SciForum
Mol2Net

HPLC-qTOF-MS Platform as Valuable Tool for the Exploratory Characterization of Phenolic Compounds in Guava Leaves at Different Oxidation States

Elixabet Díaz-de-Cerio [1,*], Vito Verardo [2], Ana María Gómez-Caravaca [1], Alberto Fernández-Gutiérrez [1] and Antonio Segura-Carretero [1]

[1] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avd. Fuentenueva s/n, 18071, Granada, Spain; and Functional Food Research and Development Center, Health Science Technological Park, Avd. del Conocimiento, Bioregion building, 18100, Granada, Spain. E-Mail: anagomez@ugr.es (A.M.G.C.); albertof@ugr.es (A.F.G.); ansegura@ugr.es (A.S.C.)

[2] Department of Chemistry and Physics (Analytical Chemistry Area) and Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence, ceiA3, University of Almería, Carretera de Sacramento s/n, E-04120 Almería, Spain.; E-Mails: vito.verardo@cidaf.es (V.V.);

* Author to whom correspondence should be addressed; E-Mail: ediazdecerio002@correo.ugr.es; Tel.: +34 958 637 206; Fax: +34- 958 637 083.

Published: 4 December 2015

Abstract: *Psidium guajava* L. is widely used like food and in folk medicine all around the world. Many studies have demonstrated that guava leaves have anti-hyperglycaemic and anti-hyperlipidemic activities, among others. The biological activity of guava leaves belongs mainly to phenolic compounds. Andalusia is one of the regions in Europe where guava is grown, thus, the aim of this work was to study the phenolic compounds present in Andalusian guava leaves at different oxidation state (low, medium and high). The phenolic compounds in guava leaves were determined by HPLC-DAD-ESI-qTOF-MS. We identified seventy-two phenolic compounds and, to our knowledge, twelve of them were determined for the first time in guava leaves in negative ionization mode. Moreover, positive ionization mode allowed the identification of the cyanidin-glucoside. To our knowledge this compound has been identified for the first time in guava leaves.

The results obtained by chromatographic analysis reported that guava leaves with low degree of oxidation have a higher content gallic and ellagic derivatives and flavonols compared to the other two guava leaf samples. Contrary, high oxidation state guava leaves reported the highest content of cyanidin-glucoside that was 2.6 and 15 times higher than guava leaves with medium and low oxidation state, respectively.

The qTOF platform permitted the determination of several phenolic compounds and provided new information about guava leaf phenolic composition that could be useful for nutraceutical production.

Keywords: Psidium guajava L.; HPLC-DAD-ESI-qTOF-MS; phenolic compounds; gallic and ellagic derivatives; flavonols; cyanidin-glucoside.

Mol2Net YouTube channel: *http://bit.do/mol2net-tube*

1. Introduction

Psidium guajava L., from the Myrtaceae family, is common throughout tropical and subtropical areas[1] and Andalusia is one of the regions in Europe where guava is grown. Guava tree shows different phenological stages throughout its vegetative period in response to environmental conditions[2]. Moreover, is widely used like food and in folk medicine all around the world. Many studies have demonstrated that guava leaves have anti-hyperglycaemic and anti-hyperlipidemic activities[3], among others. The biological activity of guava leaves belongs mainly to phenolic compounds[4].

Different analytical techniques are commonly used to characterize the bioactive present in plant extracts. LC/MS technique has opened up new approaches for the qualitative and the quantitative analysis of target compounds. LC/TOF/MS can provide tentative identification of unknown peaks, due to accurate-mass measurement[5].

Thus, the aim of this work was to study the phenolic compounds present in Andalusian guava leaves at different oxidation state (low, medium and high) by HPLC-DAD-ESI-qTOF-MS.

.

2. Results and Discussion

Negative and positive mode LC-ESI/MS conditions were optimized for the analysis of all the phenolics. To identify compounds for which no commercial standards were available, data generated by TOF analysis were checked. HPLC and mass spectral data obtained are summarized in Table 1. A total of sixty-nine phenolic compounds were characterized in negative mode, twelve of them were determined for the first time in guava leaves. In positive mode, only cyanidin-3-O-glucoside was detected.

Quantification of the extracts by HPLC-DAD-ESI-qTOF-MS revealed that the three samples showed significant differences ($p < 0.05$). Low oxidation state provided the highest content of total phenolic compounds (103 ± 2 mg/g leaf d.w.), followed by medium and high oxidation state (92.0 ± 0.4 and 87.91 ± 0.04 mg/g leaf d.w., respectively).

In terms of concentration of the different families present in leaves, the extracts reported the same trend as TPC, lowest content of different classes of phenolics compound were found in high oxidation state, whereas the highest content was found at low oxidation state (Figure 1). The major class of phenolic compounds in guava leaves samples was flavonols, ranged between 48.1 and 50.6 mg/g

leaf d.w. The second class of polar compounds was represented by flavan-3-ols (24.2 - 24.7 mg/g leaf d.w.), succeeded by gallic and ellagic acid derivatives (14.8 - 15.8 mg/g leaf d.w.) and finally, flavanone, that varied from 0.49 to 0.63 mg/g leaf d.w.

In contrast, and as was expected, in positive mode, opposite results were found (Figure 2). Greater amount of cyanidin-3-O-glucoside was determined when the oxidation state was higher (varying from 441.28 ± 0.04 to 29.5 ± 0.2 µg/g leaf d.w.).

These changes in composition could be due to the different synthesis of secondary metabolites as response to oxidative state[6]. It may happen due to a reaction between them and anthocyanins that cause the dramatic red coloration of thee leaves, decreasing in this way its concentration[7].

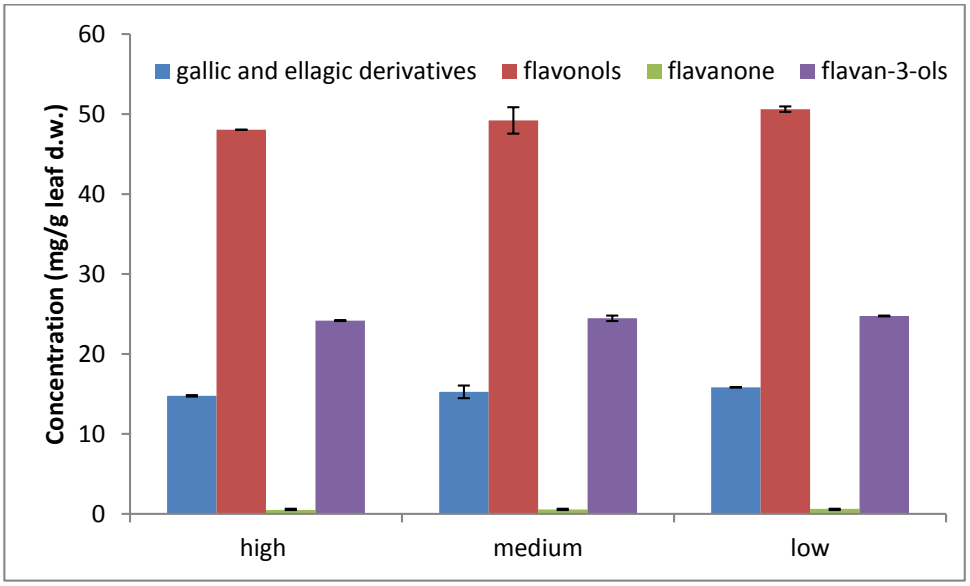

Figure 1. Quantification of different families of phenolic compounds present in guava leaves.

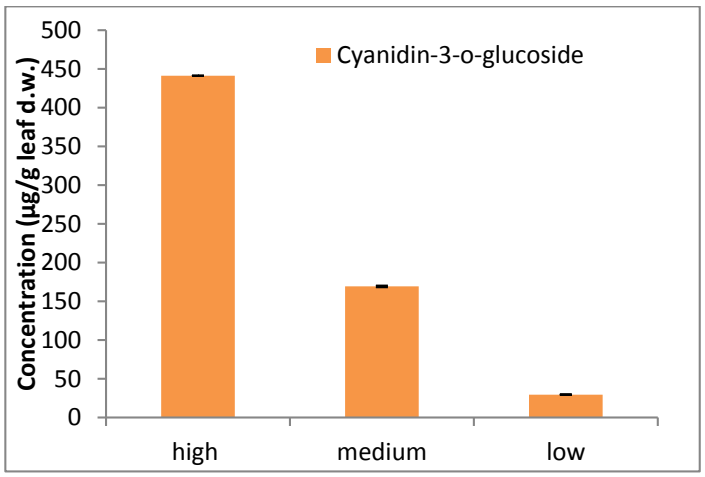

Figure 2. Quantification of cyanidin-3-O-glucoside

No.	Compound	tr (min)	m/z exp	m/z calculated	Molecular Formula	λ (nm)	Fragments	Score	error(ppm)
	Negative mode								
1	HHDP glucose Isomer 1	1.929	481.064	481.3406	C20H18O14	290	421.0406, 300.9991, 275.0202	96.51	-2.55
2	HHDP glucose Isomer 2	2.139	481.0638	481.3406	C20H18O14	290	421.0406, 300.9991, 275.0202	99.09	-0.19
3	HHDP glucose Isomer 3	2.516	481.0639	481.3406	C20H18O14	290	421.0406, 300.9991, 275.0202	97.21	-2.24
4	Prodelphinidin B Isomer	3.85	609.1276	609.5111	C30H26O14	272, 225	441.0838, 423.0701, 305.0687, 125.0226	97.84	-1.7
5	Gallic acid	4.022	169.0142	169.1116	C7H6O5	280, 360	125.0243	99.27	0.37
6	Pedunculagin/ Casuariin Isomer	5.865	783.0699	783.5332	C34H24O22	253	481.0606, 391.0307, 300.9999, 275.0191	98.57	-1.29
7	Prodelphinidin Dimer Isomer	7.272	593.1311	593.5117	C30H26O13	280, 340	425.0875, 289.0715,	96.51	-2.35
8	Gallocatechin	7.814	305.0698	305.2595	C15H14O7	270	125.0241, 179.0347, 219.0661, 261.0774	95.55	-3.32
9	Vescalagin/castalagin Isomer	7.953	933.0649	933.6216	C41H26O26	260, 280	466.0299, 300.9968	99.19	-0.79
10	Prodelphinidin Dimer Isomer	8.119	593.1316	593.5117	C30H26O13	280, 340	305.0667, 423.0719, 441.0841	96.51	-2.35
11	Uralenneoside	9.387	285.0624	285.2268	C12H14O8	270	153.0193, 109.0279	97.8	-2.69
12	Geraniin Isomer	9.497	951.0749	951.6369	C41H28O27	270	907.0825, 783.0785, 481.0606, 300.9999	99.56	-0.2
13	Pedunculagin/ Casuariin Isomer	9.536	783.0699	783.5332	C34H24O22	253	481.0606, 391.0307, 300.9999, 275.0191	98.39	-1.36
14	Geraniin Isomer	9.652	951.0752	951.6369	C41H28O27	270	907.0825, 783.0785, 481.0606, 300.9999	99.56	-0.2
15	Procyanidin B Isomer	10.018	577.1367	577.5123	C30H26O12	278	425.0881, 407.0777, 289.0718, 125.0243	95.68	-2.55
16	Galloyl(epi)catechin-(epi)gallocatechin	10.345	745.142	745.6160	C37H30O17	280, 340	593.1302, 575.1214, 423.0694, 305.0688	96.9	-0.62
17	Procyanidin B Isomer	10.356	577.1367	577.5123	C30H26O13	278	425.0881, 407.0777, 289.0718, 125.0243	99.41	-0.61
18	Tellimagrandin I Isomer	10.738	785.0851	785.5491	C34H26O22	279, 340	615.0674, 392.0396, 300.9985, 169.0144	99.13	-0.96
19	Pterocarinin A	10.998	1067.122	1067.7521	C46H36O30	280	533.0585, 377.0313, 301.0330, 249.0377	99.82	-0.11
20	Pterocarinin A Isomer	11.208	1067.122	1067.7521	C46H36O30	280	533.0585, 377.0313, 301.0330, 249.0377	98.39	-1.26
21	Stenophyllanin A	11.247	1207.1495	1207.8903	C56H40O31	278	917.0763, 603.0735	98.64	-1.08
22	Procyanidin trimer Isomer 1	11.247	865.1998	865.7645	C45H38O18	278	739.1593, 577.1337, 449.0888, 289.0745	97.53	-1.59
23	Catechin	11.258	289.0727	289.2601	C15H14O6	281	245.0821, 203.0718, 179.0349, 125.0242	96.76	-3.18
24	Procyanidin tetramer	11.336	1153.2612	1155.0246	C60H50O24	280	576.1291	99.6	-0.5
25	Procyanidin trimer Isomer 2	11.413	865.1998	865.7645	C45H38O18	278	739.1593, 577.1337, 449.0888, 289.0745	97.53	-1.59
26	Guavin A	11.496	1223.1423	1223.8897	C56H40O32	277	611.0724	99.05	0.85

27	Casuarinin/ Casuarictin Isomer	11.895	935.081	935.6375	C41H28O26	275	783.0637, 633.0735, 300.9979, 275.0189	97.67	-1.43
28	Galloyl(epi)catechin-(epi)gallocatechin	12.1	745.142	745.6160	C37H30O17	280, 340	593.1302, 575.1214, 423.0694, 305.0688	96.9	-0.62
29	Procyanidin pentamer	12.144	1441.3234	1442.2688	C75H62O30	280	720.1604	95.66	1.97
30	Galloyl-(epi)catechin trimer Isomer 1	12.166	1017.2097	1017.8687	C52H42O22	280	508.104	99.72	-0.01
31	Gallocatechin	12.327	305.0702	305.2595	C15H14O7	270	125.0241, 179.0347, 219.0661, 261.0774	95.55	-3.32
32	Tellimagrandin I Isomer	12.504	785.0855	785.5491	C34H26O22	277, 338	615.0674, 392.0396, 300.9985, 169.0144	98.44	-1.38
33	Vescalagin	12.758	933.0649	933.6216	C41H26O26	260, 280	466.0295, 457.0781, 300.9968	96.33	-0.8
34	Stenophyllanin A Isomer	12.925	1207.1472	1207.8903	C56H40O31	280	917.0763, 603.0735	98.37	0.89
35	Galloyl-(epi)catechin trimer Isomer 2	12.985	1017.2097	1017.8687	C52H42O22	280	508.104	98.17	-1.35
36	Myricetin hexoside Isomer	13.284	479.0836	479.3678	C21H20O13	261, 358	317.0294, 316.0226, 271.0255	98.36	-0.92
37	Stachyuranin A	13.412	1225.1587	1225.9055	C56H42O32	276	612.0779	95.54	1.35
38	Procyanidin gallate Isomer	13.517	729.1476	729.6166	C37H30O16	280	577.1356, 559.1226, 425.0874, 407.0798, 298.0716	96.89	-1.91
39	Myricetin hexoside Isomer	13.677	479.0835	479.3678	C21H20O13	261, 358	317.0294, 316.0226, 271.0255	97.89	-0.08
40	Vescalagin/castalagin Isomer	13.844	933.0645	933.6216	C41H26O26	260	466.0299, 300.9968	88.32	-1.57
41	Myricetin -arabinoside/ xylopyranoside Isomer	13.988	449.0728	449.3418	C20H18O12	264, 356	317.0291, 316.0241, 271.0249	98.39	-1.65
42	Myricetin -arabinoside/ xylopyranoside Isomer	14.214	449.0726	449.3418	C20H18O12	264, 357	317.0291, 316.0241, 271.0249	98.02	-1.65
43	Procyanidin gallate Isomer	14.563	729.6356	577.5123	C30H26O12	280	577.1356, 559.1226, 425.0874, 407.0798, 298.0716	98.17	-1.73
44	Myricetin -arabinoside/ xylopyranoside Isomer	14.99	449.0726	449.3418	C20H18O12	264, 356	317.0291, 316.0241, 271.0249	98.66	-1.65
45	Myricetin hexoside Isomer	15.034	479.0839	479.3678	C21H20O13	261, 358	317.0294, 316.0226, 271.0255	97.08	-1.92
46	Myricetin hexoside Isomer	15.217	479.0841	479.3678	C21H20O13	264, 356	317.0288, 316.0241, 271.0253	97.08	-1.92
47	Myricetin -arabinoside/ xylopyranoside Isomer	15.604	449.0743	449.3418	C20H18O12	264, 356	317.0291, 316.0241, 271.0249	98.39	-1.65
48	Quercetin -galloylhexoside Isomer	15.626	615.1008	615.4726	C28H24O16	268, 350	463.0886, 300.0283	99.16	-0.98
49	Ellagic acid deoxyhexoside	15.837	447.0578	447.3259	C20H16O12	265, 350	300.9974,	91.25	-3.19
50	Quercetin -galloylhexoside Isomer	16.036	615.0999	615.4726	C28H24O16	280, 345	463.0886, 300.0283	99.16	-0.98
51	Myricetin -arabinoside/ xylopyranoside Isomer	16.191	449.0736	449.3418	C20H18O12	256, 356	317.0291, 316.0241, 271.0249	98.39	-1.65
52	Morin	16.28	301.0362	301.2278	C15H10O7	257, 374	178.9978, 151.0032	97.46	-2.5
53	Myricetin -arabinoside/ xylopyranoside Isomer	16.462	449.0735	449.3418	C20H18O12	257, 356	317.0291, 316.0241, 271.0249	98.39	-1.65

54	Ellagic acid	16.507	300.9996	301.1847	C14H6O8	254, 360	283.9921, 257.0088, 229.0169, 185.0233	98.88	-1.71
55	Hyperin	16.616	463.0895	463.3684	C21H20O12	259, 355	301.0350, 300.0279, 178.9980, 151.0032	96.41	-2.65
56	Quercetin glucoronide	16.723	477.0659	477.3519	C21H18O13	265, 355	301.0359, 151.0026	98.1	-1.83
57	Isoquercitrin	16.95	463.0893	463.3684	C21H20O12	258, 355	301.0353, 300.0281, 178.9983, 151.0090	97.04	-2.33
58	Procyanidin gallate Isomer	17.038	729.1476	729.6166	C37H30O16	280	577.1356, 559.1226, 425.0874, 407.0798, 298.0716	96.89	-1.91
59	Reynoutrin	17.498	433.0792	433.3424	C20H18O11	258, 356	301.0356	95.94	-2.9
60	Guajaverin	17.802	433.0795	433.3424	C20H18O11	257, 356	301.0352	97.99	-1.91
61	Guavinoside A	17.985	543.1159	544.4610	C26H24O13	218, 288	313.0568, 229.0503, 169.0148	98.1	-1.77
62	Avicularin	18.206	433.0803	433.3424	C20H18O11	257, 355	301.0359	96.7	-2.2
63	Quercitrin	19.194	447.0947	447.3690	C21H20O11	264, 353	301.0348, 271.0247, 178.9988, 151.0028	95.23	-3.02
64	Myrciaphenone B	19.208	481.0999	481.3836	C21H22O13	280, 340	313.0570, 169.0141	97.2	-2.23
65	Guavinoside C	19.768	585.0898	585.4466	C27H22O15	265, 355	433.0757, 301.0351, 283.0449, 169.0142	97.19	-1.92
66	Guavinoside B	20.77	571.147	571.5062	C28H28O13	218, 283	313.057, 257.0829, 169.0142	97.26	-2.05
67	Guavinoside A Isomer	20.702	543.1159	543.4530	C26H24O13	218, 288	313.0568, 229.0503, 169.0148	98.1	-1.77
68	Guavinoside B Isomer	21.667	571.147	571.5062	C28H28O13	218, 283	313.057, 257.0829, 169.0142	97.26	-2.05
69	2,6-dihydroxy-3-methyl-4-O-(6″-O-galloyl-β-D-glucopyranosyl)-benzophenone	21.971	557.1318	557.4796	C27H26O13	280	313.0575, 243.0670, 169.0146	96.93	-2.12
70	Guavin B	22.237	693.111	693.5414	C33H26O17	283	391.1468	97.82	-1.67
71	Quercetin	22.314	301.0358	301.2278	C15H10O7	257, 374	178.9985, 151.0036	98.9	-1.34
72	Naringenin	26.738	271.0622	271.2448	C15H12O5	280	118.6395, 150.5022	96.09	-3.67
	Positive mode								
73	Cyanidin-3-o-glucoside	3.661	449.1089	449.3911	C21H21O11	287, 288	517, 280	96.97	-2.34

Table 1. Tentatively identified compounds in guava leaves.

3. Materials and Methods

3.1 Plant Material and Sample Preparation

Fresh guava leaves were harvested in Motril, Spain, at different oxidation states (low, medium, and high). The samples were air-dried, grounded and extracted with ethanol:water 80/20 (v/v) by ultrasonics[8].

3.2 HPLC-DAD-ESI-qTOF-MS Analysis

Chromatographic analyses were performed using an HPLC Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an online degasser, an autosampler and a thermostatically controlled column compartment, and a UV-Vis Diode Array Detector (DAD). The column was maintained at 25ºC. Phenolic compounds from *Psidium guajava* L. leaves were separated at room temperature using a method previously reported by Gómez-Caravaca et al.[9] for positive mode.

MS analyses were carried out using a 6540 Agilent Ultra-High-Definition Accurate-Mass Q-TOF-MS coupled to the HPLC, equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface. In negative ionization mode at the following conditions: drying gas flow (N2), 12.0 L/min; nebulizer pressure, 50 psi; gas drying temperature, 370°C; capillary voltage, 3500 V; fragmentor voltage and scan range were 3500 V and m/z 50-1500, respectively. Automatic MS/MS experiments were carried out using the followings collision energy values: m/z 100, 30 eV; m/z 500, 35 eV; m/z 1000, 40 eV; and m/z 1500, 45 eV. In positive mode: auto MS/MS experiments were carried out using the followings collision energy values: m/z 100, 40 eV; m/z 500, 45 eV; m/z 1000, 50 eV; and m/z 1500, 55 eV.

.

4. Conclusions

HPLC coupled to qTOF-MS detector, which provides a molecular formula and the MS/MS data, permitted the analysis of the major phenolic compounds of guava leaves. The method performed in negative mode has proven to be successful to identify 72 compounds in guava leaves. Moreover, in positive mode, the analysis with TOF analyser and the co-elution with a standard solution allowed the identification of the cyanidin-glucoside. To our knowledge twelve compounds from the negative mode, and also the cyaniding-glucoside, were identified for the first time in guava leaves.

Quantification data, in negative mode, reported that leaves with low oxidation state presented the highest concentration of these compounds and decreased when the oxidation state raised. On the contrary, the state of oxidation affected significantly the cyanidin content. In fact, highest amount was detected in the leaves with high oxidation state.

Acknowledgments

The authors Ana María Gómez-Caravaca and Vito Verardo thank the Spanish Ministry of Economy and Competitiveness (MINECO) for “Juan de la Cierva” post-doctoral contracts.

Author Contributions

EDdC carried out the experimental analyses, data interpretation and manuscript writing; AMGC and VV design the experimental plan and were involved in the data interpretation and manuscript

redaction; AFG and ASC were the responsibly of the project and founded the financial sources, moreover, they helped in the data interpretation.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1 Morton, J. F. Guava. In *Fruits of Warm Climates*, Miami, FL, 1987, pp. 356–363.

2 Salazar, D. M.; Melgarejo, P.; Martínez, R.; Martínez, J. J.; Hernández, F.; Burguera, M. *Sci. Hortic. (Amsterdam).* **2006**, *108*, 157–161.

3 Deguchi, Y.; Miyazaki, K. *Nutr. Metab. (Lond).* **2010**, *7* (9), 1–10.

4 Gutiérrez, R. M. P.; Mitchell, S.; Solis, R. V. *J. Ethnopharmacol.* **2008**, *117* (1), 1–27.

5 Heng, M. Y.; Tan, S. N.; Yong, J. W. H.; Ong, E. S. *TrAC Trends Anal. Chem.* **2013**, *50*, 1–10.

6 Vargas-Alvarez, D.; Soto-Hernández, M.; González-Hernández, V. A.; Engleman, E. M.; Martínez-Garza, Á. *Agrociencia* **2006**, *40*, 109–115.

7 Coley, P. D.; Barone, J. a. *Annu. Rev. Ecol. Syst.* **1996**, *27* (1), 305–335.

8 Díaz-de-Cerio, E.; Verardo, V.; Gómez-caravaca, A. M.; Fernández-Gutiérrez, A.; Segura-carretero, A. *J. Chem.* **2015**, *2015*, 1–9.

9 Gómez-Caravaca, A. M.; Verardo, V.; Toselli, M.; Segura-Carretero, A.; Fernández-Gutiérrez, A.; Caboni, M. F. *J. Agric. Food Chem.* **2013**, *61* (22), 5328–5337.