SUPPLEMENTARY MATERIAL

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

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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 ^1H NMR spectrum of Bianca pistachio hydroalcoholic extract at 298 K relative to the months from May (upper) to September (lower).

Figure S2 Bidimensional NMR ^1H - ^1H TOCSY spectrum of Bianca pistachio hydroalcoholic extract at 298 K relatives to the month of May

Figure S3 Bidimensional NMR ^1H - ^{13}C HSQC spectrum of Bianca pistachio hydroalcoholic extract at 298 K relatives to the month of May

Figure S4 Bidimensional NMR ^1H - ^{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 ^1H NMR spectrum of the aqueous extracts of pistachios. Diagnostic resonances employed for quantification are evidenced in bold.

COMPOUND	ASSIGNMENT	δ (ppm)	MULTIPLICITY
<u>Organic acids</u>			
Citric acid	$\alpha,\gamma\text{-CH}$ $\alpha',\gamma'\text{-CH}$	2.67 2.71	d d
Acetic acid	CH_3	1.92	s
Shikimic acid	$\text{CH}_2\text{-7}$ CH-6 CH-5 CH-4 CH-3	2.20-2.78 3.96 3.71 4.40 6.46	m dd m t m
Shikimic acid 3-phosphate	CH-3	6.48	m
Fumaric acid	$\alpha,\beta\text{-C=C}$	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	$\alpha\text{-CH}$ $\beta,\beta'\text{-CH}$	4.31 2.38,2.69	dd dd
Quinic Acid	$\text{CH}_2\text{-1}$ CH-2 CH-3 CH-4 $\text{CH}_2\text{-5}$	1.89, 2.09 4.02 3.55 4.15 2.06, 2.00	dd m m m dd
<u>Amino acids</u>			
Alanine	$\beta\text{-CH}_3$ $\alpha\text{-CH}$	1.49 3.80	d q
Threonine	$\gamma\text{-CH}_3$ $\alpha\text{-CH}$ $\beta\text{-CH}$	1.33 3.60 4.27	d m m
Arginine	$\gamma\text{-CH}_2$ $\beta\text{-CH}_2$ $\delta\text{-CH}_2$ $\alpha\text{-CH}$	1.69 1.90 3.23 3.77	m m t t
Valine	$\gamma\text{-CH}_3$ $\gamma'\text{-CH}_3$ $\beta\text{-CH}$ $\alpha\text{-CH}$	0.99 1.05 2.29 3.62	d m m m
Isoleucine	$\delta\text{-CH}_3$ $\gamma'\text{-CH}_3$ $\gamma\text{-CH}$ $\gamma'\text{-CH}$ $\beta\text{-CH}$ $\alpha\text{-CH}$	0.95 1.02 1.25 1.49 1.99 3.69	t d m m m m
Leucine	$\delta,\delta'\text{-CH}_3$ $\beta\text{-CH}_2$ $\alpha\text{-CH}$	0.97 1.73 3.74	m m m
Aspartate	$\beta\text{-CH}$ $\beta'\text{-CH}$ $\alpha\text{-CH}$	2.70 2.79 3.91	dd dd m
Asparagine	$\beta\text{-CH}$ $\beta'\text{-CH}$ $\alpha\text{-CH}$	2.86 2.98 4.01	dd dd m

Tyrosine	CH-5 CH-6	6.88 7.20	d d
Phenylalanine	CH-2,6 CH-4 CH-3,5	7.32 7.38 7.42	d d d
Glycine	α-CH₂	3.57	s
<u>Carbohydrates</u>			
α -Glucose	CH-1 CH-2 CH-3 CH-4 CH-5 CH ₂ -6,6'	5.25 3.55 3.72 3.42 3.84 3.73, 3.90	d n.d. n.d. n.d. n.d. n.d.
β -Glucose	CH-1 CH-2 CH-3 CH-4 CH-5 CH ₂ -6,6'	4.69 3.26 3.50 3.42 3.48 3.74, 3.91	d n.d. n.d. n.d. n.d. n.d.
Sucrose	GLC CH-1 CH-2 CH-3 CH-4 CH-5 CH ₂ -6 FRU CH ₂ -1' CH-3' CH-4' CH-5' CH ₂ -6	5.42 3.59 3.79 3.48 3.85 3.82 3.69 4.22 4.06 3.90 3.82	d n.d. n.d. n.d. n.d. n.d. n.d. d n.d. n.d. n.d.
Raffinose	GLC CH-1 GAL CH-1 FRU CH-3	5.45 5.01 4.22	d d d
Stachyose	GLC CH-1 GAL CH-1 FRU CH-3	5.46 5.02 4.22	d d d
Melibiose	GLC α-CH-1 GLC β -CH-1 GAL CH-1	5.22 4.62 4.97	d d d
Trehalose	GLC CH-1,1'	5.16	d
<u>Miscellaneous metabolites</u>			
N-methyl-trans-4-hydroxy-L-proline	NCH ₃ CH-2 CH-3a CH-3b CH-4 CH-5a CH-5b	3.06 4.21 2.49 2.26 4.65 3.20 3.96	s dd tttt ddd ept dt dd
Coline	N(CH₃)₃	3.20	s
Trigonelline	CH-1 CH-3,5 CH-4 NCH ₃	9.1 8.82 8.02 4.43	s d m s
Allantoin	CH	5.40	s
Glycin-betaine	N(CH₃)₃ CH ₂	3.43 3.88	s s
Ethyl-lactate	LA α -CH	4.37	q