

SUPPLEMENTARY MATERIAL

Article

New Approaches for the Extraction of Anthocyanins from Grape Skins Using Deep Eutectic Solvents

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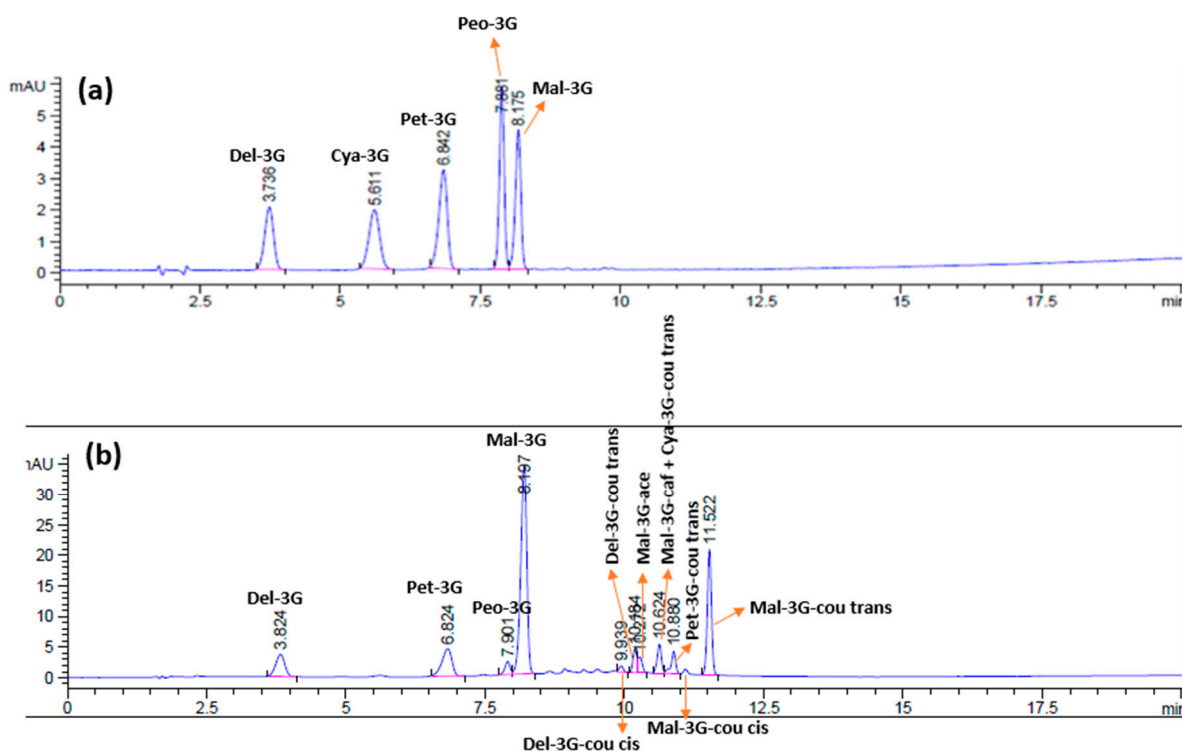


Figure S1. HPLC-DAD chromatogram corresponding to the analytical standards (a) and a grape skin extract (b).

Table S1. Regression parameters of the calibration curves and features of the HPLC method.

	Delphinidin-3G	Cyanidin-3G	Petunidin-3G	Malvidin-3G
Intercept ($\pm$ s)	0.2 $\pm$ 0.1	0.6 $\pm$ 0.4	-2.2 $\pm$ 0.9	1.6 $\pm$ 0.8
Slope ($\pm$ s)	70 $\pm$ 4	73 $\pm$ 2	111 $\pm$ 3	93 $\pm$ 3
R	0.9995	0.9994	0.9997	0.9996
LOD, μ g/mL	0.07	0.08	0.03	0.07
LOQ, μ g/ml	0.24	0.26	0.11	0.24
Rsd%(N=10)	2.25	0.34	0.94	0.22

Table S2. MRM transitions and parameters of the analysis (UPLC-QQQ).

rt	Compound	Transition	m/z (Q1)	m/z (Q3)	DP	EP	CEP	CE	CXP
9.3	Del-3-G-cou cis	T1	611	303	80	8	27.77	50	14
		T2	611	229	80	8	27.77	70	4
9.7	Del-3-G-cou trans	T1	611	303	80	8	27.77	50	14
		T2	611	229	80	8	27.77	70	4
9.8	Mal-3-G-6-Ac 1	T1	535	331	60	8	25.351	35	14
		T2	535	315	60	8	25.351	50	6
10.15	Mal-3-G-6-Caf	T1	655	331	60	8	29.171	35	14
		T2	655	315	60	8	29.171	35	14
10.2	Cya-3-G-cou trans	T1	595	287	80	8	27.261	50	3
		T2	595	213	80	8	27.261	50	3
10.3	Pet-3-G-cou trans	T1	625	317	80	8	28.216	35	14
		T2	625	302	80	8	28.216	50	6
10.6	Mal-3-G-cou cis	T1	639	331	80	8	28.662	50	14
		T2	639	315	80	8	28.662	70	6
11	Mal-3-G-cou trans	T1	639	331	80	8	28.662	50	14
		T2	639	315	80	8	28.662	70	6

Q1: Parent ion m/z, Q3: fragment ion m/z, rt (min): retention time, DP (volt): Declustering potential, EP (volt): Entrance potential, CEP (volt): Collision cell entrance potential, CE (volt): Collision cell potential, CXP (volt): Collision cell exit potential.

Table S3A. Natural logarithm of activity coefficient at infinity dilution ($\ln \gamma^\infty$) predicted by COSMO-RS at 25°C, for ratio HBA:HBD (1:1).

DES		$\ln \gamma^\infty$	
HBA	HBD	Mal-3G	Mal-3G-cou
Choline chloride (ChCl)	Citric acid (CA)	-0.13	0.40
Choline chloride	Lactic acid (LA)	1.06	-0.45
Choline chloride	1,2-Propanediol (Pro)	0.80	-2.36
Choline chloride	Glycerol (Gly)	2.73	1.07
Betaine	Citric acid	0.20	-1.76
Choline chloride	Malic acid (MA)	0.59	-0.02
Choline chloride	Oxalic acid (Ox)	-0.93	-0.97
Choline chloride	Ethyleneglycol (EG)	1.92	-0.49
Choline chloride	1,6-Hexanediol (Hex)	2.51	0.32
Sodium acetate	Glycerol	-1.75	-2.75
Sodium acetate	Lactic acid	-2.19	-3.26
*Sodium acetate (SA)	Ethyleneglycol	-2.09	-3.40
Potassium carbonate (PC)	Glycerol	-18.43	-28.61
Potassium carbonate	Ethyleneglycol	-21.44	-32.92
*Potassium carbonate	Lactic acid	-18.47	-28.76
Sodium propionate (SP)	Glycerol	-3.94	-6.39
*Sodium propionate	Ethyleneglycol	-4.50	-7.38
*Sodium propionate	Lactic acid	-3.63	-6.14

*These mixtures have not been found reported in the literature.

Table S3B. Natural logarithm of activity coefficient at infinity dilution ($\ln \gamma^\infty$) predicted by COSMO-RS at 25°C.

DES				$\ln \gamma^\infty$	
Code	HBA	HBD	Ratio	Mal-3G	Mal-3G-cou
DES 1	Choline chloride	Ethyleneglycol	1:2	1.97	0.64
DES 2	Choline chloride	1,2-Propanediol	1:3	0.40	-1.78
DES 3	Choline chloride	1,6-Hexanediol	1:2	2.22	0.71
DES 4	Choline chloride	Glycerol	1:1	2.73	1.07
DES 5	Choline chloride	Oxalic acid	1:1	-0.93	-0.97
DES 6	Choline chloride	Citric acid	2:1	-0.13	0.40
DES 7	Choline chloride	Malic acid	1:1	0.59	-0.02
DES 8	Choline chloride	Lactic acid	1:1	1.06	-0.45
DES 9	Sodium acetate	Lactic acid	1:3	-1.93	-1.41
DES 10	Choline chloride	Citric acid	1:1	-0.13	0.40
DES 11	Betaine	Citric acid	1:1	0.20	-1.76
DES 12	Sodium acetate	Glycerol	1:3	-0.09	-0.01
DES 13	Potassium carbonate	Glycerol	1:5	-3.51	-7.38
DES 14	Potassium carbonate	Glycerol	1:10	-1.64	-3.83
DES 15	Sodium propionate	Glycerol	1:3	-1.46	-2.56

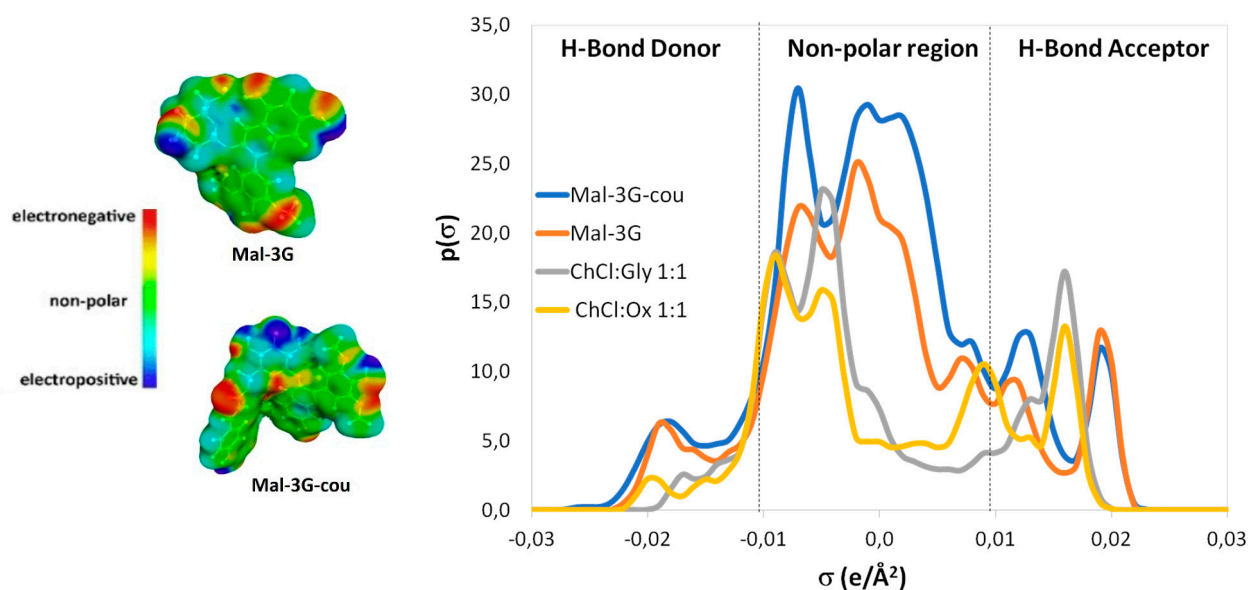


Figure S2. Representation of the α -surface for Mal-3G and Mal-3G-cou, and the α -profile for Mal-3G, Mal-3G-cou, ChCl:Gly (1:1) and ChCl:Ox (1:1).

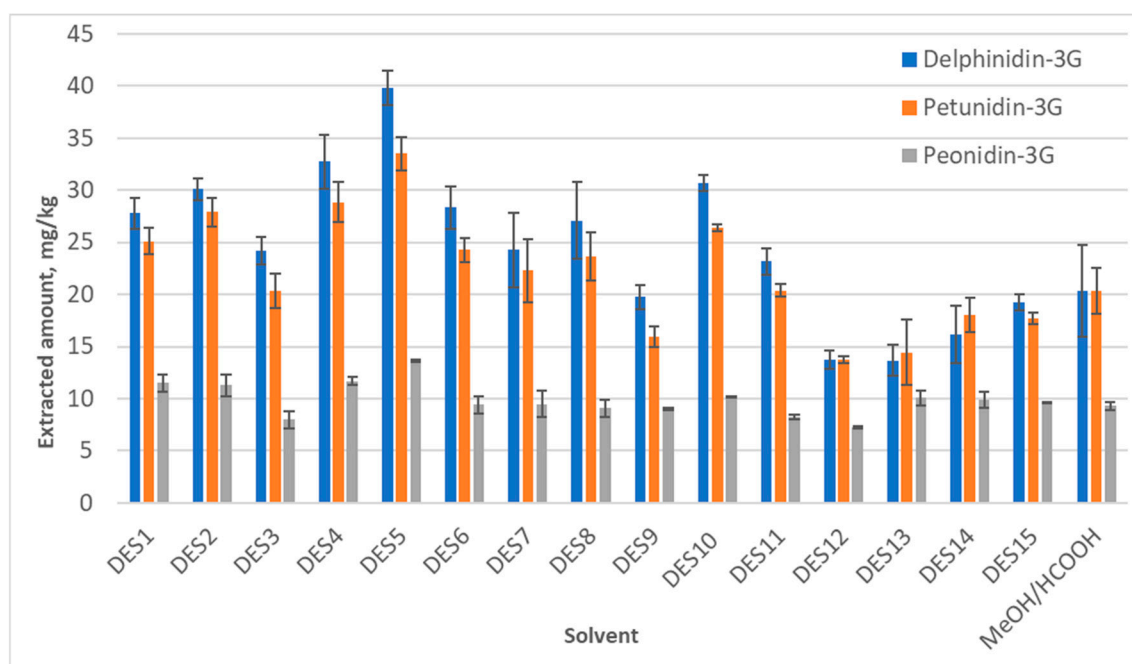


Figure S3. Del-3G, Pet-3G and Peo-3G-cou extracted amount (mg/kg) with different DES systems. The error bars have been calculated as $\pm$ twice the standard deviation. DES1 (ChCl:EG 1:2), DES2 (ChCl:Pro 1:3), DES3 (ChCl:Hex 1:2), DES4 (ChCl:Gly 1:1), DES5 (ChCl:Ox 1:1), DES6 (ChCl:CA 2:1), DES7 (ChCl:MA 1:1), DES8 (ChCl:LA 1:1), DES9 (SA:LA 1:3), DES10 (ChCl:CA 1:1), DES11 (Bet:CA 1:1), DES12 (SA:Gly 1:3), DES13 (PC:Gly 1:5), DES14 (PC:Gly 1:10), DES15 (SP:Gly 1:3). All extractions were performed at room temperature with 30 wt% water content, except for DES5, which was carried out at 50°C.

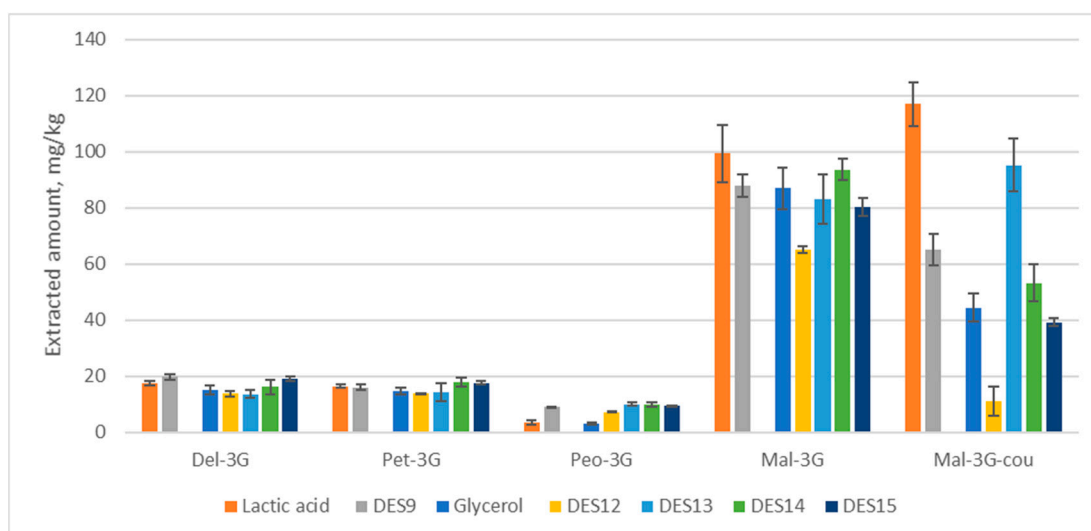


Figure S4. Comparison of the anthocyanin extraction amount using DES in relation to the solvents employed as HBD. DES9 (SA:LA 1:3), DES12 (SA:Gly 1:3), DES13 (PC:Gly 1:5), DES14 (PC:Gly 1:10), DES15 (SP:Gly 1:3). In all cases, 30 wt% water content.

Table S4. Table of experiments for ultrasound assisted extraction with DES4.

Number of Experiment	%water	Time (min)	Del-3G (mg/kg)	Pet-3G (mg/kg)	Peo-3G (mg/kg)	Mal-3G (mg/kg)	Mal-3G-cou (mg/kg)
1	29.0	13.0	24.9	20.8	6.3	98.4	46.8
2	29.0	52.0	29.3	24.8	7.6	118.8	63.6
3	71.0	13.0	21.2	18.3	5.4	86.9	15.6
4	71.0	52.0	22.3	18.0	5.7	92.7	25.6
5	20.0	32.5	26.8	23.7	7.2	119.5	85.7
6	80.0	32.5	22.7	18.3	5.8	82.7	13.7
7	50.0	5.0	24.6	20.1	6.2	92.4	27.2
8	50.0	60.0	28.6	23.1	7.0	111.7	45.2
9	50.0	32.5	24.9	20.5	6.3	96.3	43.0
10	50.0	32.5	24.4	20.9	6.4	97	42.2
11	50.0	32.5	23.7	19.7	5.9	96.4	42.1
12	50.0	32.5	24.5	20.5	6.2	96.6	42.4

Table S5. Table of experiments for microwave assisted extraction with DES 5.

Number of experiment	Temperature (°C)	Time (min)	%water	Del-3G (mg/kg)	Pet-3G (mg/kg)	Peo-3G (mg/kg)	Mal-3G (mg/kg)	Mal-3G-cou (mg/kg)
1	40.1	17.1	26.1	50.7	36.8	10.7	172.2	75.1
2	40.1	17.1	43.9	44.3	31.0	8.0	145.3	62.9
3	40.1	37.9	26.1	34.6	26.7	7.2	128.7	92.0
4	40.1	37.9	43.9	38.5	29.7	8.7	152.2	92.0
5	69.9	17.1	26.1	28.1	15.7	42.1	77.3	70.5
6	69.9	17.1	43.9	41.0	28.3	10	137.7	74.8
7	69.9	37.9	26.1	19.9	11.6	25.4	57.5	47.2
8	69.9	37.9	43.9	27.5	21.2	6.9	118.4	69.3
9	30.0	27.5	35.0	40.5	30.9	8.9	156	109.1
10	80.0	27.5	35.0	5.6	9.4	126.2	38.5	29.9
11	55.0	10.0	35.0	29.9	21.7	5.9	104.1	76.7
12	55.0	45.0	35.0	34.2	25.8	7.8	129.5	78.6
13	55.0	27.5	20.0	35.3	26.4	7.0	125.9	93.3
14	55.0	27.5	50.0	48.7	34.5	8.7	148.3	119.9
15	55.0	27.5	35.0	37.2	28.7	7.8	144.6	99.6
16	55.0	27.5	35.0	39.3	27.5	7.9	141.6	99.6
17	55.0	27.5	35.0	42.8	30	9.6	145.8	103.8
18	55.0	27.5	35.0	43.6	31	9.0	150.1	95.0

Table S6. Significance of the effects for the ultrasound assisted extraction with DES4.

Anthocyanin	Effect	p-value	Significance
Del-3G	%Water (A)	0.0017	Yes
	Time(min) (B)	0.0108	Yes
	AB	0.1825	No
	AA	0.7910	No
	BB	0.1164	No
Pet-3G	%Water (A)	0.000	Yes
	Time(min) (B)	0.0007	Yes
	AB	0.0174	Yes
	AA	0.4621	No
	BB	0.0621	No
Peo-3G	%Water (A)	0.0006	Yes
	Time(min) (B)	0.0099	Yes
	AB	0.1024	No
	AA	0.4833	No
	BB	0.2567	No
Mal-3G	%Water (A)	0.000	Yes
	Time(min) (B)	0.0003	Yes
	AB	0.0271	Yes
	AA	0.1430	No
	BB	0.0744	No
Mal-3G-cou	%Water (A)	0.000	Yes
	Time(min) (B)	0.168	Yes
	AB	0.5847	No
	AA	0.3281	No
	BB	0.0943	No

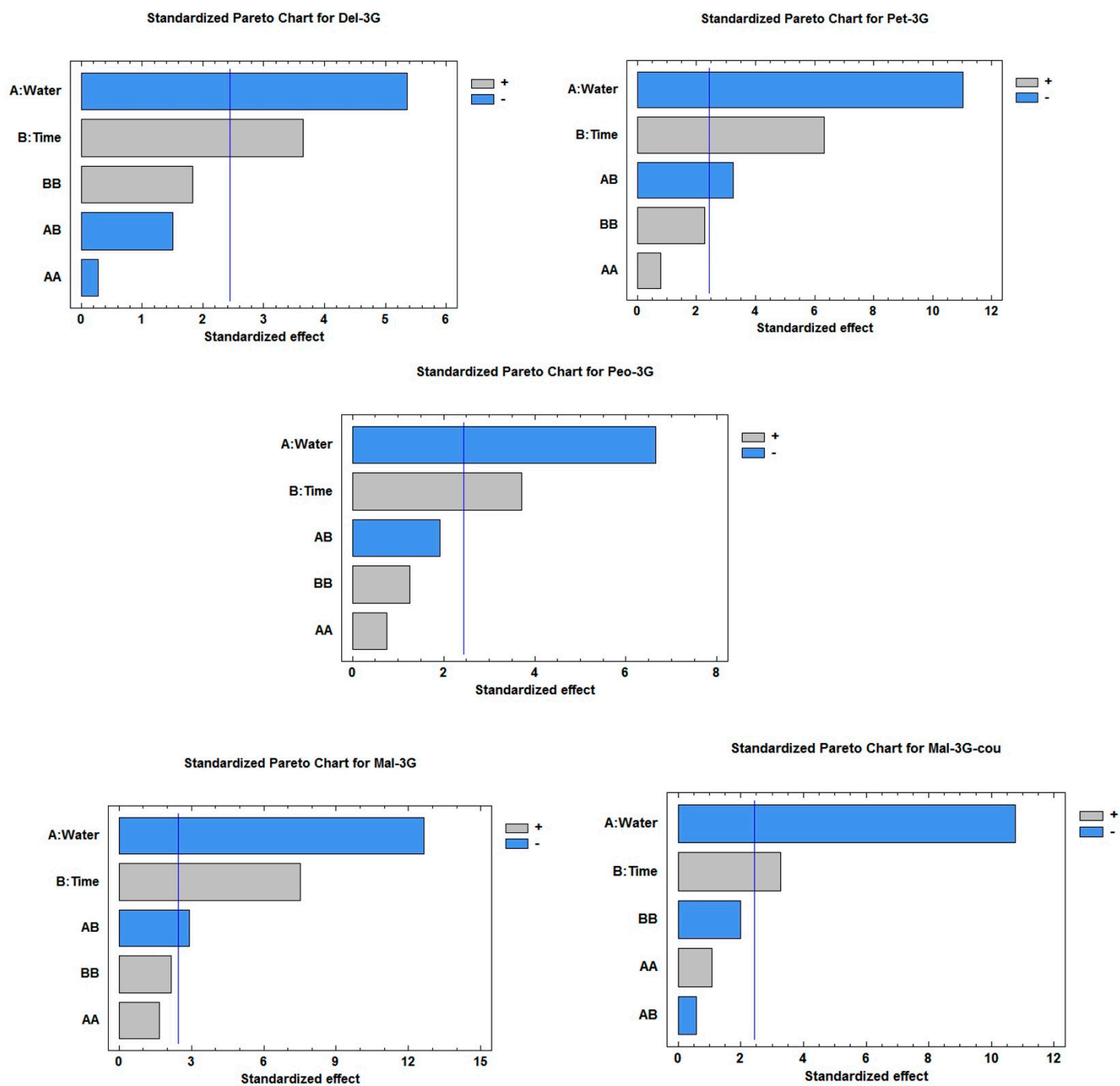


Figure S5. Pareto charts for anthocyanins extractions with ultrasound assisted extraction and DES4.

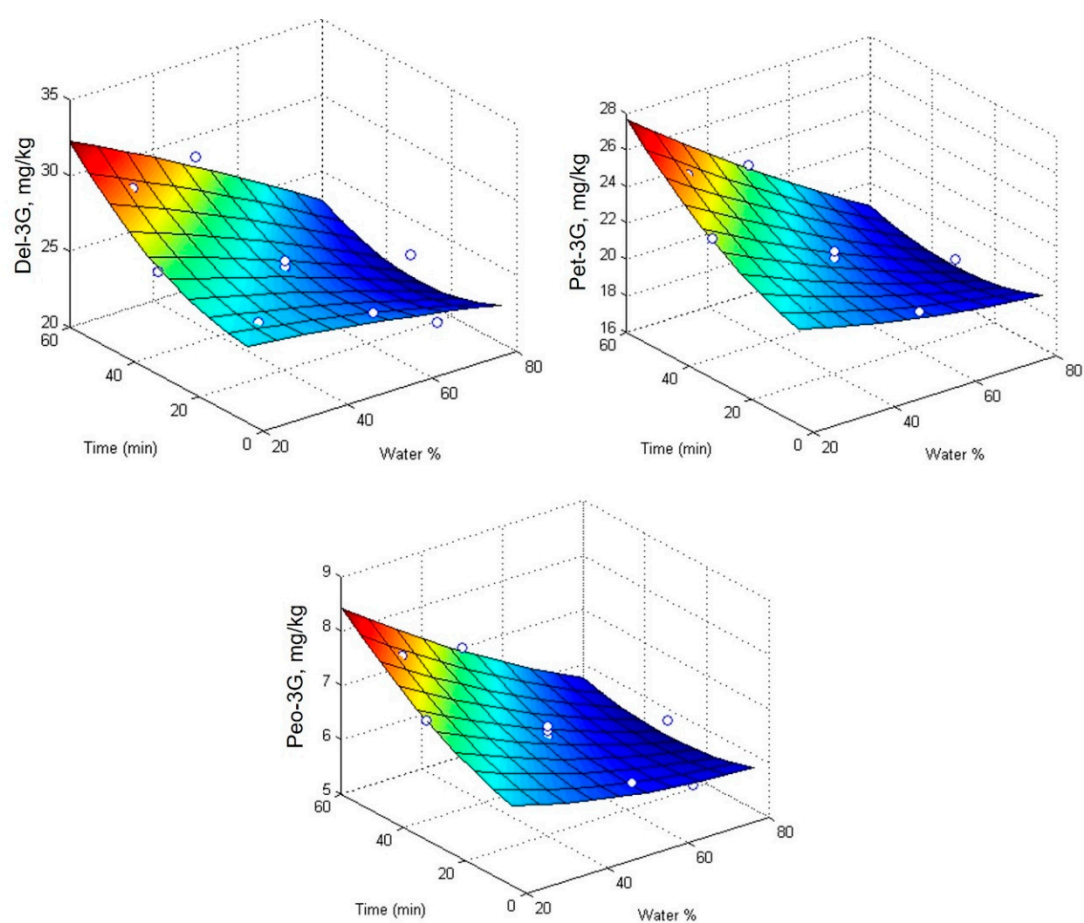


Figure S6. Response surfaces of ultrasound assisted extraction of grape skin using DES 4 for Del-3G, Pet-3G and Peo-3G.

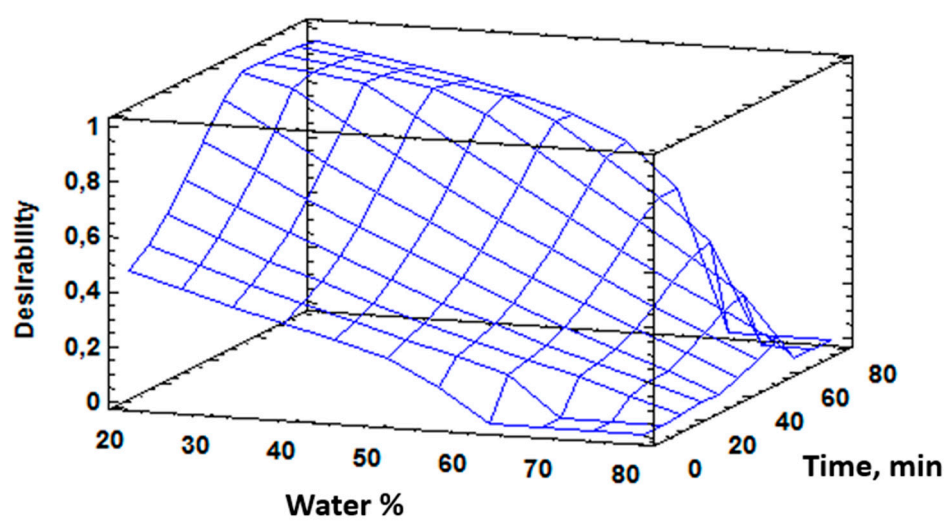


Figure S7. Desirability plot for the ultrasound assisted extraction of grape skin using DES4.

Table S7. Significance of the effects for the microwave assisted extraction with DES5.

Anthocyanin	Effect	p-value	Significance	Anthocyanin	Effect	p-value	Significance
Del-3G	Temperature (°C)(A)	0.0015	Yes	Mal-3G	Temperature (°C)(A)	0.0001	Yes
	Time(min) (B))	0.1572	No		Time(min) (B))	0.5937	No
	Water (C)	0.1211	No		Water (C)	0.0289	Yes
	AA	0.0146	Yes		AA	0.0091	Yes
	AB	0.9922	No		AB	0.9505	No
	AC	0.2341	No		AC	0.0242	Yes
	BB	0.2228	No		BB	0.0990	No
	BC	0.7870	No		BC	0.2878	No
	CC	0.5296	No		CC	0.9011	No
Pet-3G	Temperature (°C)(A)	0.0009	Yes	Mal-3G-cou	Temperature (°C)(A)	0.0043	Yes
	Time(min) (B))	0.3637	No		Time(min) (B))	0.6884	No
	Water (C)	0.0764	No		Water (C)	0.2653	No
	AA	0.0195	Yes		AA	0.0092	Yes
	AB	0.9926	No		AB	0.0817	No
	AC	0.0797	No		AC	0.3351	No
	BB	0.0977	No		BB	0.0297	Yes
	BC	0.6551	No		BC	0.4480	No
	CC	0.9812	No		CC	0.9256	No

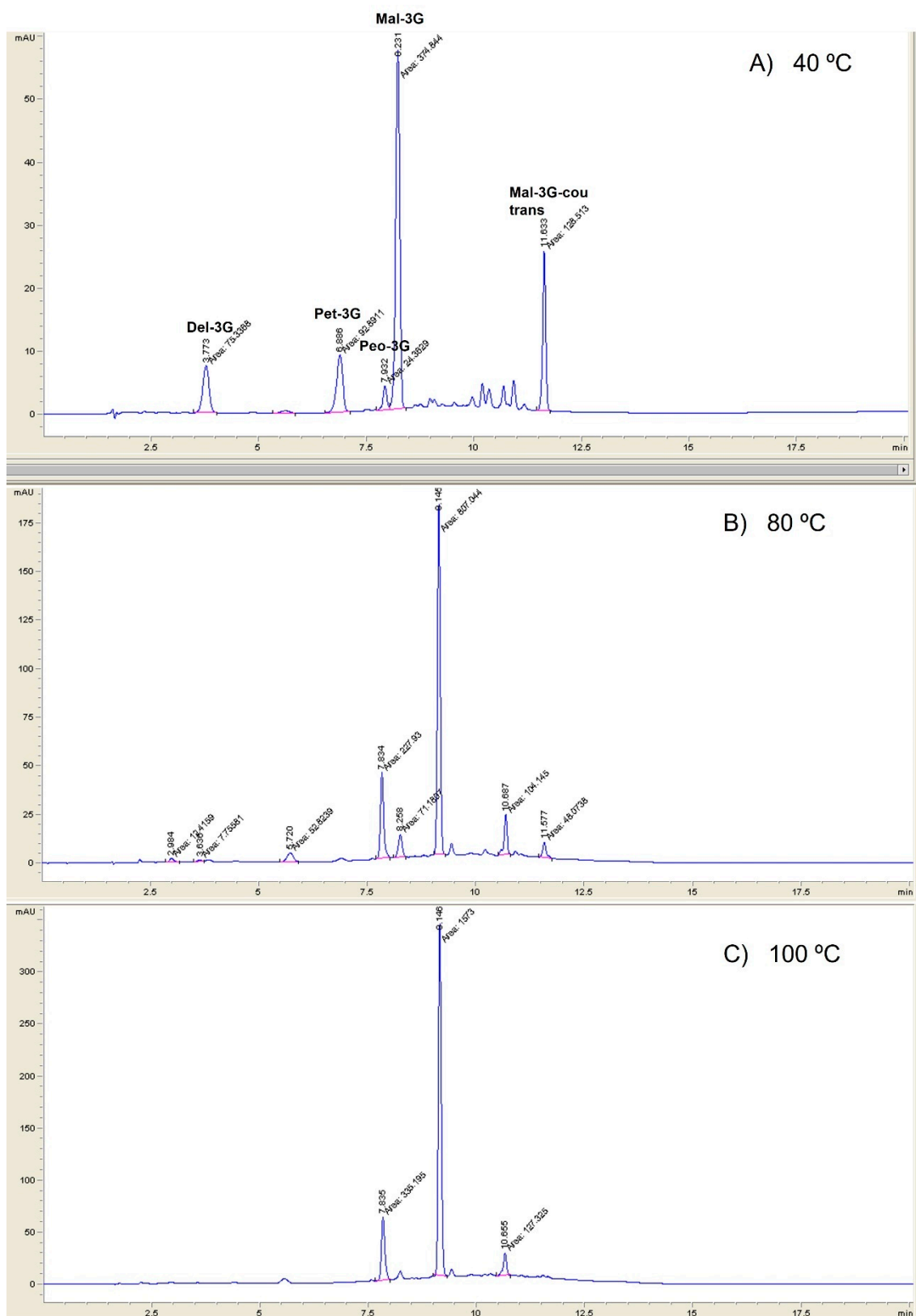


Figure S8. Chromatogram of extracts extracted at different temperatures: extract of the experiment number 10 in the microwave assisted extraction DOE, at 40 °C (A), 80 °C (B) and 100 °C (C).

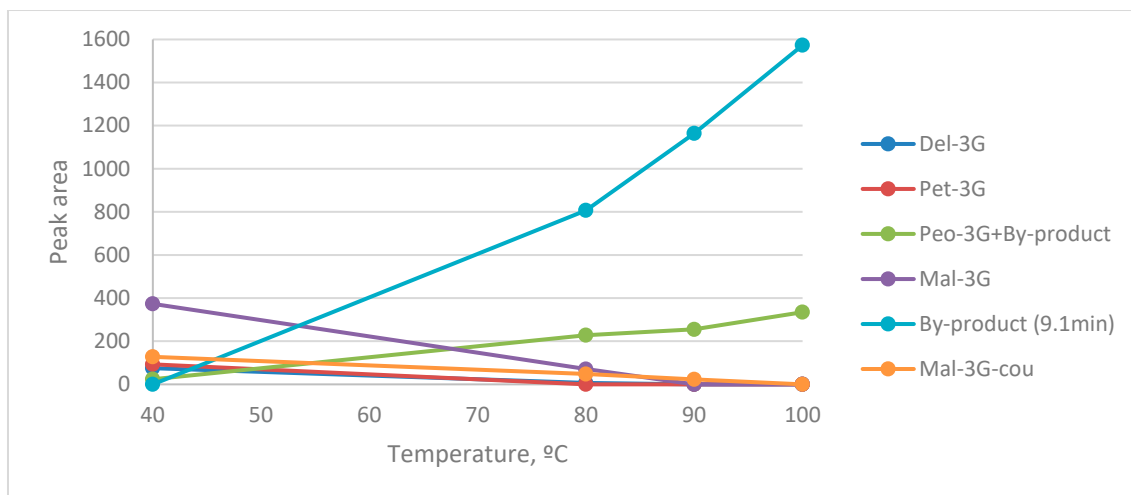


Figure S9. Evolution of peak area in the extracts obtained at different extraction temperatures.

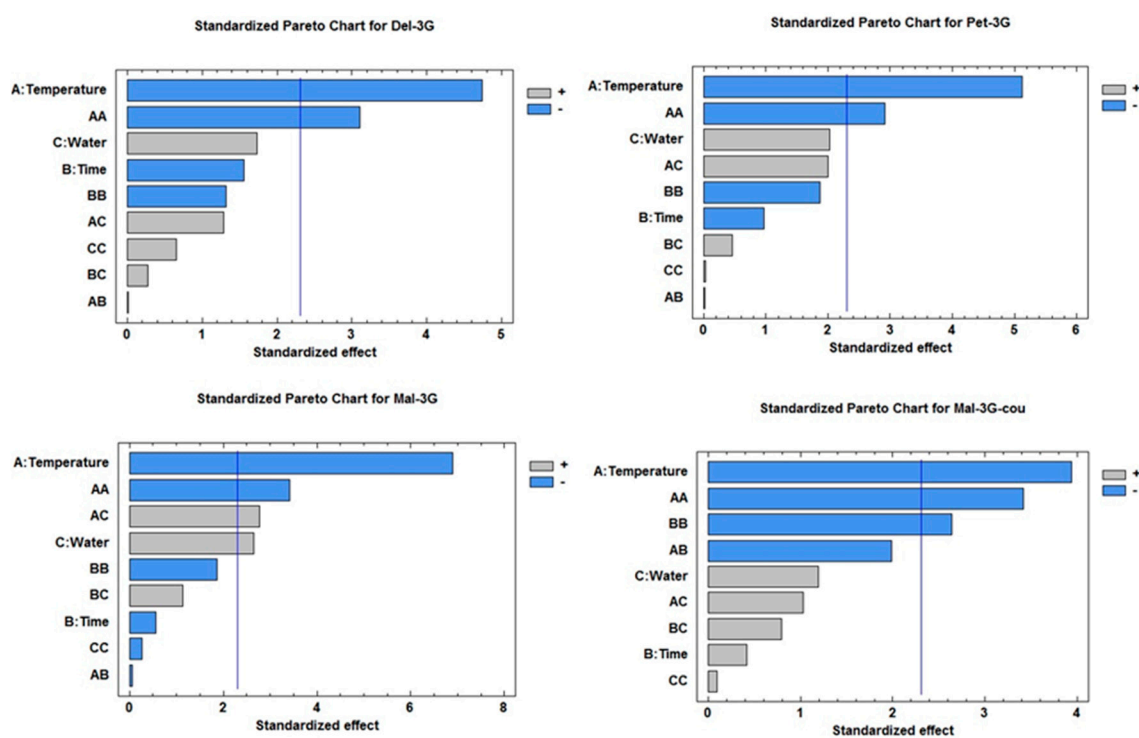


Figure S10. Pareto charts of anthocyanins with microwave assisted extraction and DES5.

Temperature (°C) - Time (min)

20 % Water

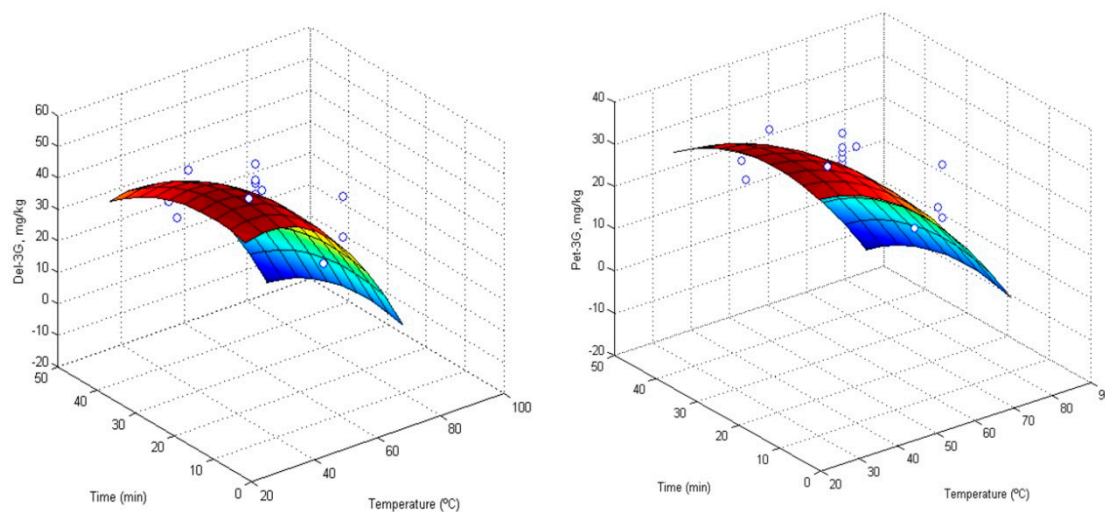


Figure S11. Response surfaces of microwave assisted extraction temperature vs. Time for 20 % of aqueous percentage.

Temperature (°C) - % Water

20 min

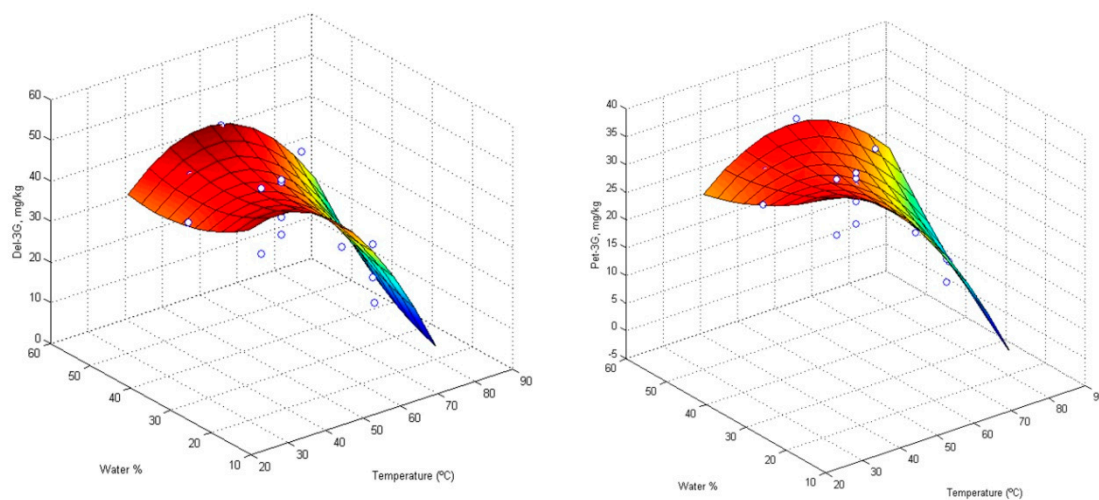


Figure S12. Response surfaces of microwave assisted extraction temperature vs. % water for 20 min.

Table S8. Experimental design results of ultrasound and microwave assisted extraction for the optimisation of %water, time and temperature.

Response	Ultrasound assisted extraction			Microwave assisted extraction			
	Time, min	% water	R ² , %	Time, min	% water	Temperature, °C	R ² , %
Del-3G	60 min	20 %	88.9	No sig	No sig	50 °C	84.0
Pet-3G	60 min	20 %	96.7	No sig	No sig	50 °C	85.3
Peo-3G	60 min	20 %	91.4	-	-	-	-
Mal-3G	60 min	20 %	97.5	No sig	20 %	50 °C	90.7
Mal-3G-cou	50 min	20 %	95.7	No sig	No sig	45 °C	83.2

No sig = No significance at 95% level.

Table S9. P-values for the comparison of MAE and USAE vs traditional methanol/H₂O/formic acid.

Anthocyanins	Microwave assisted extraction (MAE)		Ultrasound assisted extraction (USAE)	
Del-3G	4,77474E-06	<0.00001	0,00010057	<0.001
Pet-3G	0,000639109	<0.001	0,00028669	<0.001
Peo-3G	x		2,0105E-05	<0.0001
Mal-3G	1,73928E-07	<0.000001	0,00019836	<0.001
Mal-3G-cou	5,82903E-06	<0.00001	9,8591E-08	<0.0000001

p-values < 0.05; differences between compared methods

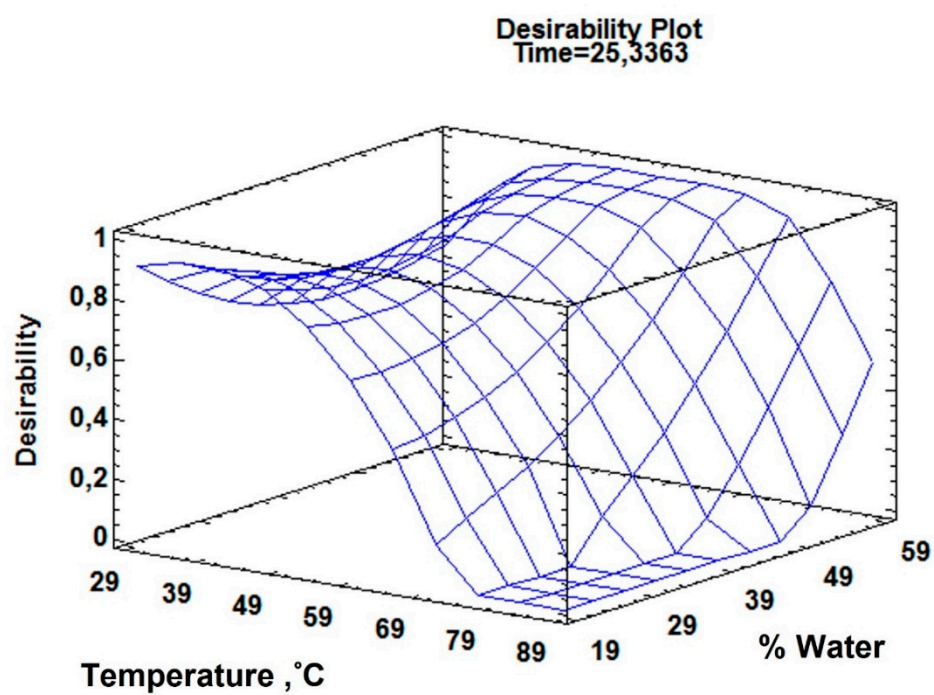
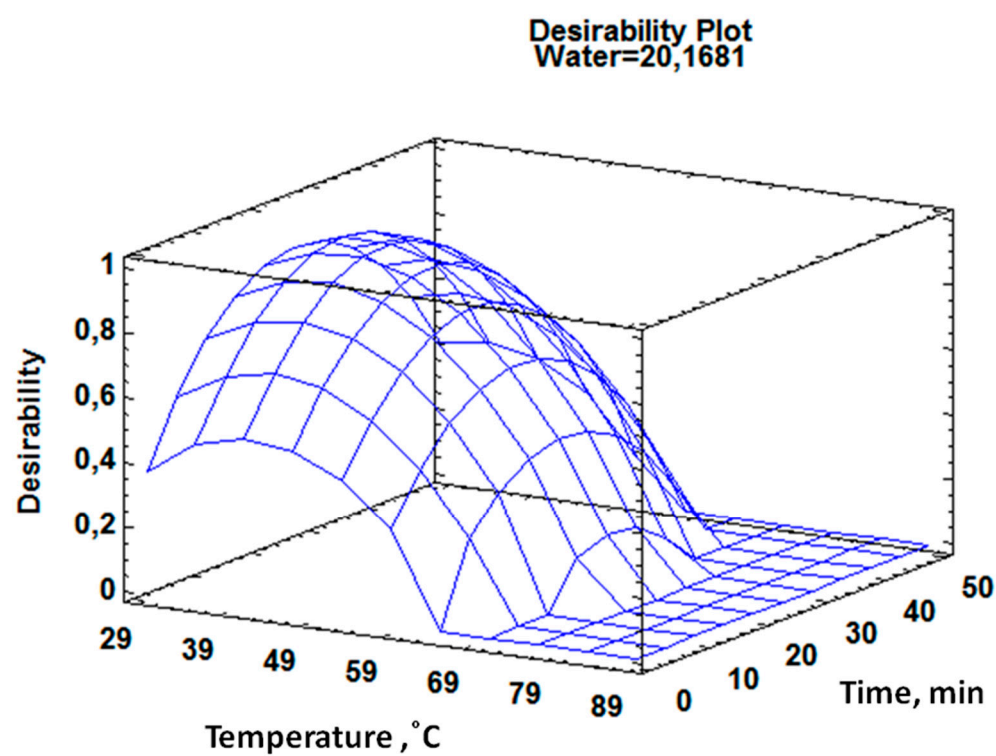


Figure S13. Desirability plot for the microwave assisted extraction experimental design (Peo-3G excluded).