Supporting information

Ascorbic acid derivatives as potential substitutes for ascorbic acid to reduce color degradation of drinks containing ascorbic acid and anthocyanins from natural extracts

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1. Evolution of UV-visible spectra of ascorbic acid and ascorbic acid derivatives in citrate buffer at pH = 3 under heat and under irradiation

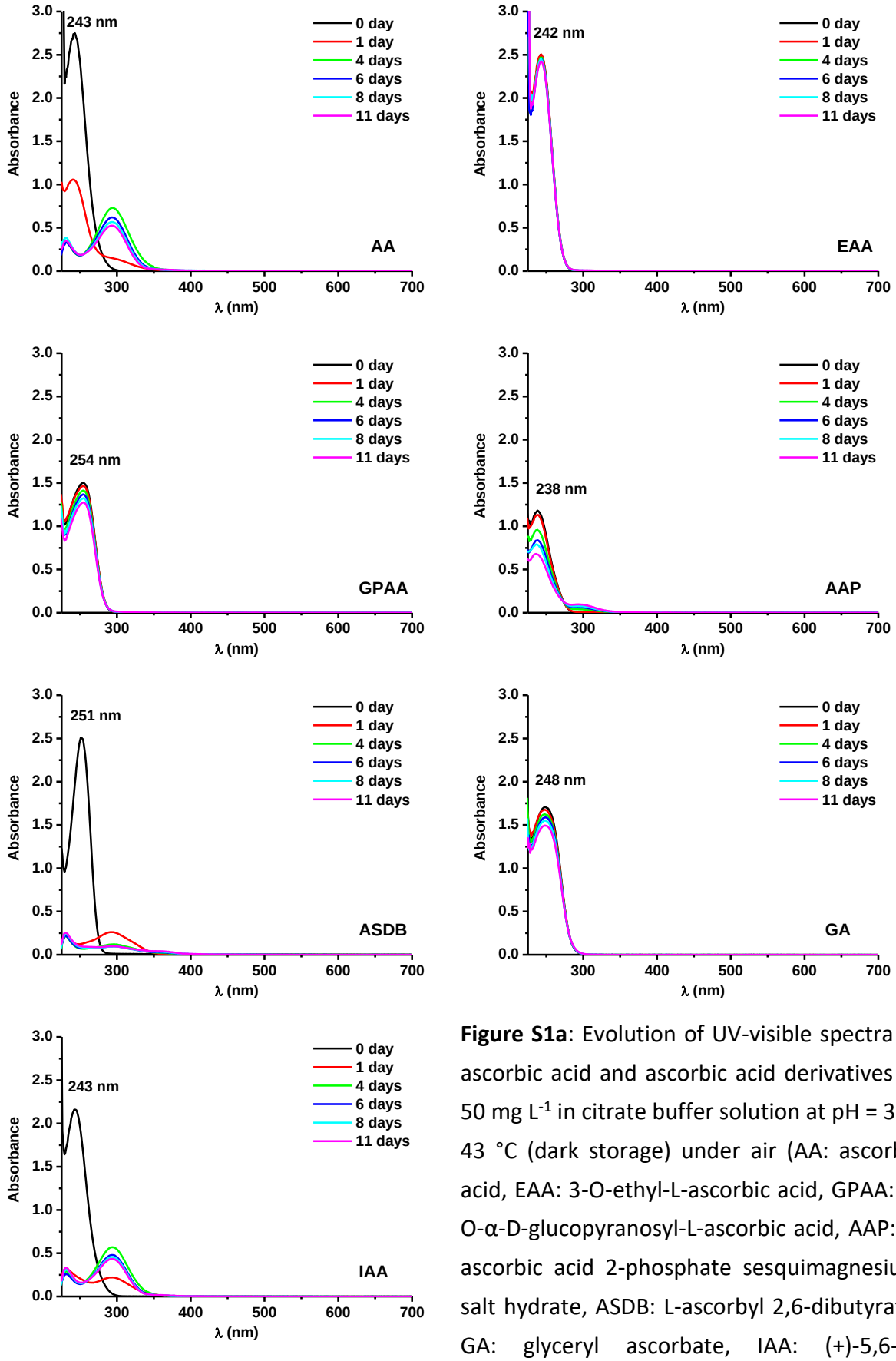
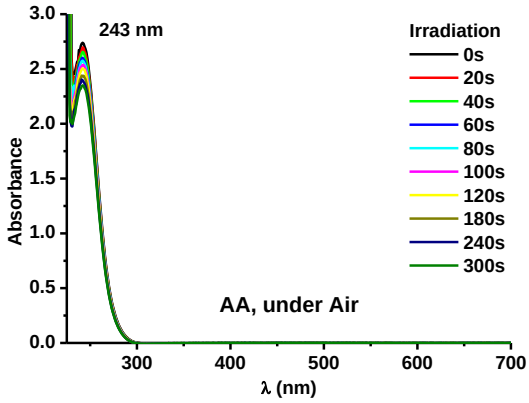
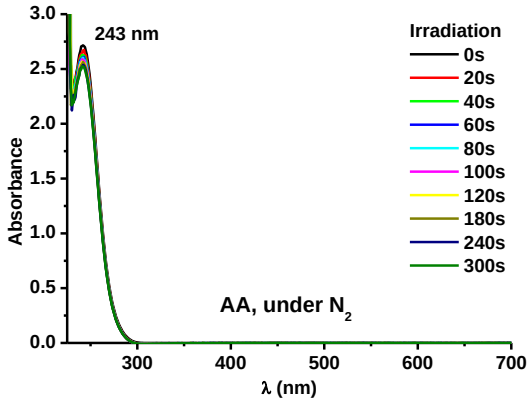


Figure S1a: Evolution of UV-visible spectra of ascorbic acid and ascorbic acid derivatives at 50 mg L^{-1} in citrate buffer solution at pH = 3 at 43 °C (dark storage) under air (AA: ascorbic acid, EAA: 3-O-ethyl-L-ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, AAP: L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, ASDB: L-ascorbyl 2,6-dibutyrate, GA: glyceryl ascorbate, IAA: (+)-5,6-*O*-isopropylidene-L-ascorbic acid).


243 nm
Irradiation
0s
20s
40s
60s
80s
100s
120s
180s
240s
300s
AA, under Air
Absorbance
λ (nm)


243 nm
Irradiation
0s
20s
40s
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120s
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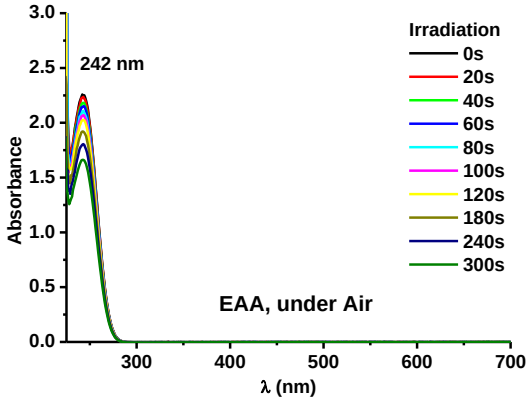
300s
AA, under N2
Absorbance
λ (nm)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Irradiation
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40s
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100s
120s
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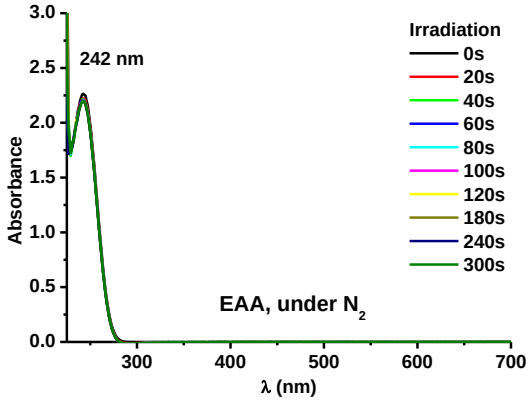
240s
300s
EAA, under Air
Absorbance
λ (nm)


242 nm
Irradiation
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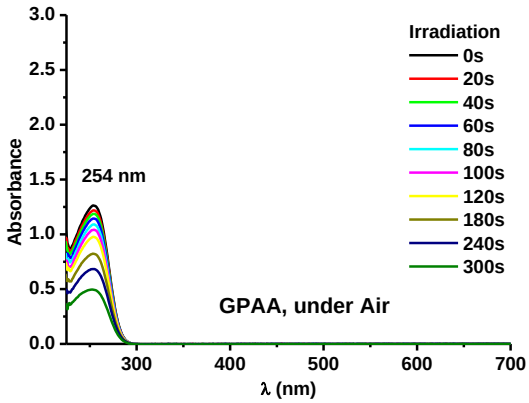
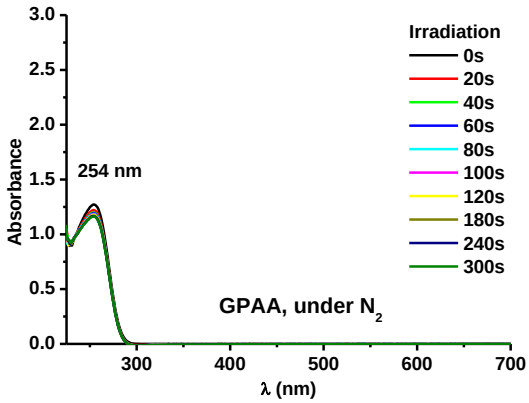
EAA, under N2
Absorbance
λ (nm)


254 nm
Irradiation
0s
20s
40s
60s
80s
100s
120s
180s
240s
300s
GPAA, under Air
Absorbance
λ (nm)


254 nm
Irradiation
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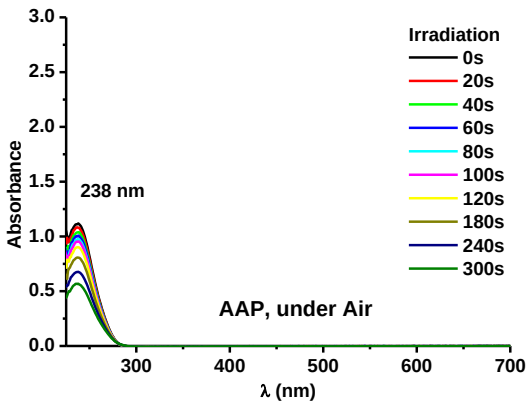
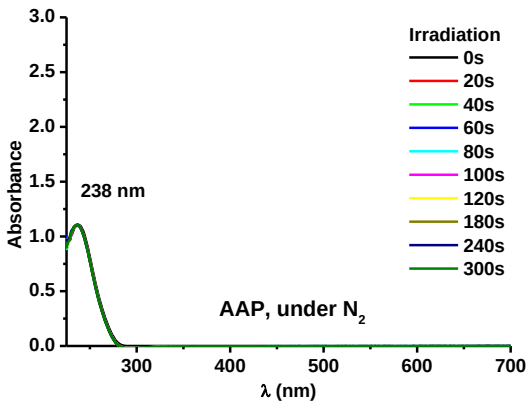
GPAA, under N2
Absorbance
λ (nm)


238 nm
Irradiation
0s
20s
40s
60s
80s
100s
120s
180s
240s
300s
AAP, under Air
Absorbance
λ (nm)


238 nm
Irradiation
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40s
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300s
AAP, under N2
Absorbance
λ (nm)

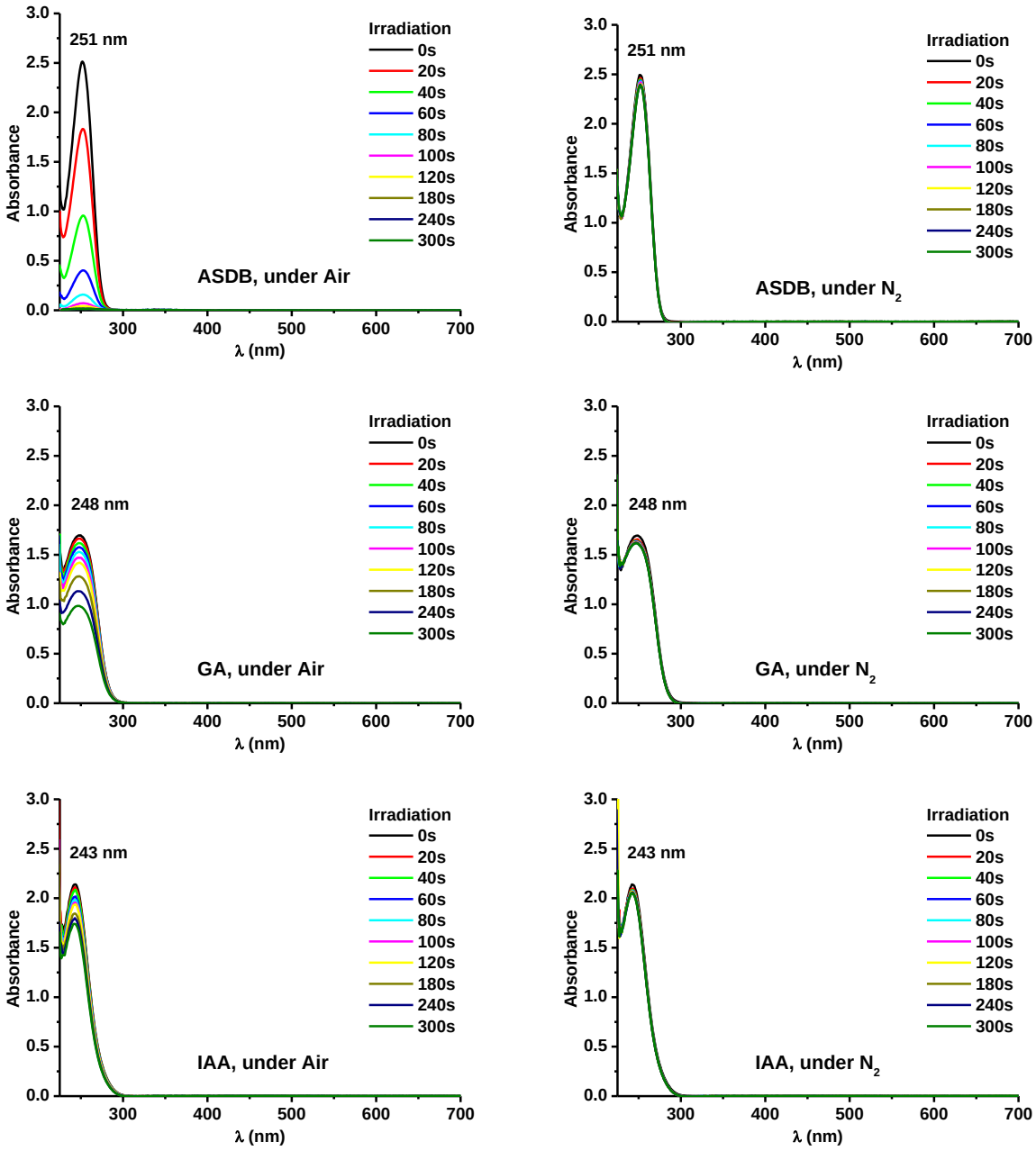


Figure S1b: Evolution of UV-visible spectra of ascorbic acid and ascorbic acid derivatives at 50 mg L^{-1} in citrate buffer solution at pH = 3 under irradiation at room temperature under air and under N_2 (AA: ascorbic acid, EAA: 3-O-ethyl-L-ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, AAP: L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, ASDB: L-ascorbyl 2,6-dibutyrate, GA: glyceryl ascorbate, IAA: (+)-5,6-*O*-isopropylidene-L-ascorbic acid).

2. Degradation mechanism of ascorbic acidic solution – identification of dehydroascorbic acid

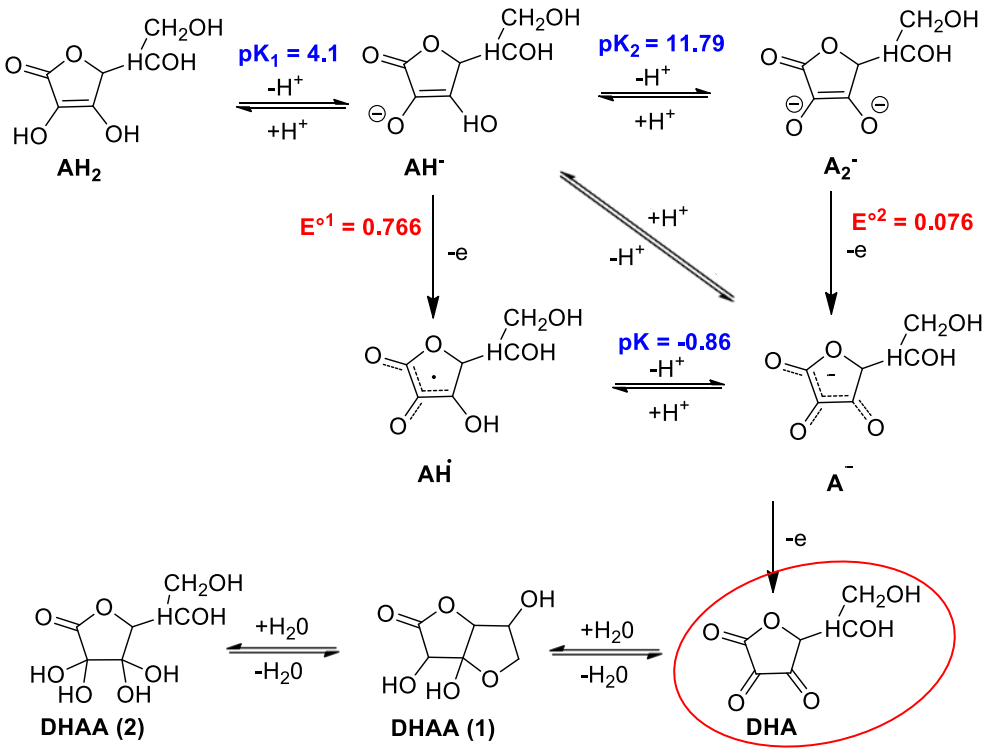


Figure S2a: Degradation mechanism of ascorbic acid in solution leading to the formation of dehydroascorbic acid (DHA) with pKa and potential E° values [Njus et al. 1991, Du et al. 2012].

Njus, D., Jalukar, J., Zu, J. A., Kelley, P.M., *Am. J. Clin. Nutr.*, **1991**, *54(6 Suppl.)*, 1179S–1183S.

Du, J., Cullen,J. J., Buettner, G. R., *Biochim. Biophys. Acta*, **2012**, *1826(2)*, 443–57.

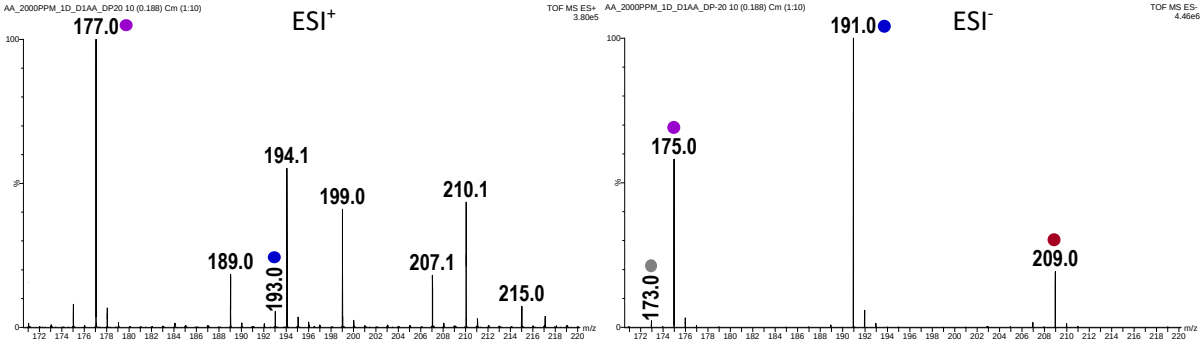
DHA corresponds to the degradation product of AA that absorbs at 294 nm (Figure S1a) according to mass spectrometry experiments (Figures S2b-c) and UV-visible spectra simulation (Figure S2d).

- **Mass spectrometry on the ascorbic acid solution at pH = 3**

Sample preparation: a solution containing 2000 ppm of ascorbic acid in an aqueous solution (HCl in water) at pH = 3 was divided into two samples placed in an oven at 43 °C (dark storage) during 1 day for one and 7 days for the other. Then the samples were diluted (1:10) in a 3 mM ammonium acetate solution in methanol solution before being introduced in the ionization source of the mass spectrometer with a flow rate of 10 µL min^{-1}.

Protocol: The analyses were performed with a mass spectrometer SYNAPT G2 HDMS (Waters) equipped with an atmospheric pressure ionization source (API). The samples were ionized in positive electrospray mode in the following conditions: electrospray voltage: + 2.8 kV; orifice voltage: + 20 V; nebulizer gas flow rate (N_2): 100 L h^{-1}. The sample were also ionized in negative electrospray mode in the following conditions: electrospray voltage: -2.27 kV; orifice voltage: -20 V; nebulizer gas flow rate (N_2): 100 L h^{-1}. The mass spectra were obtained with a time-of-flight (TOF) analyzer. The measurements of the exact masses were realized with an external calibration.

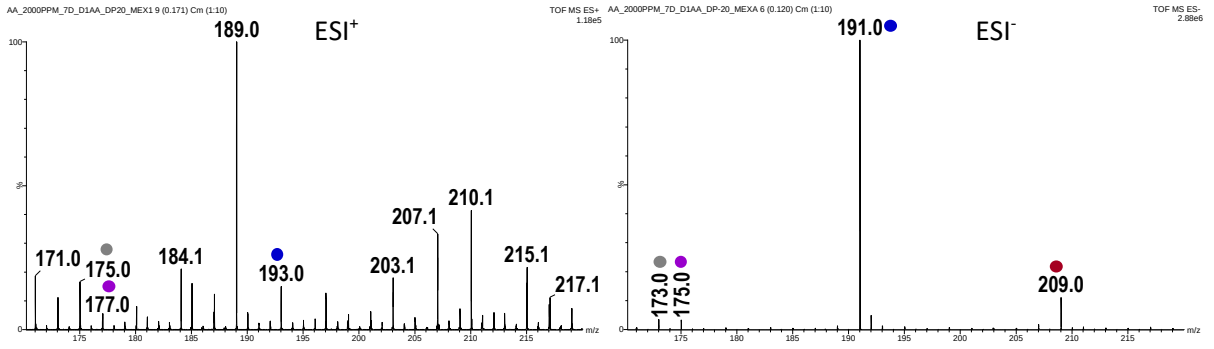
A)



B)

		Composition	m/z_{th}	m/z_{exp}	Error (mDa)	Error (ppm)
DHA	**[M-H]⁻**	$C_6H_5O_6^-$	173.0092	173.0092	0	0
				173.0092	0	0
				173.0091	- 0.1	- 0.6
AA	**[M-H]⁻**	$C_6H_7O_6^-$	175.0248	175.0250	+ 0.2	+ 1.1
				175.0250	+ 0.2	+ 1.1
				175.0251	+ 0.3	+ 1.7
DHAA(1)	**[M-H]⁻**	$C_6H_7O_7^-$	191.0197	191.0195	- 0.2	- 1.0
				191.0195	- 0.2	- 1.0
				191.0195	- 0.2	- 1.0
DHAA(2)	**[M-H]⁻**	$C_6H_9O_8^-$	209.0303	209.0302	- 0.1	- 0.5
				209.0301	- 0.2	- 1.0
				209.0301	- 0.2	- 1.0

Figure S2b: (A) Mass spectra in positive electrospray ionization mode (ESI^+) and negative electrospray ionization mode (ESI^-) of the ascorbic acid solution placed in oven at 43°C during 1 day and (B) corresponding identified masses with ESI^-.



B)

		Composition	m/z_{th}	m/z_{exp}	Error (mDa)	Error (ppm)
DHA	$[M-H]^-$	$C_6H_5O_6^-$	173.0092	173.0094	+ 0.2	+ 1.2
				173.0094	+ 0.2	+ 1.2
				173.0093	+ 0.1	+ 0.6
AA	$[M-H]^-$	$C_6H_7O_6^-$	175.0248	175.0249	+ 0.1	+ 0.6
				175.0250	+ 0.2	+ 1.1
				175.0248	0	0
DHAA(1)	$[M-H]^-$	$C_6H_7O_7^-$	191.0197	191.0201	+ 0.4	+ 2.1
				191.0201	+ 0.4	+ 2.1
				191.0200	+ 0.3	+ 1.6
DHAA(2)	$[M-H]^-$	$C_6H_9O_8^-$	209.0303	209.0304	+ 0.1	+ 0.5
				209.0304	+ 0.1	+ 0.5
				209.0304	+ 0.1	+ 0.5

Figure S2c: (A) Mass spectra in positive electrospray ionization mode (ESI^+) and negative electrospray ionization mode (ESI^-) of the ascorbic acid solution placed in oven at 43°C during 7 days and (B) corresponding identified masses with ESI^-.

The experiments (Figures S2b-c) show the presence of compounds with acidic H, with molecular formula $C_6H_6O_6$ and monoisotopic mass of 174.0164 Da (error < 2 ppm) corresponding to DHA; with molecular formula $C_6H_8O_6$ and monoisotopic mass of 176.0321 Da (error < 2 ppm) corresponding to AA; with molecular formula $C_6H_8O_7$ and monoisotopic mass of 192.0270 Da (error < 3 ppm) corresponding to DHAA(1); with molecular formula $C_6H_{10}O_8$ and monoisotopic mass of 210.0376 Da (error < 1 ppm), corresponding to DHAA(2).

We thank gratefully Valérie Monnier (Spectropole) and Frédéric Dumur (Department of Chemistry) from the University of Aix-Marseille, France, for the mass spectrometry experiments.

• **UV-visible spectra simulation of ascorbic acid degradation products in solution**

The simulated UV-visible spectra were calculated with the time dependent density functional theory uB3LYP/RTD-mPW1PW91-FC using the optimized structure simulated at uB3LYP/6-31G* level on the relaxed geometries [Foresman et al. 1996].

Foresman, J. B., Frisch, *Æ.*, *Exploring Chemistry with Electronic Structure Methods - Second Edition*, **1996**, Gaussian.

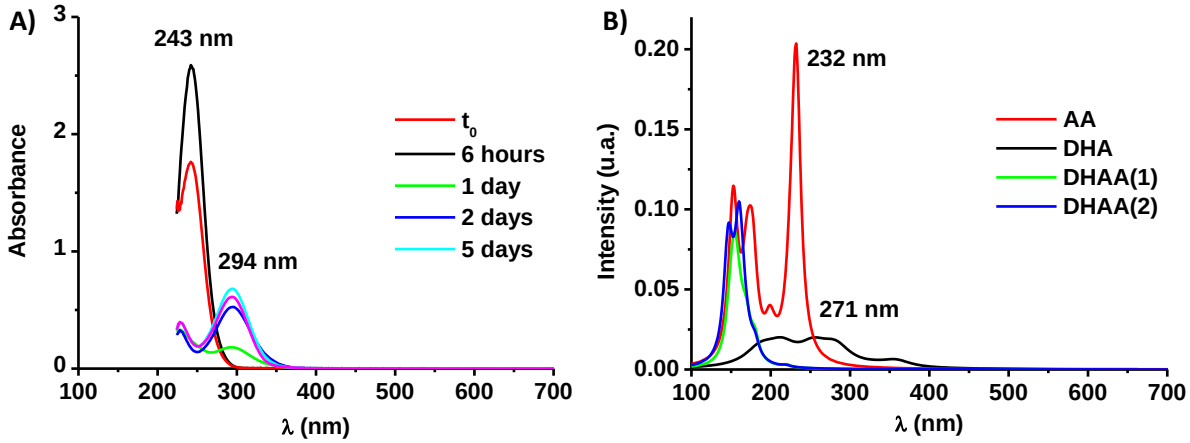


Figure S2d: (A) Experimental spectra of the thermal degradation (43°C, in dark) of ascorbic acid (AA) at 50 mg L^{-1} in citrate buffer at pH = 3 and (B) simulated spectra (without solvent) of AA and its potential degradation products DHA, DHAA(1) and DHAA(2).

The comparison of the experimental and simulated spectra suggests that the degradation product of AA that absorbs at 294 nm corresponds to the DHA.

The small difference in wavelength Δλ between the experimental and simulated values is due to the solvent used. Indeed the absorption wavelength depends on the medium in which the compounds are dissolved. Here the simulated data were calculated in the vacuum whereas the experimental values correspond to the compounds dissolved in a citrate buffer solution at pH = 3, hence the small difference.

3. Simulated pKa of ascorbic acid and ascorbic acid derivatives

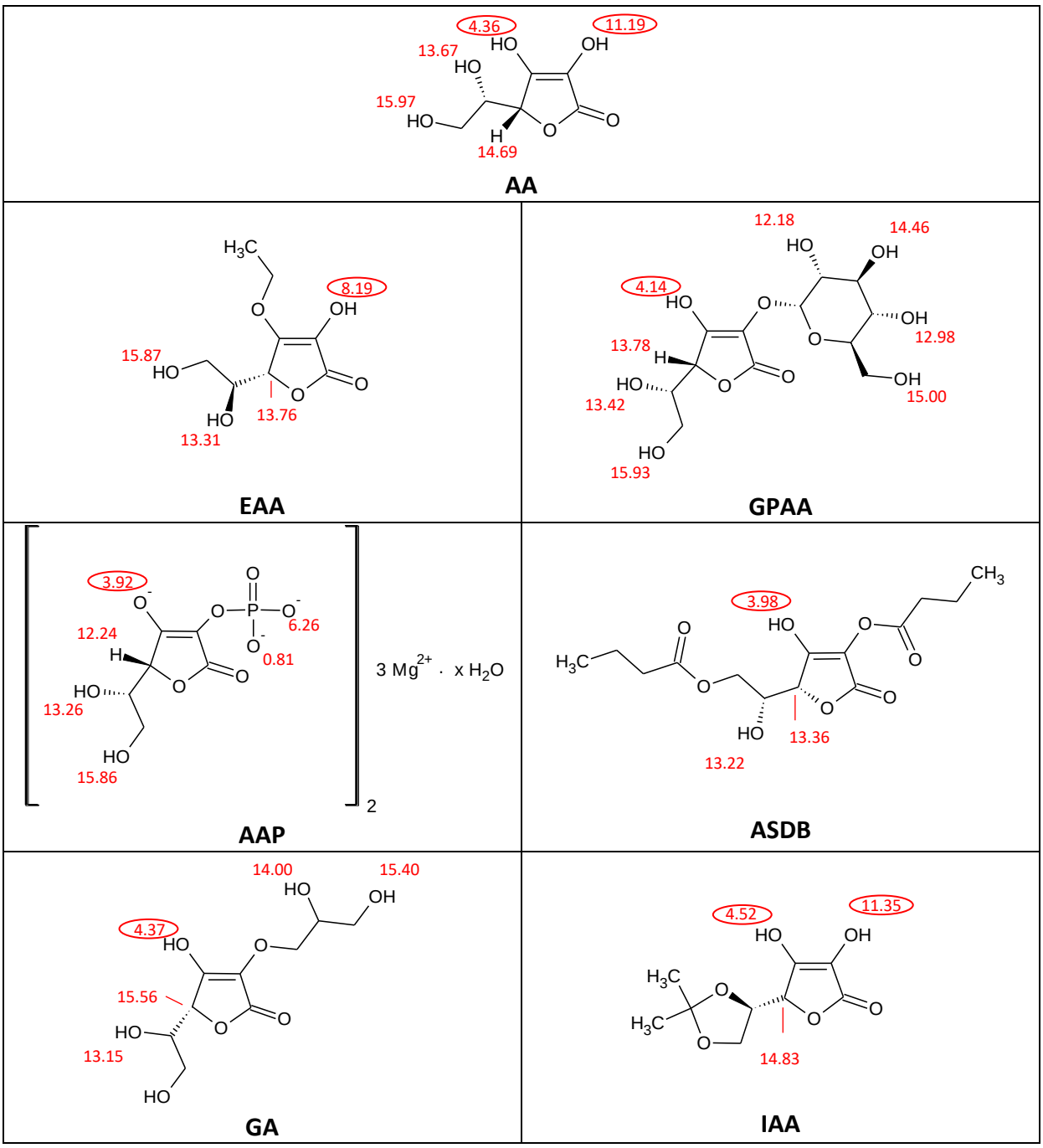
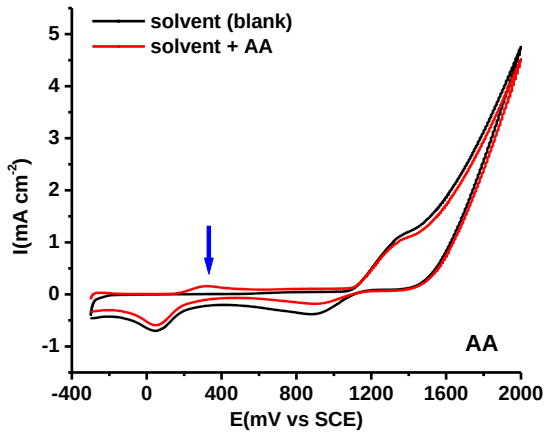
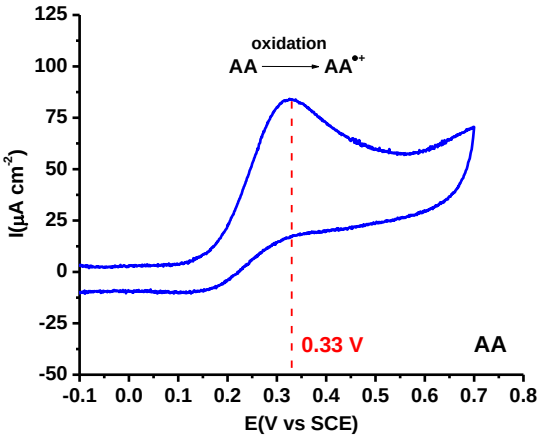
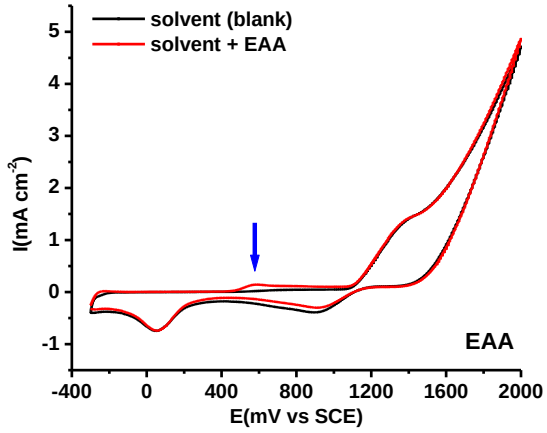


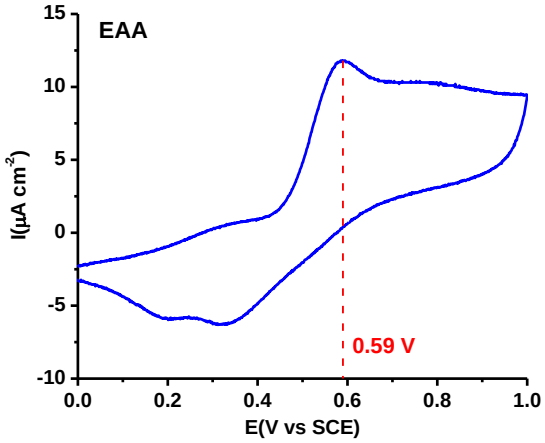
Figure S3: pKa of ascorbic acid and ascorbic acid derivatives at 25 °C simulated with Marvin Sketch 17.29.0. The pKa of the labile proton H of hydroxyl O-H bonds (bonded to the furan part of the molecule in C2 or C3) is encircled in red. (AA: ascorbic acid, EAA: 3-O-ethyl-L-ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, AAP: L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, ASDB: L-ascorbyl 2,6-dibutyrate, GA: glyceryl ascorbate, IAA: (+)-5,6-*O*-isopropylidene-L-ascorbic acid).

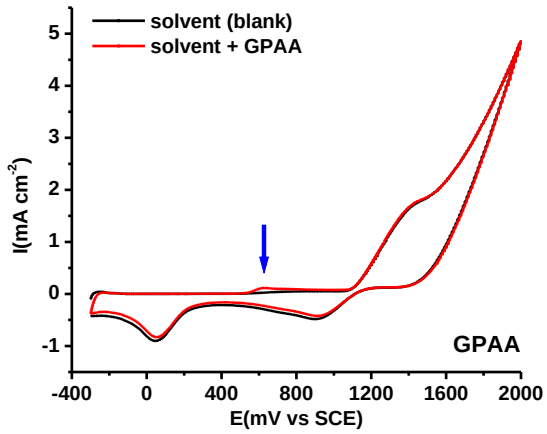
4. Oxidation potential of ascorbic acid and ascorbic acid derivatives determined from cyclic voltammetry

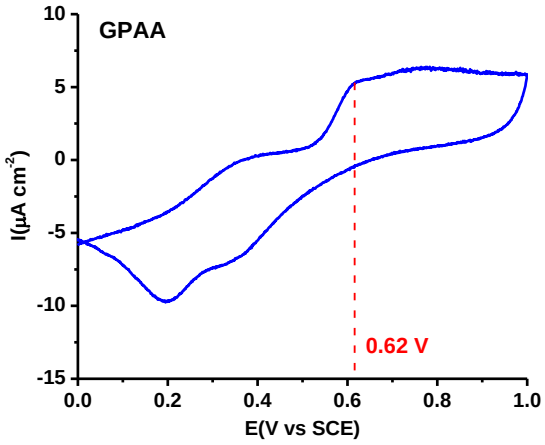


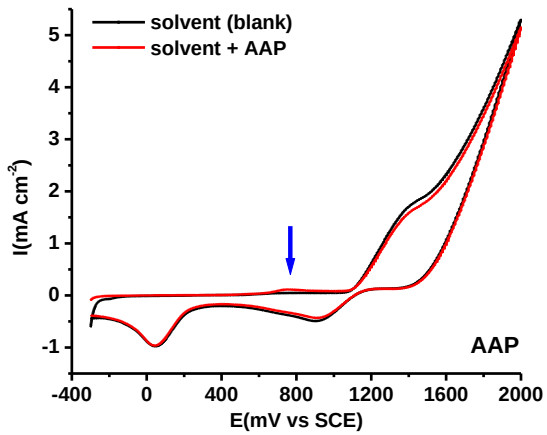


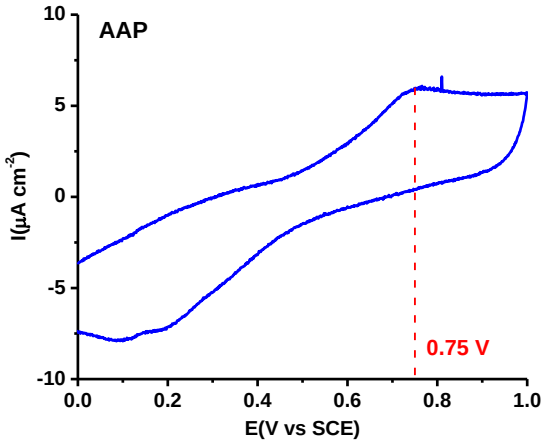












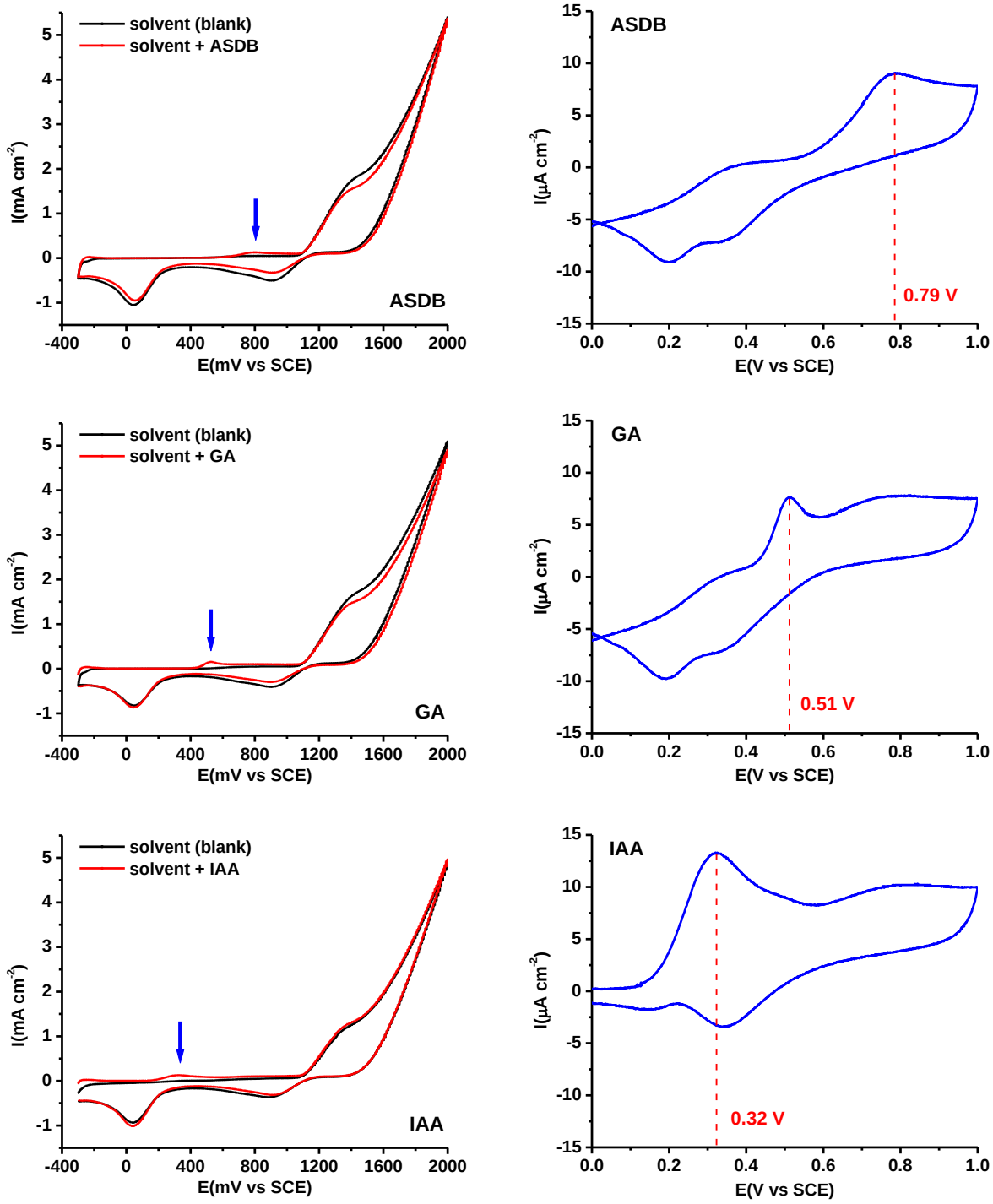
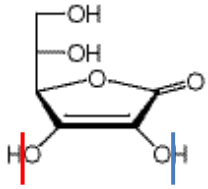
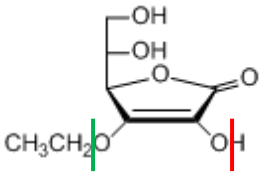
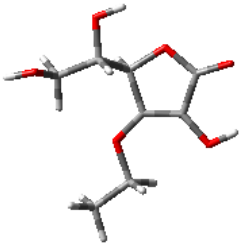
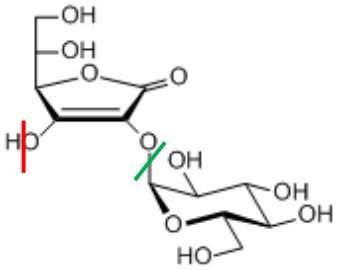
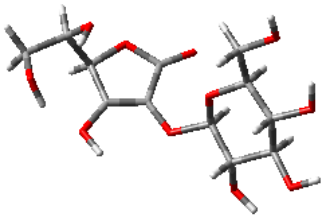
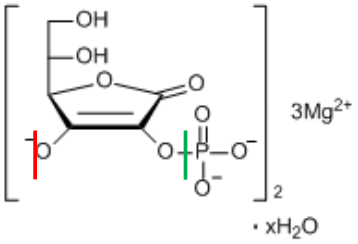
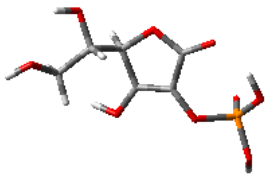
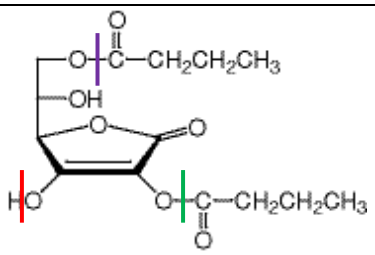
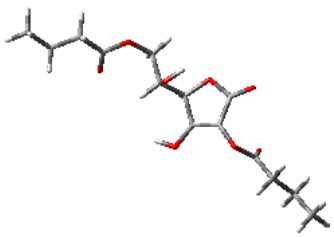
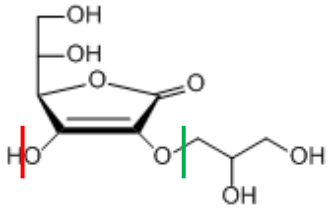
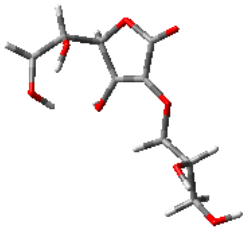
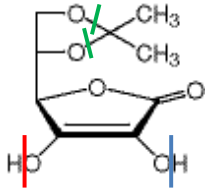
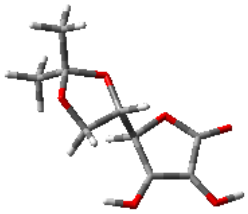


Figure S4: Cyclic voltammetry plots at room temperature under N_2 of the oxidation of ascorbic acid and ascorbic acid derivatives in aqueous KCl solutions at pH = 3 (KCl: 3.4 10^{-2} mol L^{-1}, ascorbic acid and ascorbic acid derivatives: 250 mg L^{-1}): (left) comparison between blank solvent (KCl solution in water acidified with HCl at pH = 3) and solvent with ascorbic acid or ascorbic acid derivatives; (right) ascorbic acid or ascorbic acid derivatives in the solvent at a narrower range of voltage and determination of their oxidation potential E_{Ox} (± 0.05 V) in red (AA: ascorbic acid, EAA: 3-O-ethyl-L-ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, AAP: L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, ASDB: L-ascorbyl 2,6-dibutyrate, GA: glyceryl ascorbate, IAA: (+)-5,6-*O*-isopropylidene-L-ascorbic acid).

5. Simulated Bond Dissociation Energy and Ionization potential

Table S5: Simulated bond dissociation energy (BDE) of O-H or O-substituent bonds (bonded to the furan part of the molecule in C2 or in C3) and Ionization potential (Ip) of ascorbic acid and ascorbic acid derivatives (Method UB3LYP/6-31G).

Ascorbic acid derivatives	Overview optimal molecule	BDE_{O-H} or $BDE_{O-substituent}$ (± 10 kcal mol^{-1})	Ip (± 10 kcal mol^{-1})
 **AA** $C_6H_8O_6$		71 85	185
 **EAA** $C_8H_{12}O_6$		56 81	180
 **GPAA** $C_{12}H_{18}O_{11}$		74 60	179
 **AAP** $C_6H_9O_9P$		75 79	193

 **ASDB** $C_{14}H_{20}O_8$		80 99 70	183
 **GA** $C_9H_{14}O_8$		65 61	175
 **IAA** $C_9H_{12}O_6$		72 - - 85	188

6. Correlation between properties and stability of ascorbic acid and ascorbic acid derivatives

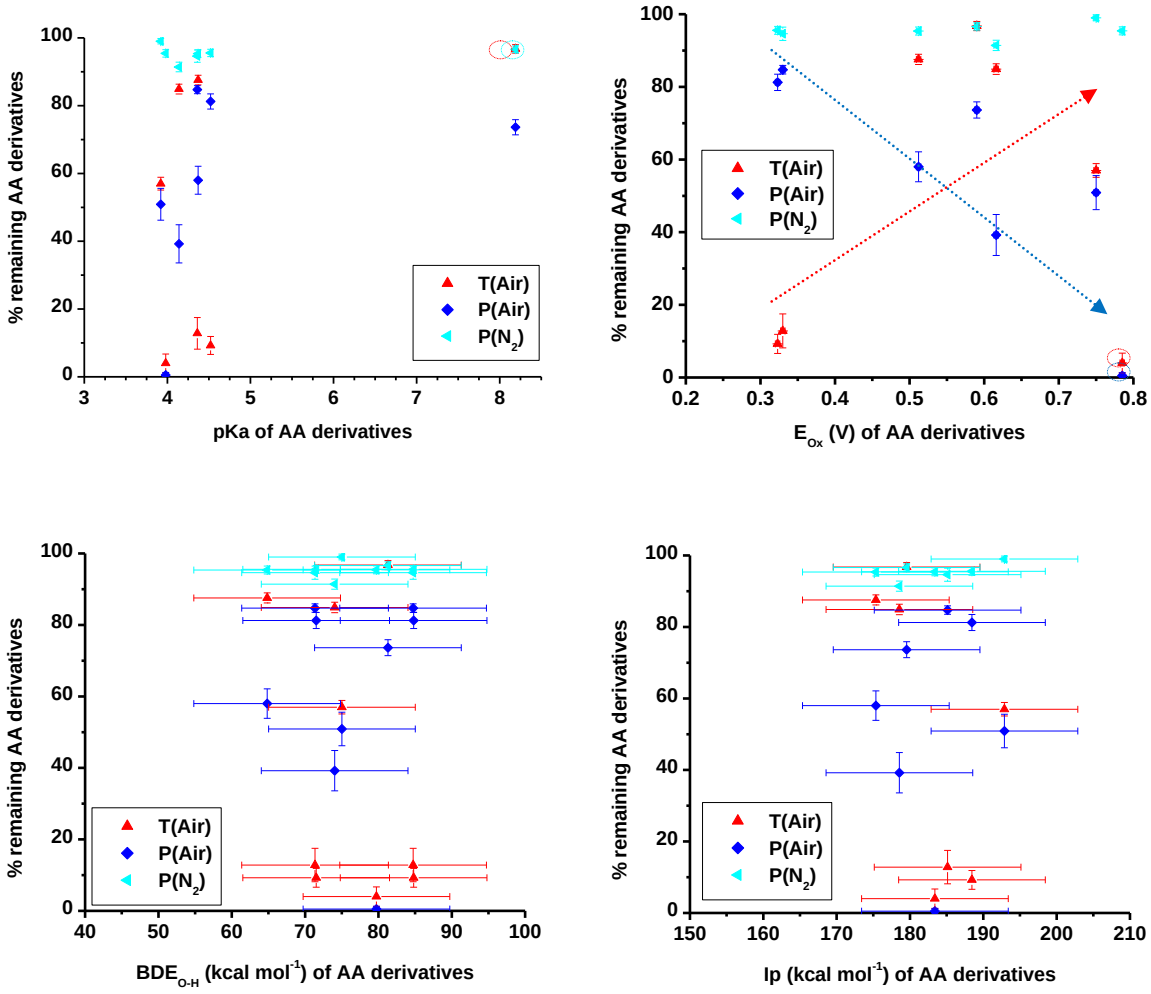


Figure S6: Thermal and photolytic stability of ascorbic acid (AA) and ascorbic acid derivatives in citrate buffer at pH = 3 after 11 days under 43 °C (dark storage) under air, T (Air), and after 300 s of irradiation at room temperature under air, P (Air), and under N_2, P (N_2), as a function of ascorbic or ascorbic acid derivatives properties: lower pKa (C3-hydroxyl group, or C2-hydroxyl group for EAA), oxidation potential E_{Ox}, bond dissociation energy BDE_{OH} and ionization potential Ip. The ascorbic acid derivatives corresponding to each value are listed in Table 1 of the article. The arrows are a guide for the eyes.

7. Correlation between stability of ascorbic acid derivatives alone and stability of anthocyanins in the presence of acid derivatives

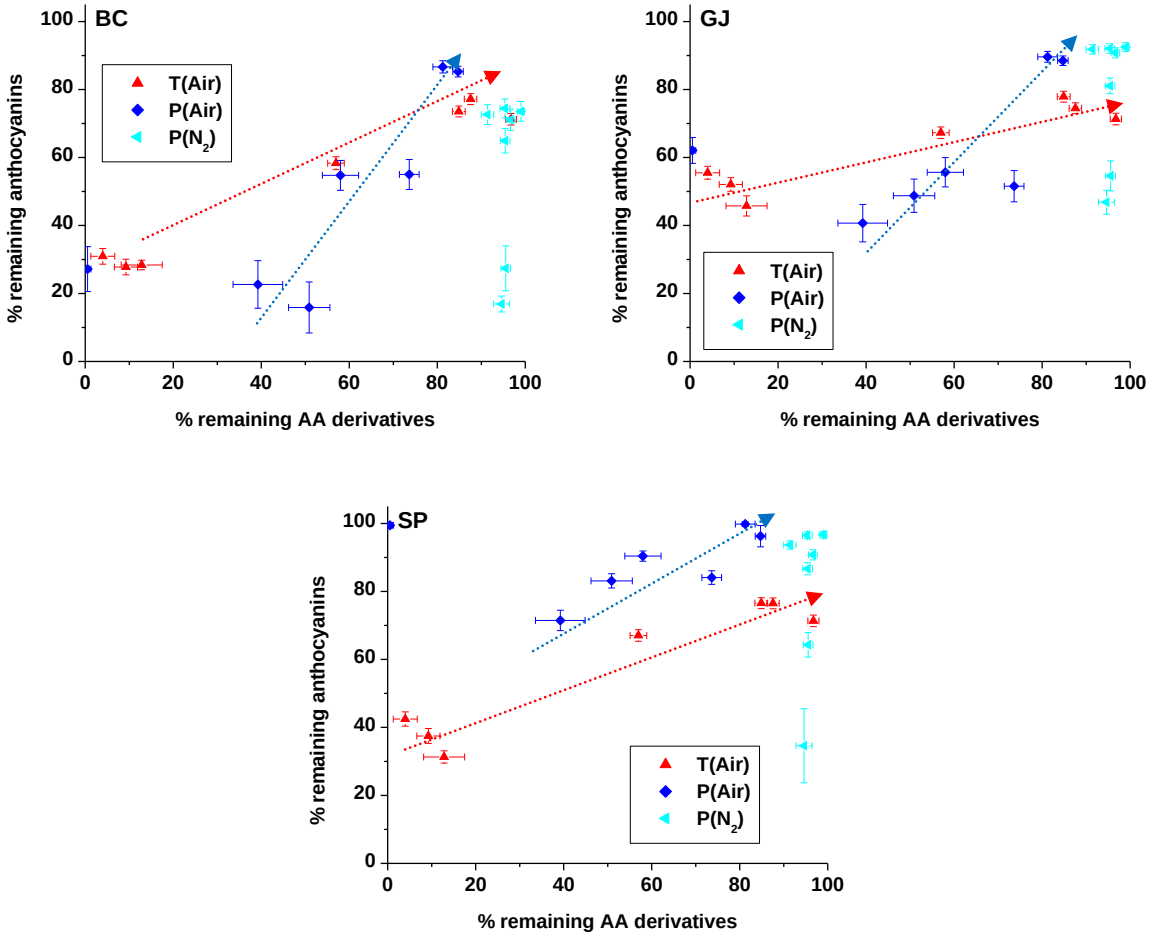


Figure S7: Thermal and photolytic stability of ascorbic acid (AA) and ascorbic acid derivatives alone in citrate buffer at pH = 3 after 11 days under 43 °C (dark storage) under air, T(Air), and after 300 s of irradiation at room temperature under air, P(Air), and under N_2, P(N_2), as a function of stability of anthocyanins from BC, GJ and SP extracts in the presence of ascorbic acid derivatives after 10 days under 43 °C (dark storage) under air, T(Air), and after 300 s of irradiation under air, P(Air), and under N_2, P(N_2). (BC: black carrot, GJ: grape juice, SP: purple sweet potato, AA: ascorbic acid, EAA: 3-O-ethyl-L-ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, AAP: L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, ASDB: L-ascorbyl 2,6-dibutyrate, GA: glyceryl ascorbate, IAA: (+)-5,6-*O*-isopropylidene-L-ascorbic acid). The arrows are a guide for the eyes.

8. Correlation between properties of ascorbic acid derivatives and stability of anthocyanins in the presence of ascorbic acid derivatives

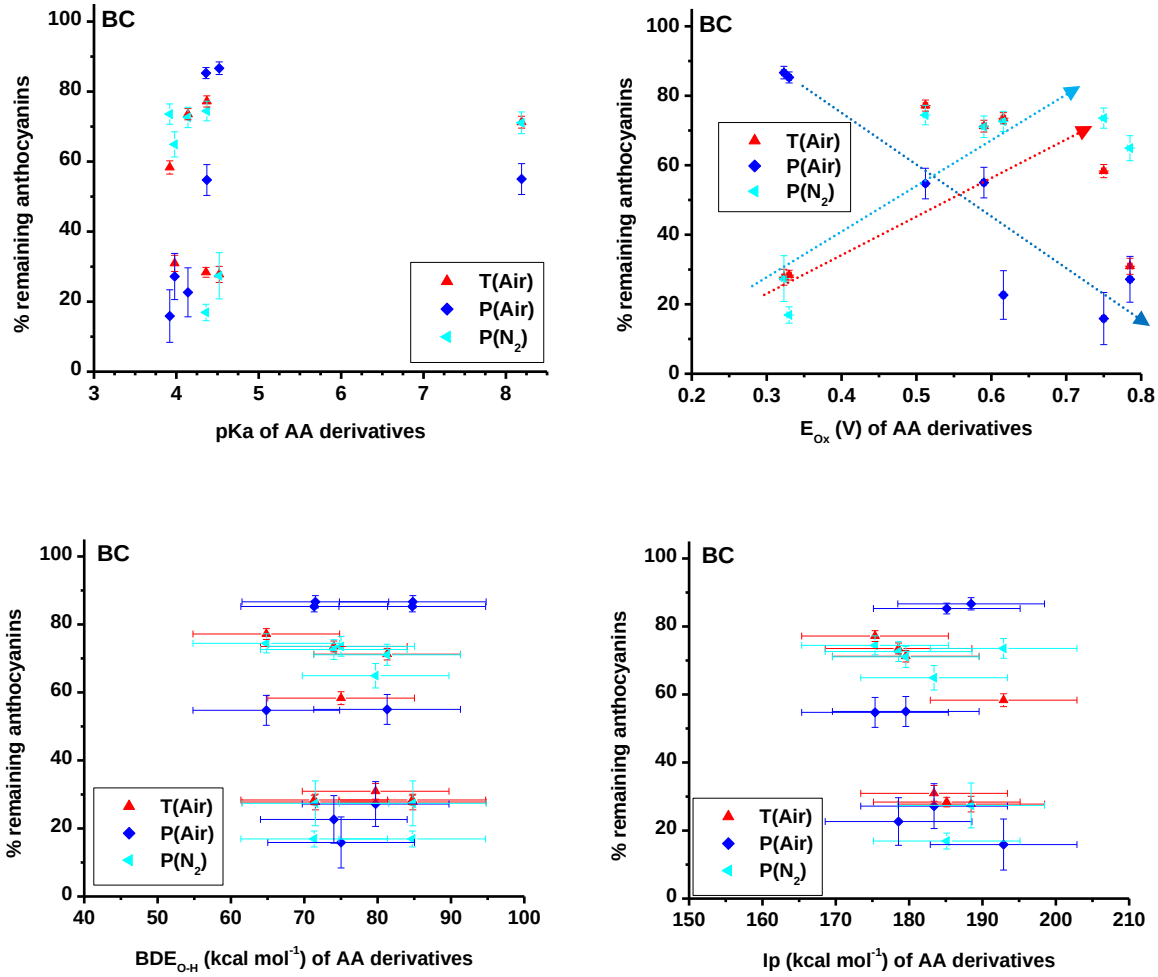


Figure S8a: Thermal and photolytic stability of anthocyanins from black carrot (BC) extract in the presence of ascorbic acid (AA) or ascorbic acid derivatives in citrate buffer at pH = 3 after 10 days under 43 °C (dark storage) under air, T(Air), and after 300 s of irradiation under air, P(Air), and under N_2, P(N_2), as a function of ascorbic acid or ascorbic acid derivatives properties: pKa (C3-hydroxyl group, or C2-hydroxyl group for EAA), oxidation potential E_{Ox}, bond dissociation energy BDE_{OH} and ionization potential Ip. The ascorbic acid derivatives corresponding to each value are listed in Table 1 of the article. The arrows are a guide for the eyes.

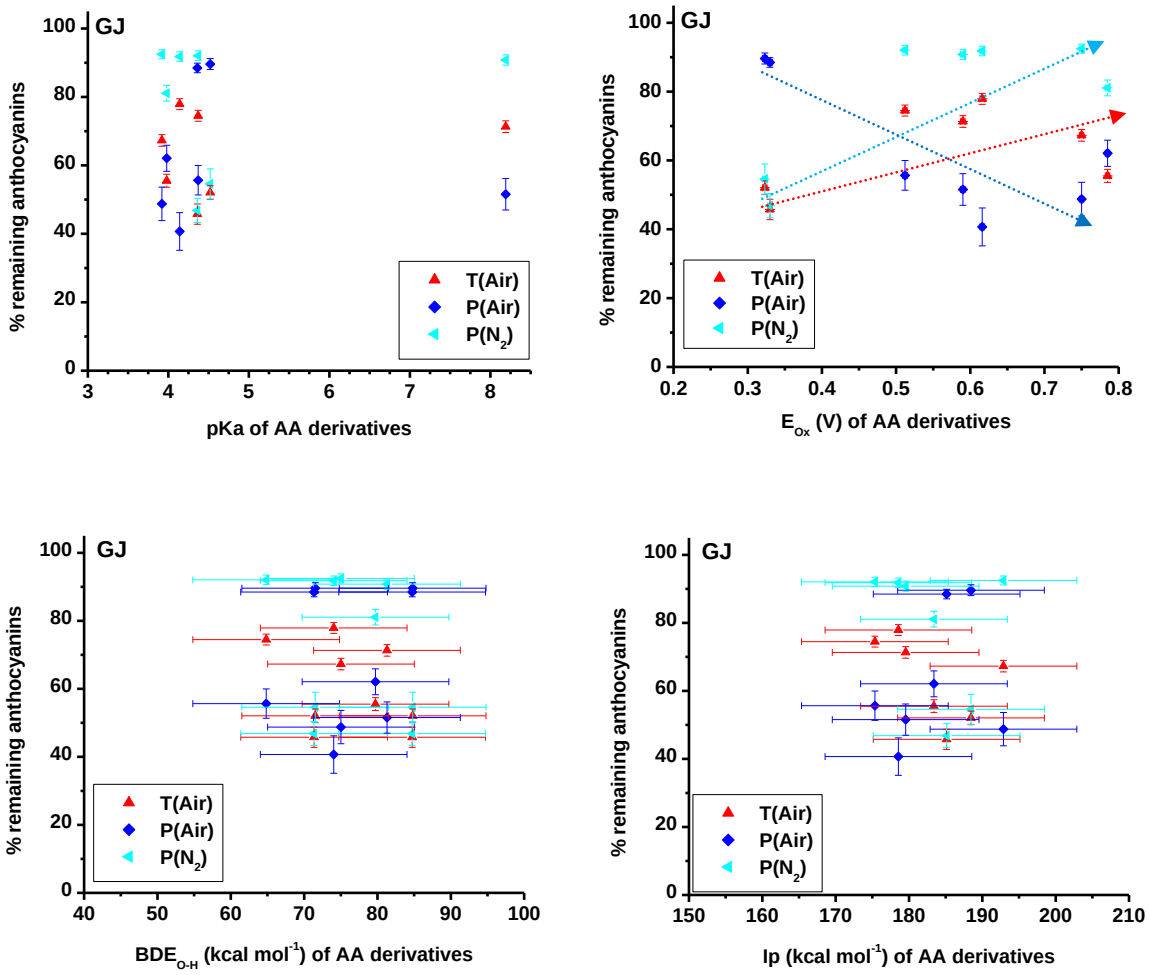


Figure S8b: Thermal and photolytic stability of anthocyanins from grape juice (GJ) extract in the presence of ascorbic acid (AA) or ascorbic acid derivatives in citrate buffer at pH = 3 after 10 days under 43 °C (dark storage) under air, T(Air), and after 300 s of irradiation under air, P(Air), and under N_2, P(N_2), as a function of ascorbic acid or ascorbic acid derivatives properties: pKa (C3-hydroxyl group, or C2-hydroxyl group for EAA), oxidation potential E_{Ox}, bond dissociation energy BDE_{OH} and ionization potential Ip. The ascorbic acid derivatives corresponding to each value are listed in Table 1 of the article. The arrows are a guide for the eyes.

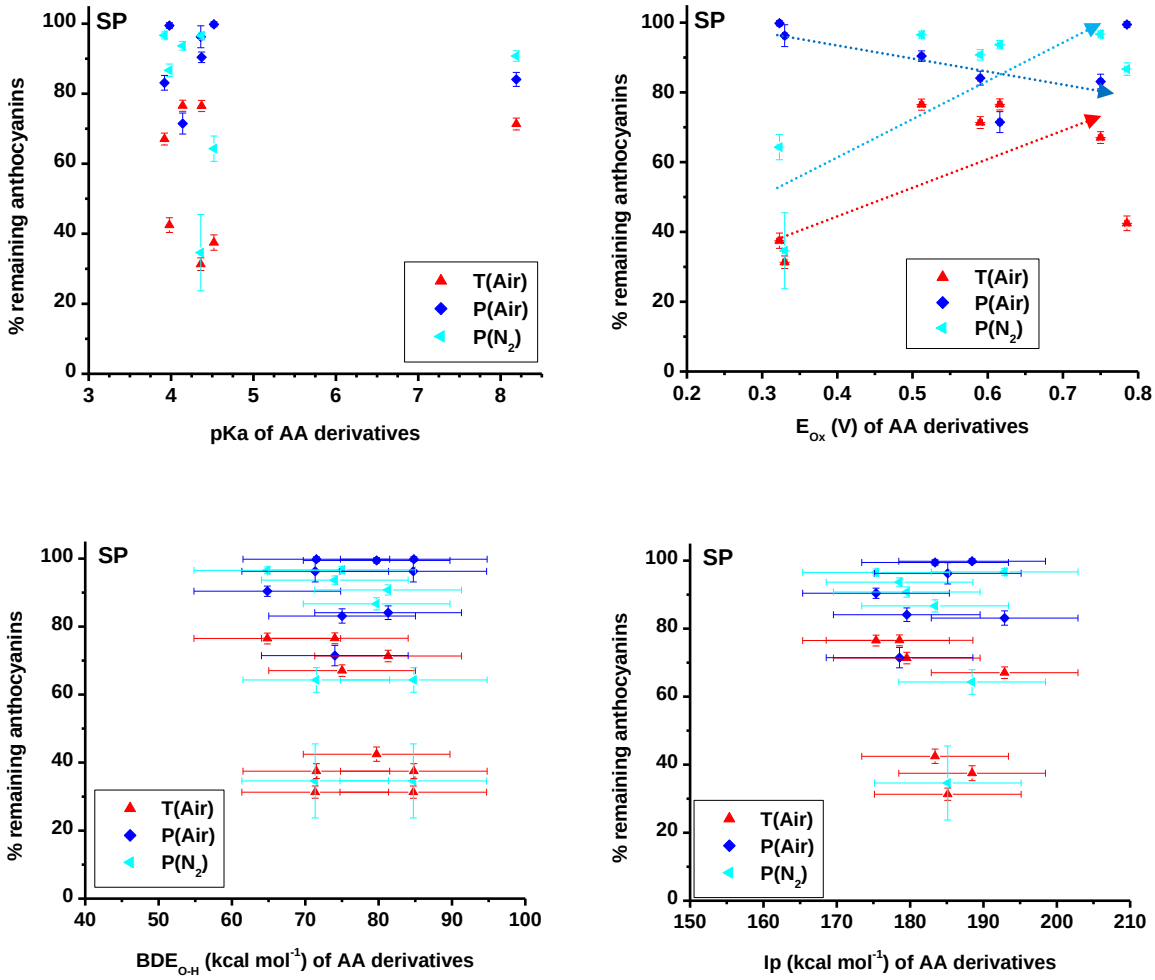


Figure S8c: Thermal and photolytic stability of anthocyanins from purple sweet potato (SP) extract in the presence of ascorbic acid (AA) or ascorbic acid derivatives in citrate buffer at pH = 3 after 10 days under 43 °C (dark storage) under air, T(Air), and after 300 s of irradiation under air, P(Air), and under N_2, P(N_2), as a function of ascorbic acid or ascorbic acid derivatives properties: pKa (C3-hydroxyl group, or C2-hydroxyl group for EAA), oxidation potential E_{Ox}, bond dissociation energy BDE_{OH} and ionization potential Ip. The ascorbic acid derivatives corresponding to each value are listed in Table 1 of the article. The arrows are a guide for the eyes.

9. Effect of the chlorogenic acid on the thermal and photolytic stability of anthocyanins in the presence of ascorbic acid or ascorbic acid derivative GPAA in citrate buffer at pH = 3

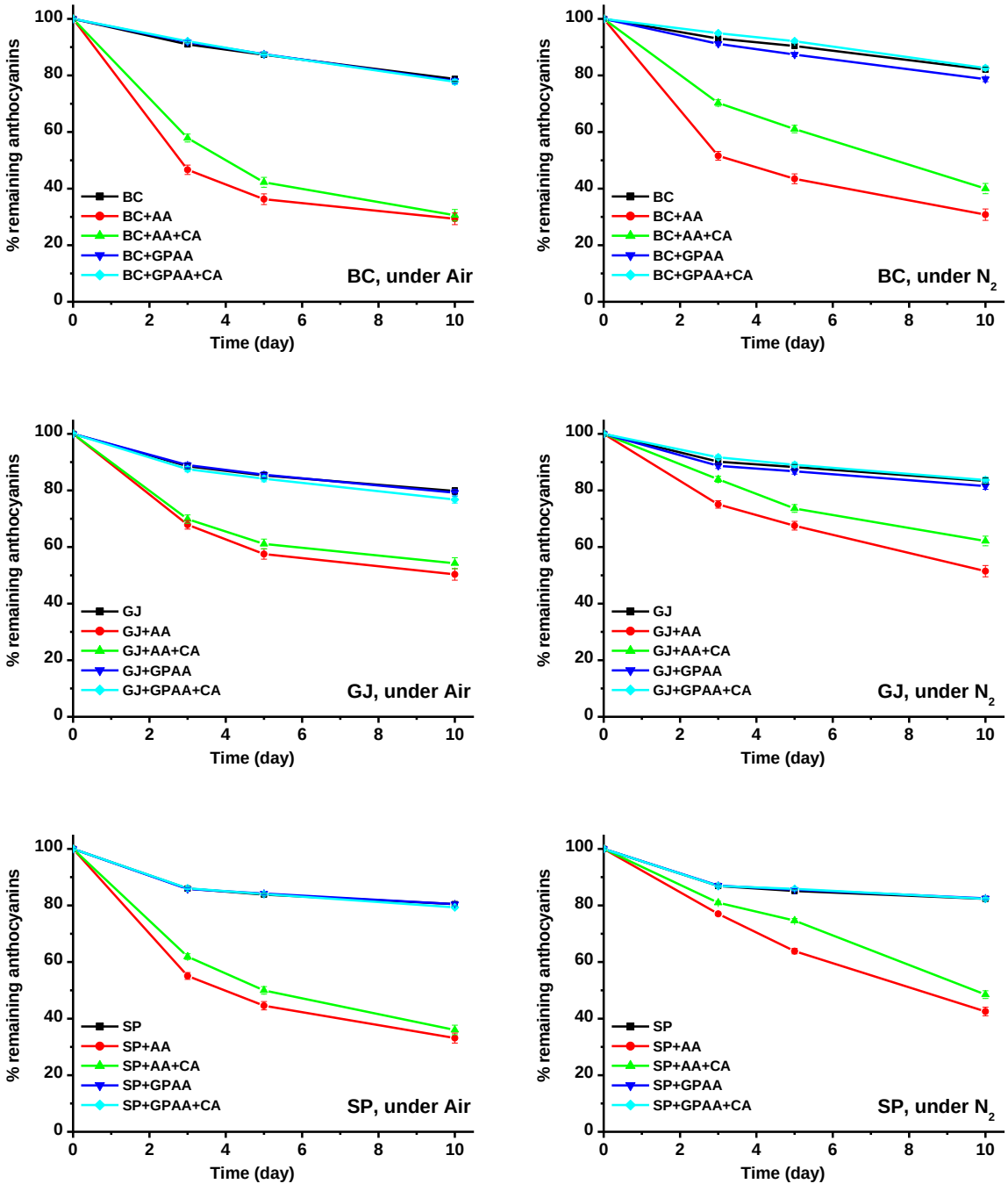


Figure S9a: Effect of ascorbic acid AA (200 mg L^{-1}) and ascorbic acid derivative GPAA (200 mg L^{-1}) in the presence or absence of a supplementary antioxidant, the chlorogenic acid (200 mg L^{-1}), on the stability of the anthocyanins from the BC, GJ and SP extracts (at 500 mg L^{-1}) in citrate buffer at pH = 3 under thermal stability at 43 °C (dark storage) under air and under N_2 (BC: black carrot, GJ: grape juice, SP: purple sweet potato, AA: ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, CA: chlorogenic acid).

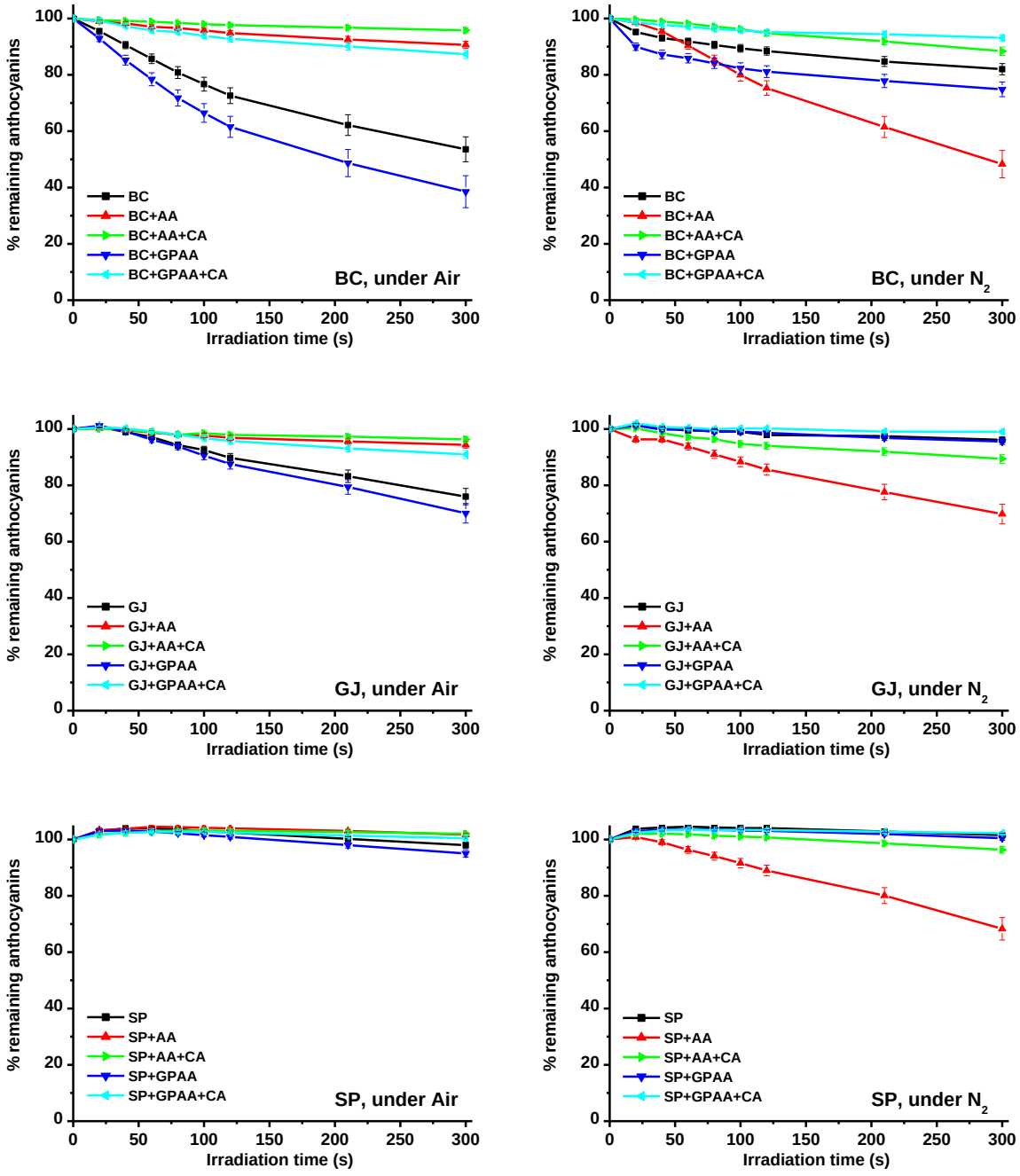


Figure S9b: Effect of ascorbic acid AA (200 mg L^{-1}) and ascorbic acid derivative GPAA (200 mg L^{-1}) in the presence or absence of a supplementary antioxidant, the chlorogenic acid (200 mg L^{-1}), on the stability of the anthocyanins from the BC, GJ and SP extracts (at 500 mg L^{-1}) in citrate buffer at pH = 3 under irradiation at room temperature under air and under N_2 (BC: black carrot, GJ: grape juice, SP: purple sweet potato, AA: ascorbic acid, GPAA: 2-O-α-D-glucopyranosyl-L-ascorbic acid, CA: chlorogenic acid).