Supplementary Materials: Comprehensive chemical characterization of the Pistacia Vera fruits by an innovative NMR approach

Archimede Rotondo, Francesca Rigano and Luigi Mondello

1. Supplementary data

After the extraction throughput described in the main text, the obtained samples look like Figure S1 below.

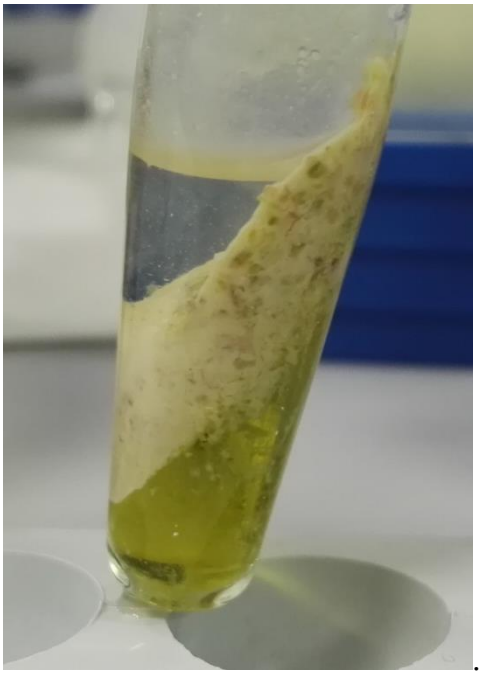

Figure S1. Sample coming from the treatment with deuterated chloroform and water after the centrifuge cycles. Samples for the NMR analysis are taken from the upper transparent solution called PWS and from the light green bottom standing solution called PO.

2. Materials and Methods

Table S1. Assignment of many resonances (ppm) related to specific metabolites of the water soluble fraction of pistacia vera (PWS). These data were definitely confirmed by 2D-NMR experiments.

Compounds	Identification Symbol	Chemical Group	Protons per Residue	cs (ppm)	Multiciplity	J (Hz)
Aminoacids and Metabolites						
Isoleucine	ISO	CH_2-CH_3		0.916	t	7.4
		CH-CH_3		0.986	d	7
		γ-CH2	2	1.236–1.440	m,m	-
		β-CH		1.953	bs	-
		α-CH		3.621		
Leucine	LEU					
		CH_3/CH_3'	6	0.937	t	4
		γ-CH/β-CH2	2	1.663	om	-
		a-CH	1	3.685	t	-
Valine	VAL					

		CH_3	3	0.967	d	6.9
		CH_3'	3	1.019	d	7.2
		β-CH	1	2.258	m	-
		α-CH	1	3.593	d	4.2
Arginine	ARG					
		γ-CH_2'	1	1.602	m	-
		γ-CH_2''	1	1.701	m	-
		β-CH_2	2	1.896	m	-
		δ-CH2	2	3.225	t	7
		α-CH	1	3.753	t	6
Glutamate	GLU					
		β-CH2	2	2.955–2.050	m	
		γ-CH_2	2	2.329	m	
		α-CH	1	3.744	t	
Asparagine	ASN					
		β-CH2'	1	2.859	dd	13.4-5.8 (abx system)
		β-CH2''	1	2.939	dd	17.0, 4.1
		α-CH	1	3.993	m	-
Lysine	LYS					
		α-CH2	2	3.740	t	-
		β-CH2	2	1.882	m	-
		γ-CH2	2	1.460–1.510	m	-
		δ-CH2	2	1.660–1.730	m	-
Proline	PRO					
		γ-CH2	2	1.991–2.043	m	
		β'-CH2	1	2.05	m	
		β-CH2	1	2.325	m	
		δ'-CH2	1	3.310	m	
		δ-CH2	1	3.397	m	
		α-CH	1	4.056	t	
Threonine	THR					
		CH_3	3	1.31	d	7
		β–CH	1	4.24	p	-
		α-CH	1	3.575	d	7
Alanine	ALA					
		CH3	3	1.459	d	6
		α-CH	1	3.769	q	6
Tyrosine	TYR					
		CH_2	1	2.05	m	
		3,5-CH	2	6.876	d	8.5
		2,6-CH	2	7.172	d	8.5
3-Indole Acetic acid	3-IAA					
		3-CH	1	7.463	s	
		4-CH	1	7.600	d	
		5-CH	1	7.239	t	
		6-CH	1	7.313	t	
		7-CH	1	7.636	d	
Propanediol	PDO					
		CH3	3	1.120	d	
		CH2'	1	3.428	dd	

		CH2"	1	3.528	dd	
		CH	1	3.864	m	
N-Methyl-4-hydroxy-proline	MHP					
		3-CH2-anti-OH	1	2.230	td	
		3-CH2-syn-OH	1	2.47	ddt	
		5-CH2-syn-OH	1	3.17	d	
		5-CH2-anti-OH	1	3.93	dd	
		2-CH	1	4.17	dd	
		4-CH	1	4.62	p	
g-ammino-butirric acid	GABA					
		α-CH2	2	2.276	t	7.5
		β-CH2	2	1.884	p	-
		γ-CH2	2	2.997	t	7.4
Trigonelline	TRG					
		1-CH	1	9.103	s	
		3,5-CH	2	8.824–8.808	od	
		4-CH	1	8.07	t	
		N-Me	3	-	s	
Organic Acids and Similar Compounds						
Lactate	LAC					
		CH_3	3	1.31	d	7
		α-CH	1	4.1	q	7
Malic Acid	MA					
		α-CH	1	4.294	t	
		β-CH2	1	2.650	dd	
		β'-CH2	1	2.360	dd	
Citric Acid	CA					
		CH2'	2	2.545	d	16
		CH2"	2	2.689	d	16
Shikimic Acid	SHA					
		3-CH	1	6.423	m	
		4-CH	1	4.380	t	
		6-CH	1	3.996		
		5-CH	1	3.691		
		7-CH2	2	2.747–2.179	mm	
Formic Acid						
		HCOO	1	8.329	s	
Sugars and Related Compounds						
α-D-Glucose	α-GLC					
		1-CH	1	5.220	d	3.6
		2-CH	1	3.52	dd	3.6 and 9.6
		3-CH	1	3.694	t	9.6
		4-CH	1	3.395	t	9.8
		5-CH	1	3.810	m	
		6-CH_2	2	3.799–3.998	m	
β-D-Glucose	β-GLC					
		1-CH	1	4.628	d	9.1
		2-CH	1	3.225	t	9.1

		3-CH	1	3.470	t	9
		4-CH	1	3.382	t	9
		5-CH	1	3.443	m	-
		6-CH_2	2	3.746–3.881	mm	-
β-D-Galactose	β-GAL					
		1-CH	1	4.565	d	-
		2-CH	1	3.474	t	-
		3-CH	1	3.630	dd	-
		4-CH	1	3.911	d	-
		5-CH	1	-	m	-
		6-CH_2	2	-	m	-
α-D-Galactose	α-GAL					
		1-CH	1	5.25	D	3.7
		2-CH	1	3.786	Dd	4 and 12
		3-CH	1	3.837	dd	3.4 and 12
		4-CH	1	3.973	broad	
		5-CH	1	3.670	M	
		6-CH_2	2	3.70–3.72	mm	
Rafinose	RAF					
		GAL1-CH	1	4.974	d	4
		GAL2-CH	1	3.819	dd	10 & 4
		GAL3-CH	1	3.881	dd	10 & 3
		GAL4-CH	1	3.990	dd	3 & 0.5
		GAL5-CH	1	4.028	m	
		GAL6-CH_2	2	-		
		GLC1-CH	1	5.409	d	
		GLC2-CH	1	3.558	dd	
		GLC3-CH	1	3.457	t	
		GLC4-CH	1	3.740	t	
		GLC5-CH	1	4.030	m	
		GLC6-CH_2	2	3.810	m	
		FRU1-CH_2	2	3.661	bs	
		FRU3-CH	1	4.208	d	8.80
		FRU4-CH	1	4.045	t	8
		FRU5-CH	1	3.880	m	
		FRU6-CH_2	2	3.810–3.759	mm	
Sucrose	SUC					
		GLC1-CH	1	5.389	d	
		GLC2-CH	1	3.541	dd	
		GLC3-CH	1	3.743	t	
		GLC4-CH	1	3.456	t	
		GLC5-CH	1	3.834	m	
		GLC6-CH_2	2	3.804	m	
		FRU1-CH_2	2	3.663	bs	
		FRU3-CH	1	4.198	d	9.00
		FRU4-CH	1	4.032	t	8
		FRU5-CH	1	3.876	m	
		FRU6-CH_2	2	3.807–3.865	mm	
Myoinolsitol	MYI					
		2-CH	1	4.040	t	4
		4,6-CH	2	3.600	t	10
		1,3-CH	2	3.510	dd	10, 4

		5-CH	1	3.260	t	10
Uridine	URI					
		U-6-CH	1	7.853	d	
		U-5-CH	1	5.877	d	
		R-1-CH	1	5.791	d	
		R-2-CH	1	4.288	t	
		R-3-CH	1	4.208	t	
		R-4-CH	1	4.197	m	
		R-5-CH_2	2	4.186–4.109	m	
Adenosine	ADE					
		A-2-CH	1	8.318	s	
		A-8-CH	1	8.243		
		R-1-CH	1	6.056		
		R-2-CH	1	3.828		
		R-3-CH	1	3.904		
		R-4-CH	1	4.333		
		R-5-CH'	1	4.286		
		R-5-CH''	1	4.425		
Inosine	INO					
		I-2-CH	1	8.243	bd	
		I-8-CH	1	8.176	bs	
		R-1-CH	1	6.040		
		R-2-CH	1	3.814		
		R-3-CH	1	3.887		
		R-4-CH	1	4.263		
		R-5-CH	1	4.411		
		R-5-CH	1	4.411		

Table S2. Quantification of all the 47 variables (39 from PWS and 8 from PO) according to the NMR integration of water soluble (PWS) and $CDCl_3$ soluble (PO) fractions. Data and relative uncertainty are recovered by the innovative MARA-NMR processing: a) from 65 PWS ^{1}H integrations, b) from 20 PO ^{1}H and 19 PO ^{13}C neat integrations.

Provenience	California	California	California	California	California	California	California	California	California	California	Turkey	Turkey	Turkey	Turkey	Turkey
Sample	**S_1_1**	**S_1_2**	**S_1_3**	**S_1_4**	**S_1_5**	**S_2_1**	**S_2_2**	**S_2_3**	**S_2_4**	**S_2_5**	**S_3_1**	**S_3_2**	**S_3_3**	**S_3_4**	**S_3_5**
TRG	0.24	0.23	0.24	0.23	0.23	0.26	0.20	0.22	0.18	0.23	0.24	0.24	0.23	0.22	0.23
FA	0.21	0.20	0.23	0.21	0.20	0.24	0.18	0.19	0.11	0.20	n.d	n.d	n.d	n.d	n.d
INO	0.57	0.57	0.63	0.57	0.58	0.76	0.61	0.60	0.33	0.60	0.23	0.21	0.26	0.22	0.24
UDP	0.87	0.92	1.00	0.88	0.88	1.07	1.02	1.02	0.65	1.00	0.22	0.22	0.33	0.25	0.26
URI	0.53	0.56	0.72	0.55	0.52	0.72	0.38	0.62	0.18	0.51	0.54	0.49	0.56	0.39	0.49
I3AA	0.79	0.76	0.81	0.79	0.79	0.81	0.79	0.68	0.45	0.72	0.57	0.57	0.58	0.55	0.56
PHE+TRP	0.26	0.28	0.25	0.28	0.27	0.21	0.31	0.21	0.33	0.32	0.19	0.19	0.18	0.18	0.18
TYR	0.66	0.65	0.66	0.65	0.66	0.73	0.72	0.80	0.90	0.83	0.40	0.38	0.46	0.47	0.43
GA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.13	n.d	0.25	0.23	0.22	0.23	0.24
FUMA	0.57	0.56	0.60	0.57	0.57	0.46	0.47	0.43	0.41	0.44	0.36	0.35	0.40	0.39	0.38
SHA	0.45	0.48	0.51	0.47	0.48	0.38	0.42	0.53	0.48	0.45	2.26	2.26	2.55	2.49	2.39
Q3G	0.43	0.46	0.57	0.43	0.44	0.36	0.30	0.39	0.20	0.32	0.45	0.43	0.52	0.37	0.44
RAF	24.15	23.76	24.47	24.17	24.14	24.07	23.79	22.98	22.70	23.39	20.27	20.30	19.69	19.67	19.98
SUC	91.86	92.36	91.45	92.29	91.99	66.93	67.84	62.14	59.41	64.08	36.73	36.34	40.26	41.69	38.75
GAL	5.69	5.42	5.56	5.21	5.31	5.99	6.19	6.22	6.23	6.16	4.52	4.95	4.60	4.64	4.68
GLC	7.47	7.55	8.01	8.86	8.63	26.24	26.80	31.12	32.41	29.14	5.31	5.22	5.36	5.29	5.29
XIL	1.75	1.72	1.82	1.73	1.75	1.84	1.83	1.72	1.58	1.74	1.84	1.67	2.11	2.11	1.93
FRC	23.89	23.45	25.28	23.48	23.82	25.46	22.83	53.37	55.85	40.20	12.55	12.27	10.45	6.98	10.21
PRO	3.62	3.74	3.91	3.81	3.77	4.24	4.43	4.60	4.24	4.33	5.80	5.78	6.41	6.80	6.20
MYI	6.42	6.53	7.09	6.77	6.70	7.46	7.52	9.12	8.74	8.50	7.19	7.13	8.75	8.78	7.94
CAR	0.77	0.76	0.79	0.77	0.77	0.52	0.50	0.47	0.46	0.49	0.25	0.24	0.27	0.25	0.25
CHN	2.39	2.30	2.38	2.27	2.33	3.40	3.06	3.32	3.23	3.26	2.74	2.75	2.80	2.78	2.77
MHP	25.66	25.48	25.48	25.48	25.53	24.60	24.60	24.60	24.61	24.60	12.47	12.47	12.47	12.47	12.47
GABA	4.02	4.05	4.10	4.10	4.06	5.63	5.70	5.78	5.79	5.72	2.74	2.70	3.05	3.11	2.90
ASN	0.84	0.89	0.95	0.84	0.85	0.70	0.75	1.05	0.81	0.91	1.50	1.67	1.94	2.10	1.89
ASP	3.27	3.29	3.43	3.37	3.34	3.15	3.01	3.57	3.88	3.64	2.99	3.14	2.99	2.86	2.94
CA	10.38	10.29	10.70	10.50	10.47	8.09	7.67	7.75	7.66	7.85	13.07	12.96	13.18	13.09	13.08
MAL	3.81	3.84	3.89	3.77	3.82	2.53	4.09	3.11	2.39	2.64	2.75	2.81	3.15	3.45	3.10
GLU	7.95	7.52	8.14	7.46	7.77	8.68	7.27	8.08	8.04	8.25	2.71	2.74	2.84	2.41	2.65
ARG	5.83	5.76	5.77	5.71	5.74	6.93	6.89	6.63	6.65	6.85	4.35	4.40	4.78	4.85	4.60
ACA	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.68	0.23	1.50	1.46	1.79	1.73	1.64
ILE	0.60	0.60	0.62	0.59	0.61	0.73	0.73	0.76	0.78	0.75	0.27	0.28	0.30	0.30	0.29
LEU	1.93	1.93	1.95	1.99	1.97	2.40	2.42	2.56	2.67	2.46	0.82	0.76	0.83	0.87	0.82

VAL	0.62	0.60	0.63	0.60	0.61	1.00	0.97	1.08	1.10	1.04	0.45	0.45	0.50	0.49	0.47
ALA	5.70	5.61	5.75	5.63	5.68	6.03	5.62	5.62	5.32	5.56	3.23	3.06	3.84	3.88	3.55
PDO	1.46	1.46	1.47	1.47	1.46	0.51	0.52	0.62	0.78	0.69	0.52	0.51	0.50	0.51	0.51
PA	0.95	0.97	1.00	0.96	0.97	0.97	1.06	0.89	0.68	0.89	0.47	0.44	0.50	0.51	0.48
ACLC	0.62	0.57	0.62	0.58	0.60	0.75	0.92	0.70	0.47	0.76	0.48	0.41	0.50	0.51	0.48
LAC+THR	1.10	1.05	1.10	1.06	1.08	0.15	1.23	1.16	1.27	1.35	0.87	0.83	0.97	0.95	0.91
TG%	94.58	94.50	94.14	94.95	94.54	94.41	94.48	89.08	89.17	91.79	96.40	95.98	95.94	95.94	96.06
1,2DG%	2.64	2.73	2.42	2.01	2.45	2.49	2.51	7.31	7.20	4.88	1.82	2.10	2.10	2.10	2.03
1,3DG%	2.78	2.76	3.43	3.04	3.01	3.10	3.02	3.61	3.63	3.34	1.78	1.92	1.96	1.96	1.90
SAT%	16.62	16.68	17.31	17.05	16.92	17.53	17.58	15.49	15.43	16.51	14.01	13.10	16.17	16.17	14.86
O%	53.39	53.25	54.56	54.88	54.02	54.94	54.91	59.09	59.25	57.05	71.09	71.69	72.46	72.43	71.92
L%	29.15	29.20	27.55	27.59	28.37	26.84	26.79	24.80	24.74	25.79	14.54	14.83	11.02	11.06	12.86
Ln%	0.85	0.87	0.57	0.48	0.69	0.70	0.71	0.62	0.59	0.65	0.36	0.39	0.34	0.34	0.36
STR	11454	11726	8413	8881	10118	9791	7849	7741	8158	8385	4515	5619	6662	6662	5865
Provenience	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Turkey**	**Iran**	**Iran**	**Iran**	**Iran**	**Iran**
Sample	**S_4_1**	**S_4_2**	**S_4_3**	**S_4_4**	**S_4_5**	**S_5_1**	**S_5_2**	**S_5_3**	**S_5_4**	**S_5_5**	**S_6_1**	**S_6_2**	**S_6_3**	**S_6_4**	**S_6_5**
TRG	0.34	0.33	0.38	0.40	0.35	0.31	0.26	0.27	0.27	0.28	0.42	0.42	0.41	0.41	0.41
FA	0.11	0.09	0.13	0.12	0.11	n.d	n.d	n.d	n.d	n.d	0.10	0.11	0.11	0.11	0.11
INO	0.36	0.34	0.45	0.41	0.38	0.29	0.24	0.30	0.24	0.27	0.47	0.49	0.48	0.50	0.47
UDP	0.27	0.22	0.59	0.61	0.41	0.26	0.16	0.33	0.16	0.22	0.64	0.76	0.77	0.74	0.72
URI	0.52	0.54	0.87	0.58	0.63	1.05	0.73	0.69	0.55	0.73	0.60	0.73	0.77	0.76	0.76
I3AA	0.71	0.69	0.75	0.75	0.72	1.01	0.92	0.81	0.88	0.90	0.91	0.90	0.88	0.88	0.89
PHE+TRP	0.25	0.26	0.26	0.26	0.26	0.34	0.37	0.34	0.29	0.34	0.17	0.19	0.18	0.18	0.18
TYR	0.79	0.78	0.77	0.78	0.78	0.42	0.45	0.46	0.46	0.45	0.48	0.47	0.47	0.47	0.47
GA	0.23	0.28	0.33	0.26	0.27	0.35	0.37	0.33	0.31	0.34	n.d	n.d	n.d	n.d	n.d
FUMA	0.85	0.87	0.87	0.85	0.86	0.78	0.76	0.76	0.75	0.76	1.36	1.33	1.35	1.37	1.35
SHA	4.89	4.83	5.09	4.78	4.90	3.74	3.86	3.55	3.44	3.65	0.73	0.72	0.80	0.74	0.75
Q3G	0.48	0.54	0.63	0.57	0.55	1.24	0.86	0.63	0.65	0.83	0.49	0.63	0.61	0.61	0.60
RAF	24.90	25.02	27.51	28.07	26.38	25.53	25.03	23.84	24.19	24.65	26.35	26.21	25.23	25.23	25.75
SUC	63.23	63.59	63.83	65.30	63.99	56.03	55.68	53.28	54.28	54.82	65.14	65.79	62.29	62.06	63.82
GAL	4.85	4.92	5.35	5.54	5.16	5.00	5.05	4.68	4.69	4.86	4.66	4.72	4.46	4.52	4.59
GLC	6.29	6.35	6.78	6.56	6.49	6.59	6.95	6.35	6.26	6.54	4.80	4.85	4.76	4.60	4.75
XIL	2.53	2.54	2.62	2.68	2.59	3.11	3.01	2.84	2.84	2.95	1.49	1.53	1.69	1.65	1.59
FRC	9.64	8.67	17.26	15.02	13.52	12.99	12.38	13.72	13.03	13.19	21.44	21.10	20.20	20.19	20.81
PRO	10.25	10.42	10.60	10.85	10.54	10.16	10.15	9.74	9.84	9.97	3.42	3.53	3.94	3.80	3.67
MYI	13.80	14.23	13.41	13.26	13.71	10.45	10.51	10.28	10.46	10.46	4.30	4.48	4.86	4.85	4.63
CAR	0.62	0.59	0.58	0.56	0.59	0.34	0.33	0.32	0.32	0.33	0.33	0.32	0.33	0.33	0.33
CHN	3.86	3.95	4.06	4.02	4.03	3.15	3.22	3.16	3.14	3.15	2.99	3.02	2.99	3.00	3.00

MHP	19.71	19.71	19.71	19.71	19.71	15.04	15.04	15.04	15.04	15.04	30.95	30.95	30.95	30.95	30.95
GABA	7.42	7.57	7.76	7.79	7.63	4.80	4.74	4.64	4.79	4.74	7.40	7.51	7.22	7.18	7.33
ASN	2.09	2.50	2.05	1.91	2.16	2.67	2.55	2.73	2.43	2.65	0.95	1.01	1.07	1.02	1.00
ASP	3.67	3.31	3.64	4.16	3.73	3.95	3.98	3.89	3.88	3.92	4.22	4.26	3.93	4.04	4.11
CA	14.56	14.60	15.44	15.77	15.08	17.85	17.90	17.22	17.31	17.58	16.02	15.91	15.15	15.35	15.60
MAL	5.88	6.23	5.81	5.27	5.82	7.49	7.58	6.83	6.74	7.13	4.10	4.17	4.57	4.58	4.36
GLU	5.97	5.51	5.73	5.83	5.73	2.17	1.76	2.37	2.32	2.15	7.30	6.92	6.69	6.83	6.93
ARG	11.47	11.88	11.09	11.67	11.53	8.87	8.61	8.25	8.49	8.56	8.94	8.94	9.30	9.65	9.21
ACA	3.81	3.59	3.75	3.16	3.69	2.77	2.74	2.73	2.67	2.74	n.d	n.d	n.d	n.d	n.d
ILE	0.52	0.53	0.53	0.53	0.53	0.31	0.31	0.31	0.31	0.31	0.46	0.44	0.44	0.45	0.45
LEU	1.56	1.48	1.53	1.50	1.51	0.74	0.93	0.94	0.88	0.87	1.72	1.72	1.65	1.64	1.68
VAL	0.89	0.88	0.88	0.87	0.88	0.58	0.59	0.58	0.58	0.58	0.52	0.51	0.52	0.51	0.52
ALA	9.07	9.23	8.03	7.50	8.45	4.88	4.86	4.88	4.97	4.92	2.39	2.39	2.43	2.44	2.41
PDO	0.70	0.71	0.75	0.82	0.74	0.68	0.69	0.65	0.68	0.68	0.13	0.14	0.14	0.14	0.14
PA	1.00	1.03	0.98	1.07	1.01	0.62	0.58	0.53	0.60	0.59	0.71	0.72	0.70	0.70	0.71
ACLC	0.53	0.53	0.61	0.67	0.58	0.39	0.41	0.41	0.43	0.42	0.57	0.55	0.57	0.61	0.57
LAC+THR	1.64	1.75	1.77	1.92	1.72	1.89	1.17	1.18	1.18	1.20	0.82	0.77	0.81	0.79	0.80
TG%	96.25	96.25	96.18	96.18	96.21	95.95	95.95	95.77	95.78	95.86	97.86	97.84	96.28	96.28	97.24
1,2DG%	1.81	1.81	2.07	2.07	1.94	2.07	2.07	2.32	2.34	2.20	2.14	2.16	2.03	2.03	2.08
1,3DG%	1.94	1.94	1.74	1.74	1.84	1.99	1.98	1.90	1.88	1.94	n.d	n.d	1.70	1.70	0.67
SAT%	14.36	14.36	15.29	15.29	14.83	15.19	15.19	14.95	14.97	15.07	17.79	17.82	15.05	15.05	16.43
O%	71.29	71.32	71.10	71.10	71.20	71.00	71.00	69.96	69.92	70.47	51.01	50.98	60.32	60.08	55.60
L%	13.94	13.91	13.13	13.13	13.53	13.38	13.38	14.61	14.63	14.00	30.60	30.59	24.16	24.40	27.44
Ln%	0.42	0.42	0.48	0.48	0.45	0.43	0.43	0.47	0.48	0.45	0.60	0.61	0.47	0.47	0.54
STR	6224	6224	5792	5792	6008	6085	5979	6984	6373	6355	10423	8907	8402	8402	9033
Provenience	**Iran**	**Iran**	**Iran**	**Iran**	**Iran**	**Greece**	**Greece**	**Greece**	**Greece**	**Greece**	**Sicily**	**Sicily**	**Sicily**	**Sicily**	**Sicily**
Sample	**S_7_1**	**S_7_2**	**S_7_3**	**S_7_4**	**S_7_5**	**S_8_1**	**S_8_2**	**S_8_3**	**S_8_4**	**S_8_5**	**S_9_1**	**S_9_2**	**S_9_3**	**S_9_4**	**S_9_5**
TRG	0.44	0.42	0.41	0.40	0.41	0.27	0.27	0.27	0.27	0.27	0.19	0.19	0.19	0.19	0.19
FA	0.12	0.11	0.11	0.10	0.11	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
INO	0.47	0.49	0.52	0.48	0.49	0.32	0.32	0.37	0.34	0.33	0.19	0.19	0.19	0.19	0.19
UDP	0.71	0.70	0.78	0.72	0.72	0.15	0.30	0.29	0.34	0.31	0.23	0.21	0.23	0.24	0.26
URI	0.76	0.75	0.79	0.74	0.76	0.32	0.42	0.39	0.40	0.39	0.40	0.39	0.39	0.41	0.43
I3AA	0.91	0.91	0.92	0.88	0.91	0.36	0.39	0.41	0.41	0.40	0.44	0.45	0.43	0.42	0.43
PHE+TRP	0.25	0.28	0.26	0.28	0.27	0.23	0.22	0.22	0.22	0.22	0.10	0.10	0.11	0.11	0.11
TYR	0.58	0.57	0.56	0.56	0.57	0.53	0.55	0.57	0.57	0.56	0.35	0.35	0.35	0.35	0.34
GA	n.d	n.d	n.d	n.d	n.d	0.08	n.d	0.09	0.09	0.08	0.09	0.09	0.09	0.09	0.09
FUMA	1.49	1.46	1.36	1.35	1.41	0.51	0.53	0.60	0.60	0.56	0.80	0.81	0.77	0.77	0.79
SHA	1.18	1.03	1.16	1.34	1.18	2.92	2.78	2.87	2.83	2.85	1.49	1.49	1.68	1.68	1.59

Q3G	0.68	0.67	0.67	0.70	0.68	0.36	0.32	0.34	0.34	0.33	0.31	0.31	0.32	0.33	0.35
RAF	35.88	36.48	34.21	35.21	35.45	19.77	19.39	18.89	19.10	19.29	21.54	21.48	20.75	20.61	21.10
SUC	41.50	42.03	39.42	40.23	40.79	38.94	39.73	40.87	40.57	40.03	35.62	35.77	36.33	36.44	36.04
GAL	6.97	6.50	6.00	6.24	6.43	4.58	4.78	4.70	4.71	4.69	4.02	4.01	3.88	3.87	3.94
GLC	3.84	3.66	3.64	3.86	3.75	6.21	6.00	6.56	6.42	6.30	4.24	4.24	4.28	4.22	4.24
XIL	1.87	1.90	1.99	1.96	1.93	2.22	2.15	2.37	2.37	2.28	1.27	1.27	1.28	1.27	1.28
FRC	19.33	19.11	17.98	17.27	18.37	12.24	12.52	13.02	12.95	12.66	12.20	12.12	11.42	11.50	11.75
PRO	3.56	3.67	3.81	3.75	3.70	4.93	4.87	5.10	5.15	5.01	3.43	3.43	3.70	3.68	3.56
MYI	6.50	6.62	6.80	6.56	6.63	5.43	5.38	5.76	5.71	5.57	3.19	3.19	3.58	3.57	3.38
CAR	0.39	0.38	0.39	0.37	0.38	0.57	0.58	0.55	0.55	0.56	0.25	0.25	0.27	0.27	0.26
CHN	3.66	3.71	3.57	3.55	3.62	2.19	2.19	2.23	2.24	2.21	1.86	1.86	1.87	1.87	1.87
MHP	27.95	27.95	27.95	27.95	27.95	13.64	13.64	13.64	13.64	13.64	9.72	9.72	9.72	9.72	9.72
GABA	7.89	8.18	7.84	7.97	7.97	3.47	3.59	3.75	3.77	3.65	4.27	4.27	4.12	4.11	4.19
ASN	2.22	2.11	2.23	2.29	2.22	0.56	0.54	0.58	0.61	0.56	1.02	1.04	1.09	1.08	1.12
ASP	5.96	5.69	5.79	6.05	5.87	1.81	1.79	1.99	1.97	1.89	1.51	1.51	1.40	0.91	1.33
CA	15.01	15.19	14.83	14.99	15.01	6.70	6.76	7.38	7.38	7.06	9.39	9.40	9.08	9.09	9.24
MAL	5.22	5.11	4.25	4.44	4.75	4.83	4.70	5.17	5.29	5.00	5.48	5.50	5.58	6.04	5.65
GLU	8.36	8.51	8.04	7.48	8.10	3.53	3.84	3.80	3.55	3.68	3.43	3.42	3.06	2.58	3.13
ARG	12.31	12.72	11.95	12.41	12.35	4.27	4.37	4.76	4.67	4.51	3.24	3.24	3.19	3.19	3.21
ACA	0.96	0.92	1.06	1.15	1.02	1.07	0.94	1.19	1.21	1.10	0.40	0.39	0.51	0.52	0.46
ILE	0.60	0.57	0.57	0.57	0.58	0.43	0.44	0.47	0.47	0.45	0.29	0.29	0.28	0.28	0.29
LEU	1.88	1.99	1.82	1.87	1.89	0.91	0.96	1.01	0.99	0.97	0.79	0.79	0.75	0.75	0.77
VAL	0.84	0.82	0.85	0.84	0.84	0.54	0.56	0.59	0.57	0.57	0.38	0.38	0.37	0.36	0.37
ALA	4.82	4.73	4.68	4.68	4.73	3.85	3.91	4.23	4.18	4.04	1.89	1.88	1.99	1.95	1.93
PDO	0.16	0.17	0.17	0.17	0.17	0.36	0.36	0.39	0.39	0.38	0.17	0.17	0.18	0.18	0.18
PA	0.73	0.76	0.71	0.69	0.72	0.49	0.54	0.53	0.53	0.52	0.58	0.57	0.56	0.56	0.57
ACLC	0.75	0.76	0.78	0.74	0.76	0.19	0.21	0.25	0.23	0.22	0.30	0.30	0.27	0.26	0.28
LAC+THR	1.08	1.09	1.08	1.04	1.07	1.00	1.00	1.13	1.04	1.04	0.85	0.85	0.86	0.80	0.83
TG%	96.54	96.54	96.54	96.54	96.54	96.27	96.37	96.88	96.83	96.59	96.58	96.04	95.29	96.08	96.00
1,2DG%	1.91	1.91	1.79	1.80	1.85	2.02	1.97	2.61	2.60	2.30	2.47	2.18	2.38	2.07	2.28
1,3DG%	1.55	1.55	1.67	1.67	1.61	1.71	1.65	0.50	0.57	1.11	0.95	1.78	2.32	1.85	1.72
SAT%	13.49	13.49	14.66	14.67	14.08	13.66	13.67	20.70	20.67	17.17	15.26	13.17	14.65	14.61	14.42
O%	60.31	60.31	57.15	57.14	58.73	76.88	77.07	62.88	62.98	69.95	67.49	70.12	71.32	71.48	70.10
L%	25.68	25.68	27.67	27.67	26.67	9.13	8.94	15.88	15.83	12.45	16.41	16.28	13.64	13.55	14.97
Ln%	0.52	0.52	0.52	0.52	0.52	0.33	0.32	0.54	0.53	0.43	0.84	0.42	0.39	0.36	0.50
STR	8337	8337	8029	7945	8162	6705	6026	7265	10031	7507	9130	8157	6327	5697	7328

Table 3. Total assignment of many spectral regions exploited by the MARA-NMR algorithm to infer the quantitative panel. This excerption shows the integrations for the first three samples, obtained results and deviation of the theoretical 0 value of equations from the experimental output.

Chemical Code and Number of H Resonances	Integration Number	Integral Range	S_a_1	S_a_2	S_a_3
TRG(1H)	I_1	9.08–9.12	1.34	1.85	1.77
TRG(2H)	I_2	8.79–8.85	2.80	3.68	3.78
FA(1H)	I_3	8.42–8.44	2.66	3.40	3.71
INO(1H)	I_4	8.31–8.34	3.16	4.48	4.73
INO(1H)	I_5	8.23–8.26	3.58	4.79	5.09
UDP(1H)	I_6	7.97–8.01	4.77	8.96	6.63
URI(1H)	I_7	7.90–7.95	3.39	3.69	5.02
I3AA(2H)	I_8	7.58–7.65	8.44	12.95	11.47
I3AA(1H)	I_9	7.45–7.47	3.34	3.31	4.22
PHE(5H)+TRP(2H)	I_10	7.29–7.42	8.30	22.20	10.79
I3AA(1H)	I_11	7.21–7.27	10.88	14.28	14.73
TYR(2H)+I3AA(1H)	I_12	7.15–7.21	10.02	13.51	12.63
GA	I_13	7.01–7.03	0.00	0.00	0.00
TYR(2H)	I_14	6.86–6.90	10.96	18.01	13.55
FUMA	I_15	6.49–6.52	6.69	9.88	9.99
SHA	I_16	6.40–6.44	1.93	4.69	4.71
Q3G	I_17	5.58–5.63	2.23	3.61	3.90
RAF+SUC	I_18	5.37–5.42	694.28	1168.79	809.56
a-GAL(35%)	I_19	5.24–5.26	7.08	15.10	12.43
a-GLC(41%)	I_20	5.20–5.23	17.01	35.26	32.21
XIL	I_21	5.11–5.14	10.36	15.37	15.03
RAF	I_22	4.95–4.99	145.77	220.06	184.17
b-GLC(59%)+MHP(1H)	I_23	4.59–4.64	139.34	214.82	161.37
b-GAL(65%)	I_24	4.55–4.58	17.96	33.02	26.92
SHA(1H)	I_25	4.36–4.40	33.41	42.55	47.78
(1H)MAL	I_26	4.24–4.32	142.70	193.54	203.82
(RAF)(1H)+SUC(1H)+MHP(1/4H)	I_27	4.18–4.23	829.67	1306.41	952.67
MHP(3/4H)	I_28	4.14–4.18	191.05	284.45	237.61
1H-FRC(0.68H)+GAL(0.35H*0.75)+PRO(1H)	I_29	4.07–4.14	126.94	202.48	163.67
(2H)b-GLC+(1H)SUC	I_30	3.42–3.48	616.53	1080.81	693.73
a-GLC+b-GLC+(1H)PRO	I_31	3.35–3.42	41.92	78.88	73.92
MeOH (3H) ref	I_32	3.33–3.35	300.00	300.00	300.00
(1H)PRO	I_33	3.29–3.33	15.81	33.53	30.31
(3/4H)MYI	I_34	3.25–3.29	22.95	43.93	48.02
b-GLC+(1/4H)MYI	I_35	3.20–3.25	65.42	107.87	86.17
(9H) CAR	I_36	3.19–3.20	40.72	62.09	53.72
(9H)CHN+MHP(1/2H)	I_37	3.17–3.19	205.00	337.23	234.93
MHP(1/2H)	I_38	3.15–3.17	80.17	125.77	91.63
MHP(3H)	I_39	3.01–3.05	475.36	763.06	533.85
GABA(2H)	I_40	2.97–3.01	46.88	89.35	59.08
ASN(1H)+compE	I_41	2.89–2.97	11.61	24.71	11.49
ASN(1H)	I_42	2.82–2.89	4.84	8.22	6.27
SHA(1H)+ASP(1H)	I_43	2.71–2.82	25.66	41.25	40.98
CA(2H)+MAL(1H)+ASP(1H)	I_44	2.62–2.71	153.15	252.51	215.84
CA(2H)	I_45	2.50–2.58	125.57	195.06	176.33

MHP(1H)+CAR(2H)	I_46	2.43–2.50	187.38	295.38	225.23
PA(3H)	I_47	2.41–2.43	15.00	28.68	25.11
MAL(1H)+PRO(1H)+GLU(2H)	I_48	2.30–2.40	146.62	202.12	204.51
GABA(3/2H)	I_49	2.27–2.30	26.44	38.73	55.00
MHP(1H)+GABA(2/4H)+SHA(1H)	I_50	2.17–2.27	167.94	253.17	225.64
PRO(3H)+GLU(2H)+ILE(1H)	I_51	1.93–2.17	169.96	216.57	209.74
ARG(2H)+GABA(2H)+ACA(3H)	I_52	1.84–1.93	91.87	155.98	123.98
ARG(2H)+LEU(3H)	I_53	1.55–1.79	106.62	170.50	122.23
ALA(3H)+ILE(1H)	I_54	1.43–1.55	108.82	166.10	136.70
ACLC(3H)	I_55	1.37–1.40	12.97	13.53	15.88
unknownC	I_56	1.32–1.34	6.79	6.03	8.88
THR(3H)+LAC(3H)	I_57	1.29–1.32	21.80	28.14	26.53
unkownB	I_58	1.22–1.24	20.41	28.94	23.80
ILE(1H)+unknown	I_59	1.14–1.22	61.40	102.33	85.08
PDO(3H)	I_60	1.10–1.14	26.10	43.47	31.47
VAL(3H)	I_61	1.00–1.04	15.28	19.91	19.30
ILE(3H)+VAL(3H)	I_62	0.95–1.00	28.83	42.67	37.17
ILE(3H)+LEU(6H)	I_63	0.89–0.95	71.56	121.53	94.47
Quantified Variables			**S_a_1**	**S_a_2**	**S_a_3**
Trigonelline	TRG	v_1	1.39	1.84	1.87
Formiate	FA	v_2	1.33	1.70	1.85
Inosine	INO	v_3	3.37	4.63	4.91
Uridine-di-phosphate	UDP	v_4	4.77	8.96	6.63
Uridine	URI	v_5	3.39	3.69	5.02
Indole-3-Acetic acid	I3AA	v_6	4.69	6.34	6.37
Phenylalanine and Triptophan	PHE+TRP	v_7	1.19	3.17	1.54
Tyrosine	TYR	v_8	4.02	6.30	4.95
Gallate	GA	v_9	0.00	0.00	0.00
Fumarate	FUMA	v_10	3.35	4.94	5.00
Shikimic acid	SHA	v_11	1.93	4.69	4.72
Quercetin-3-O-Glucoside	Q3G	v_12	2.23	3.61	3.90
Rafinose	RAF	v_13	145.76	220.04	184.18
Sucrose	SUC	v_14	548.53	948.76	625.39
Galactose	GAL	v_15	26.48	49.60	40.49
Glucose	GLC	v_16	33.05	68.47	66.14
Xylose	XIL	v_17	10.36	15.37	15.03
Fructose	FRC	v_18	151.96	227.78	180.76
Proline	PRO	v_19	16.62	36.87	31.93
Myo-inositol	MYI	v_20	29.98	57.51	63.16
Carnitine	CAR	v_21	4.65	7.07	6.18
Choline derivates	CHN	v_22	13.97	23.34	16.22
N-Methyl-4-trans-hydroxy proline	MHP	v_23	158.44	254.33	177.94
◎-amino butirrate	GABA	v_24	21.29	37.62	32.43
Asparagine	ASN	v_25	4.84	8.22	6.27
Aspartate	ASP	v_26	18.90	29.51	27.82
Citrate	CA	v_27	60.38	94.01	83.94
Malate	MAL	v_28	18.32	42.03	28.57
Glutamate	GLU	v_29	55.92	61.11	71.29
Arginine	ARG	v_30	36.65	57.33	39.72
Acetate	ACA	v_31	0.00	0.00	1.49
Isoleucine	ILE	v_32	3.61	6.07	4.76
Leucine	LEU	v_33	11.11	18.61	14.26

Valine	VAL	v_34	4.08	5.31	5.15
Alanine	ALA	v_35	35.07	53.34	43.98
Propan-1,2-diol	PDO	v_36	8.70	14.49	10.49
Piruvate	PA	v_37	5.00	9.57	8.36
Acetyl-lactate	ACLC	v_38	4.32	4.53	5.29
Lactate+ Threonine	LAC+THR	v_39	7.28	9.37	8.84
total SUMM			1466.91	2410.13	1846.84
MARA-NMR Equations					
TRG	eq_int_1		0.00	0.00	0.00
TRG_2	eq_int_2		0.00	0.00	0.00
FA	eq_int_3		0.00	0.00	0.00
INO	eq_int_5+4		0.00	0.00	0.00
UDP	eq_int_6		0.00	0.00	0.00
URI(1H)	eq_int_7		0.00	0.00	0.00
I3AA(2H)	eq_int_8		0.00	0.00	0.00
I3AA(1H)	eq_int_9		0.00	−0.01	−0.01
PHE+TRP	eq_int_10		0.00	0.00	0.00
I3AA	eq_int_11		0.02	0.03	0.03
2TYR+I3AA	eq_int_12		−0.01	−0.02	−0.01
GA	eq_int_13		0.00	0.00	0.00
2TYR	eq_int_14		0.01	0.02	0.01
FUMA	eq_int_15		0.00	0.00	0.00
SHA	eq_int_16		0.00	0.00	−0.01
Q3G	eq_int_17		0.00	0.00	0.00
SUC+RAF	eq_int_18		0.00	0.00	0.00
a-GAL	eq_int_19		−0.29	−0.30	−0.23
a-GLC	eq_int_20		0.46	0.96	0.68
XYL	eq_int_21		0.00	0.00	0.00
RAF	eq_int_22		0.00	0.00	0.00
b-GLC+MHP	eq_int_23		−1.29	−2.66	−1.85
b-GAL	eq_int_24		0.13	0.13	0.10
MAL	eq_int_26		0.41	0.51	0.58
RAF+SUC+MHP	eq_int_27		0.32	0.25	0.33
MHP	eq_int_28		0.24	0.31	0.35
FRC	eq_int_29		0.00	0.00	0.00
2b-GLC+SUC	eq_int_30		0.10	0.17	−0.03
GLC(a+b)+PRO	eq_int_31		−0.03	−0.09	−0.08
PRO	eq_int_33		0.14	0.26	0.27
3/4MYI	eq_int_34		0.01	0.01	0.01
1/4MYI+59%GLC+2ARG	eq_int_35		−0.12	−0.21	−0.16
CAR 9H	eq_int_36		−0.04	−0.05	−0.06
CHN+MHP	eq_int_37		0.00	0.00	0.00
1/2 MHP	eq_int_38		0.03	−0.05	0.09
3 MHP	eq_int_39		0.02	0.04	0.02
GABA	eq_int_40		0.11	0.38	−0.15
ASN	eq_int_42		0.00	0.00	0.00
SHA+ASP	eq_int_43		0.08	0.12	0.14
CA+MAL+ASP	eq_int_44		−0.08	−0.12	−0.14
CA	eq_int_45		0.08	0.12	0.14
MHP+CAR	eq_int_46		0.33	0.45	0.58
PA	eq_int_47		0.00	0.00	0.00

MAL+PRO+GLU	eq_int_48	0.00	0.02	0.02
GABA 1.5	eq_int_49	−0.15	−0.47	0.17
MHP+0.5GABA+SHA(1/2)+1Val	eq_int_50	−0.03	−0.23	0.28
PRO(2)+GLU(2)+ILE(1)+SHA(1/2)	eq_int_51	0.01	−0.08	−0.12
ARG+GABA+ACA	eq_int_52	−0.02	−0.01	0.00
ARG+LEU	eq_int_53	0.00	0.00	0.00
ALA+ILE	eq_int_54	0.00	0.00	0.00
ACLC	eq_int_55	0.00	0.00	0.00
THR+LAC	eq_int_57	0.00	0.00	0.00
PDO	eq_int_60	0.00	0.00	0.00
VAL	eq_int_61	0.00	0.00	−0.01
ILE+VAL	eq_int_62	0.00	0.00	0.00
ILE+LEU	eq_int_63	−0.07	−0.11	−0.08
MHP/PRO	eq_intprovsmhp	−0.05	−0.13	−0.09
RHO Function	**square summ of all the equations**	**2.52**	**9.38**	**5.21**

MARA-NMR is a simple program running on excel cells like table 1S with these virtual basic instructions.

@@@@@@@@@@@@@@@@@@@

```
Sub PWS()
'
' PWS Macro
'
' Scelta rapida da tastiera: CTRL+MAIUSC+P
'
   SolverReset
   SolverOk SetCell:=ActiveCell, MaxMinVal:=2, ValueOf:=0, ByChange:= _
   ActiveCell.Offset(−102, 0).Range("A1:A40"), Engine:=1, EngineDesc:="GRG Nonlinear"
   SolverAdd CellRef:=ActiveCell.Offset(−102, 0).Range("A1:A40"), _
   Relation:=3, FormulaText:="0"
   SolverOk SetCell:=ActiveCell, MaxMinVal:=2, ValueOf:=0, ByChange:= _
   ActiveCell.Offset(−102, 0).Range("A1:A40"), Engine:=3, EngineDesc:="GRG Nonlinear"
   SolverSolve
End Sub
```

@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@