.85  | n.d.|
|                | CH₂-6 | 3.82  | n.d.|
|                | FRU CH₂-1’| 3.69 | n.d.|
|                | CH-3’ | 4.22  | d  |
|                | CH-4’ | 4.06  | n.d.|
|                | CH-5’ | 3.90  | n.d.|
|                | CH₂-6 | 3.82  | n.d.|
| Raffinose      | GLC CH-1 | 5.45 | d  |
|                | GAL CH-1| 5.01 | d  |
|                | FRU CH-3 | 4.22 | d  |
| Stachyose      | GLC CH-1 | 5.46 | d  |
|                | GAL CH-1| 5.02 | d  |
|                | FRU CH-3 | 4.22 | d  |
| Melibiose      | GLC α-CH-1| 5.22 | d  |
|                | GAL β-CH-1| 4.62 | d  |
|                | GAL CH-1| 4.97 | d  |
| Trehalose      | GLC CH-1,1’| 5.16 | d  |
| Miscellaneous metabolites |       |       |    |
| N-methyl-trans-4-hydroxy-L-proline | NCH₃ | 3.06 | s  |
|                | CH-2  | 4.21  | d  |
|                | CH-3a | 2.49  | tttt|
|                | CH-3b | 2.26  | ddd |
|                | CH-4  | 4.65  | ept |
|                | CH-5a | 3.20  | dt  |
|                | CH-5b | 3.96  | ddd |
| Coline         | N(CH₃)₃| 3.20  | s  |
| Trigonelline   | CH-1  | 9.1   | s  |
|                | CH-3,5| 8.82  | d  |
|                | CH-4  | 8.02  | m  |
|                | NCH₃ | 4.43  | s  |
| Allantoin      | CH    | 5.40  | s  |
| Glycin-betaine | N(CH₃)₃| 3.43  | s  |
|                | CH₂  | 3.88  | s  |
| Ethyl-lactate  | LA α-CH | 4.37 | q  |
Figure S5 Amount of metabolites observed in pistachio hydroalcoholic extracts as a function of specie (Bianca on the left and Gloria on the right) and ripening (From May to September starting from the left).
