Supporting Information for

Aquatic photochemistry of isoflavone phytoestrogens

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Chemicals and Supplies. All of the studied phytoestrogens were purchased from Indofine Chemical Company. Perinaphthenone, *p*-nitroanisole, deuterium oxide, sodium azide, potassium superoxide, and sodium deuteroxide were purchased from Acros Organics. Deuterium chloride was supplied by Cambridge Isotope Labs. Baker supplied sodium molybdate dihydrate. Hydrogen peroxide (30%) was supplied by Integra. Furfuryl alcohol (FFA) was purchased from Aldrich. Solvents were HPLC grade and supplied by Fisher. The International Humic Substances Society supplied Suwannee River fulvic acid (SRFA) and Pony Lake fulvic acid (PLFA).

HPLC Analysis. An Agilent 1120 Compact LC (controlled by EZChrom Elite software) with a UV detector was used to quantify the phytoestrogens, FFA, and the *p*-nitroanisole actinometer. The phytoestrogens were analyzed on a C_{18} column (Agilent Eclipse Plus, 2.1 × 150 mm, 3.5 μm particle size) using a flow rate of 0.5 mL/min with the column temperature set to 40 °C. The injection volume was 20 μL. The detector wavelength was set to 310 nm for daidzein and formononetin, 280 nm for genistein and equol, and 330 nm for biochanin A. Isocratic binary solvent mixtures of acetonitrile (MeCN) and pH 5 ammonium acetate (10 mM) buffer were used for all phytoestrogens. The percent MeCN was 20 for daidzein, 25 for genistein and equol, 30 for formononetin, and 35 for biochanin A. The *p*-nitroanisole actinometer and FFA were also analyzed by HPLC according to literature precedent.[1]

[1] Latch, D. E.; Stender, B. L.; Packer, J. L.; Arnold, W. A.; McNeill, K. Photochemical fate of pharmaceuticals in the environment: Cimetidine and ranitidine. *Environ. Sci. Technol.* **2003**, *37*, 3342.

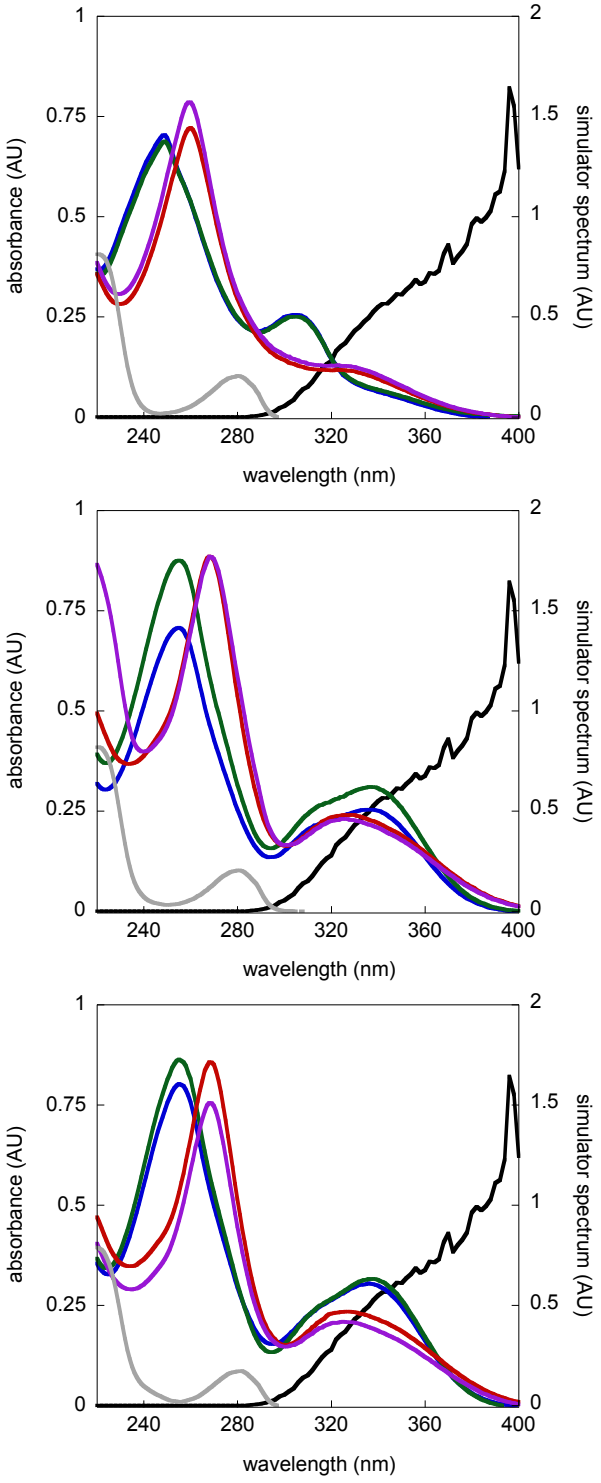

Figure S1. Absorption spectra for 20 μM solutions of daidzein (blue), formononetin (green), biochanin A (red), genistein (violet), and equol (gray). The black spectrum on each panel is the spectral output of the solar simulator (with filter) used in this work. Top panel: pH 7; middle panel: pH 8; bottom panel: pH 10.

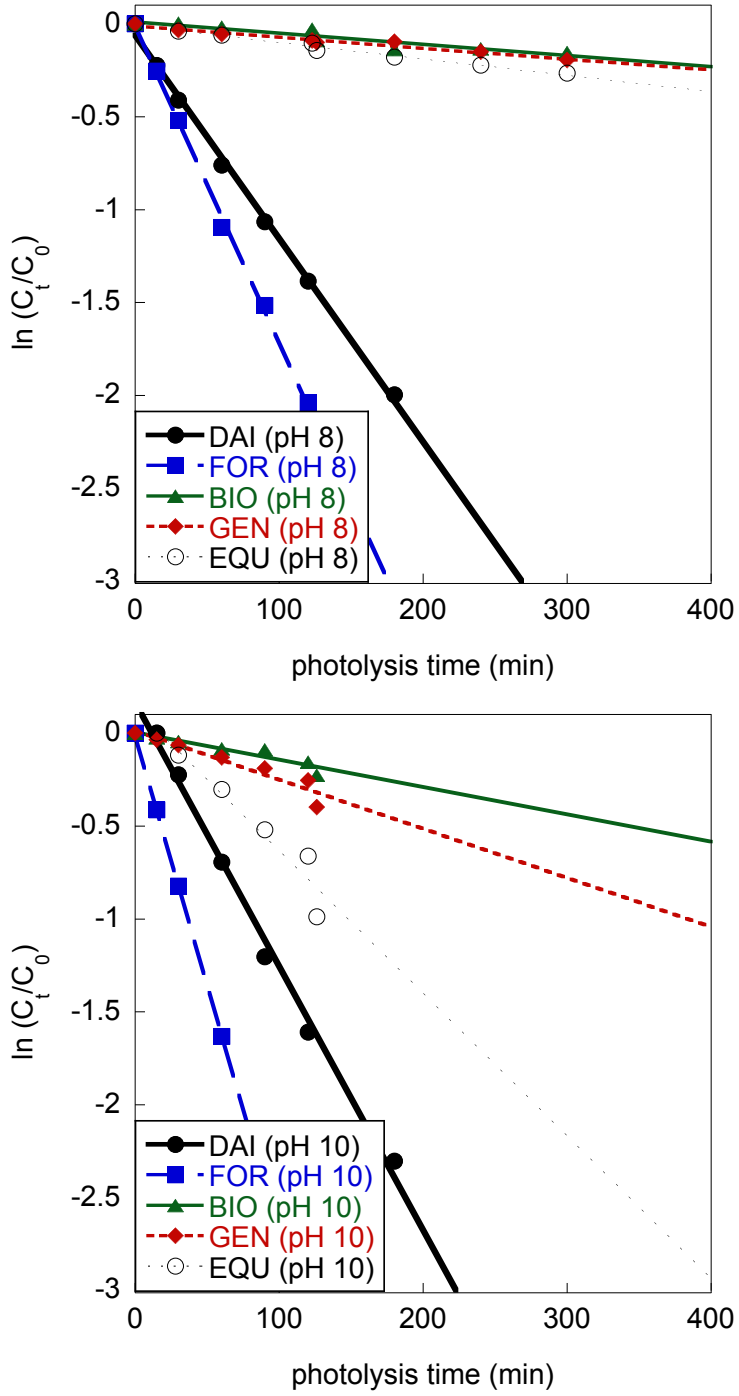

Figure S2. Direct photolysis of 2 μM phytoestrogens in pH 8 (top panel) and pH 10 (bottom panel) buffered DI water in a solar simulator employing a filtered Xe lamp operated at an intensity of 765 W/m^2. The legend for this figure (and subsequent plots) includes the following abbreviations: daidzein = DAI; formononetin = FOR; biochanin A = BIO; genistein = GEN; and equol = EQU.

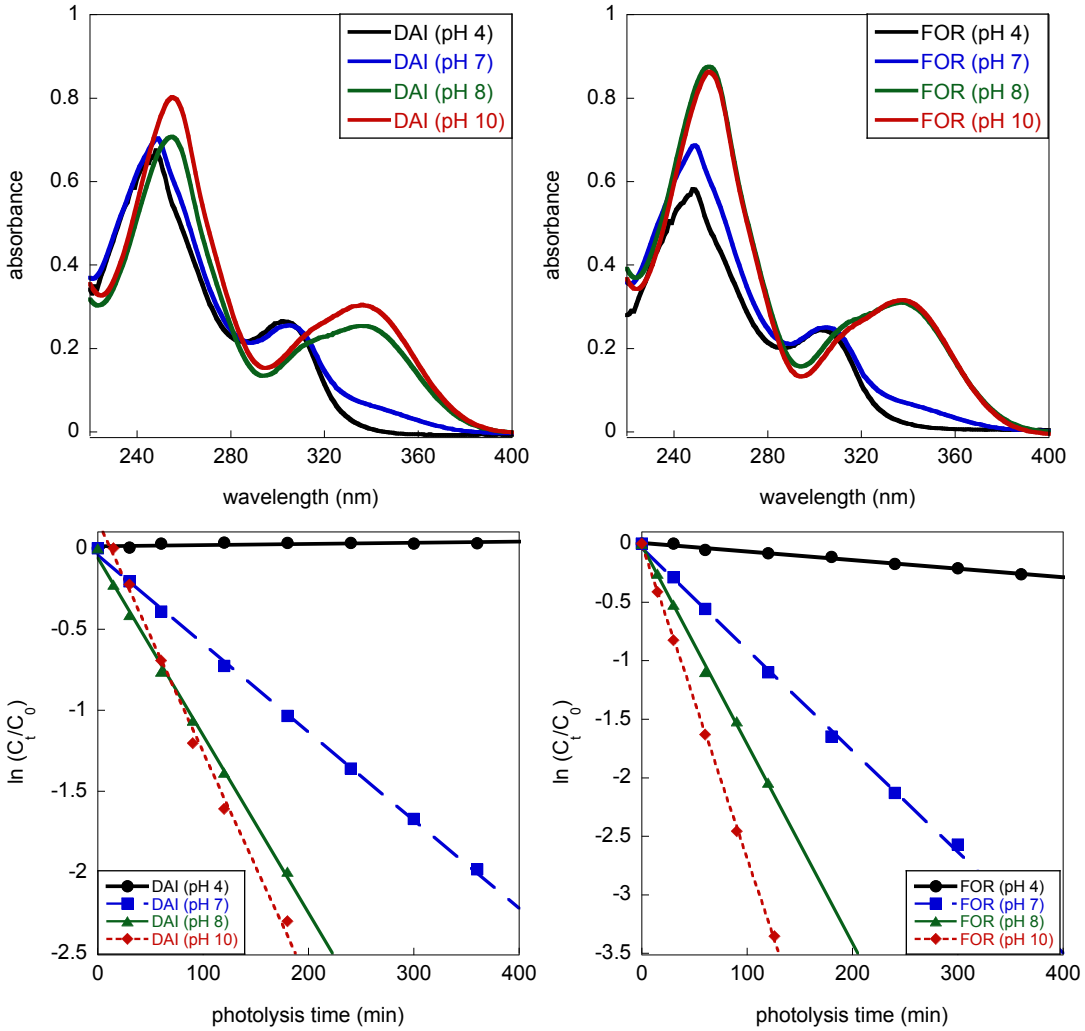

Figure S3. Absorption spectra of 20 μM daidzein (top left) and formononetin (top right) in buffers of various pH and direct photolysis kinetic plots for 2 μM daidzein (bottom left) and formononetin (bottom right) in the same pH buffers. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2.

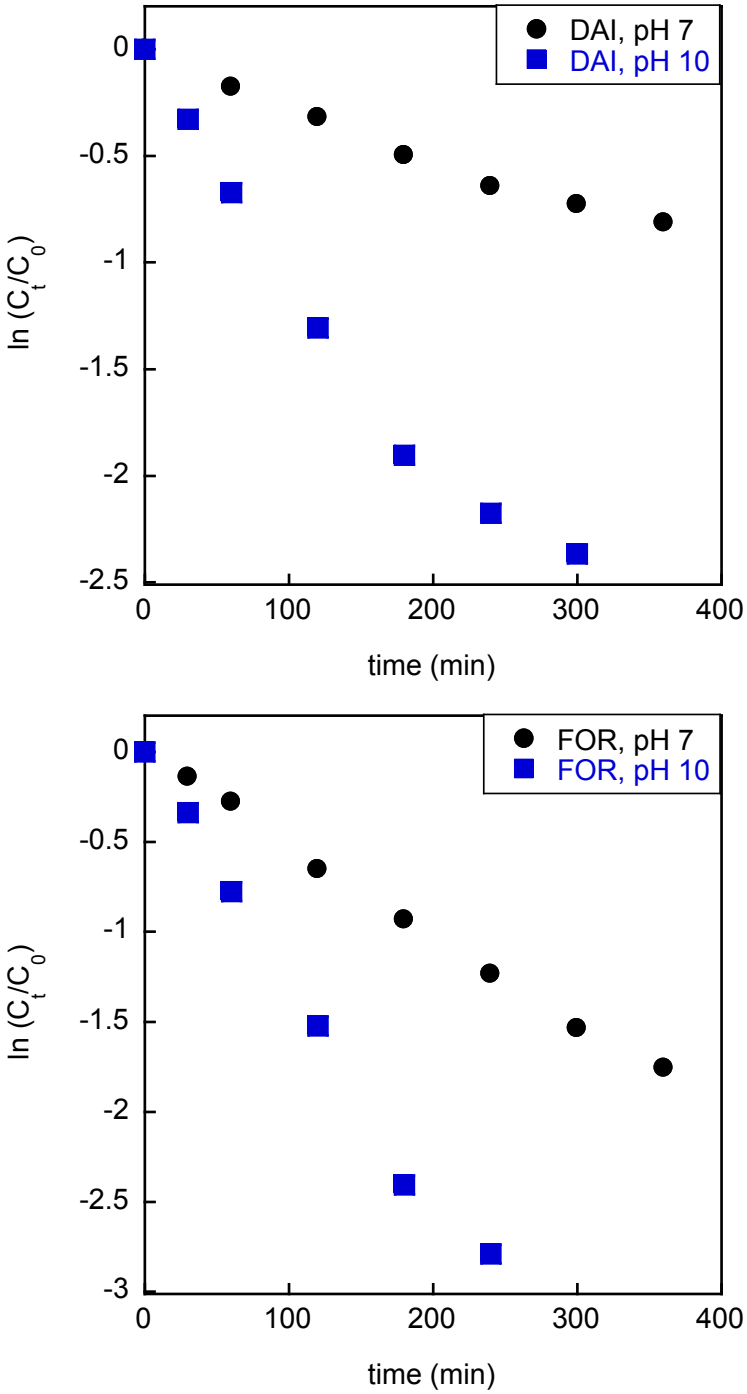

Figure S4. Outdoor direct photolysis kinetic plots for 2 μM daidzein (top panel) and formononetin (bottom panel) in pH 7 (black circles) and pH 10 (blue squares) buffered solutions. Experiments were conducted in Seattle, WA, USA on August 11, 2010.

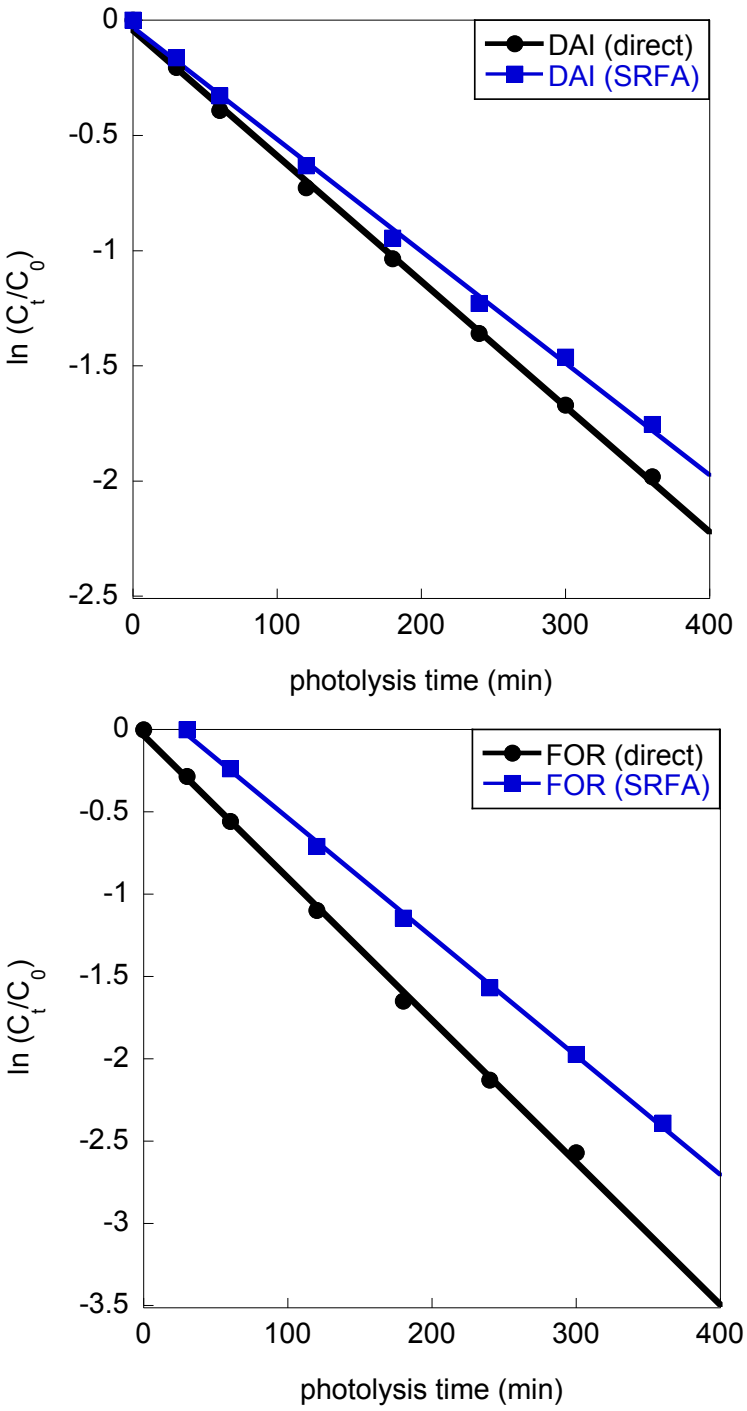

Figure S5. Effect of 10 mg/L SRFA on the photodegradation rates of 2 μM daidzein (top panel) and formononetin (bottom panel) at pH 7. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2. Data from experiments with 10 mg/L added SRFA (blue squares) are plotted alongside data from direct photolysis experiments with no added SRFA (black circles).

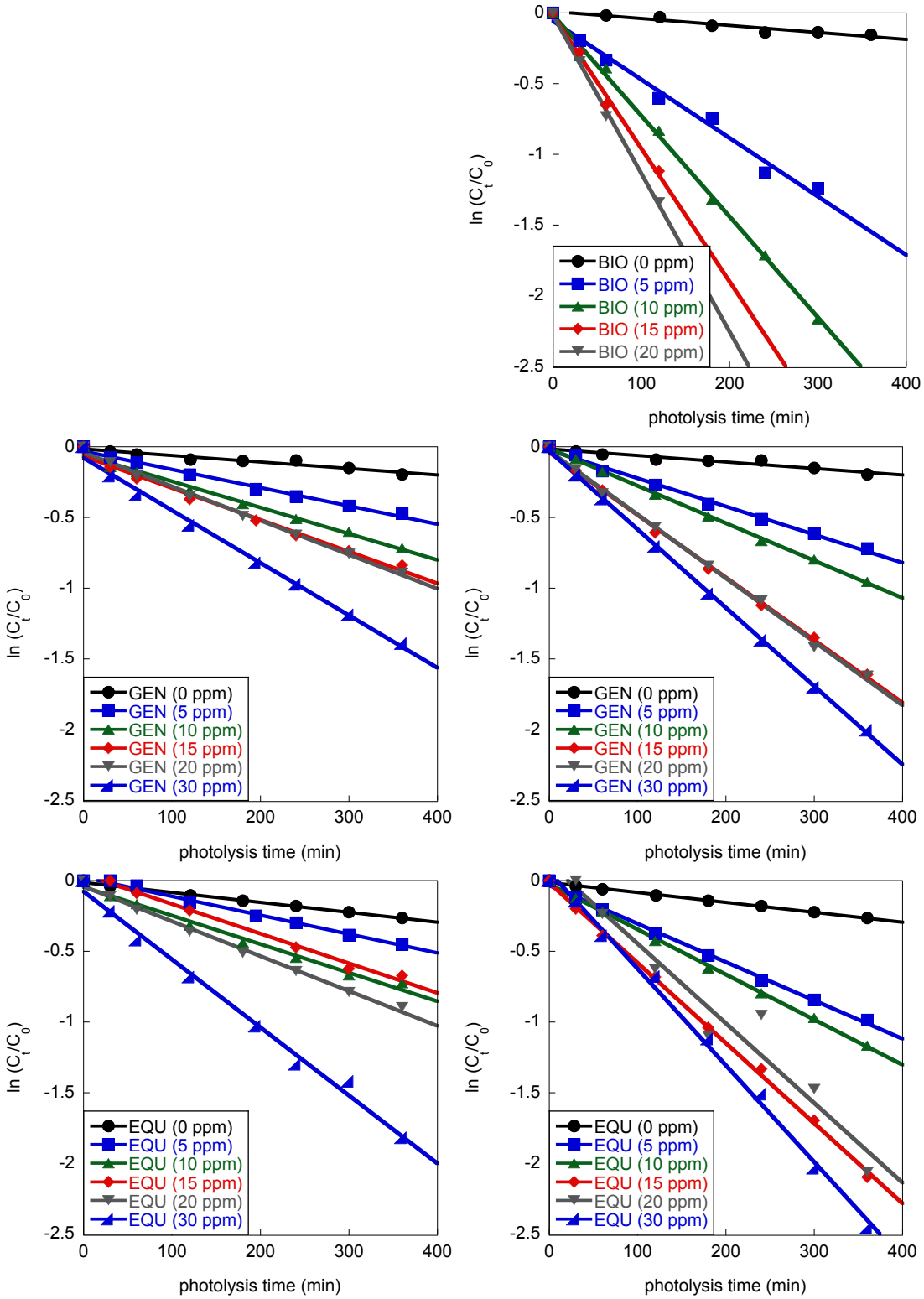

Figure S6. Effect of various concentrations of SRFA (left panels) and PLFA (right panels) on the photodegradation rate of 2 μM solutions of biochanin A (top panel), genistein (middle panels), and equol (bottom panels) at pH 8. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2. Note that Figure 2 in the main text shows biochanin A photodegradation in SRFA solutions.

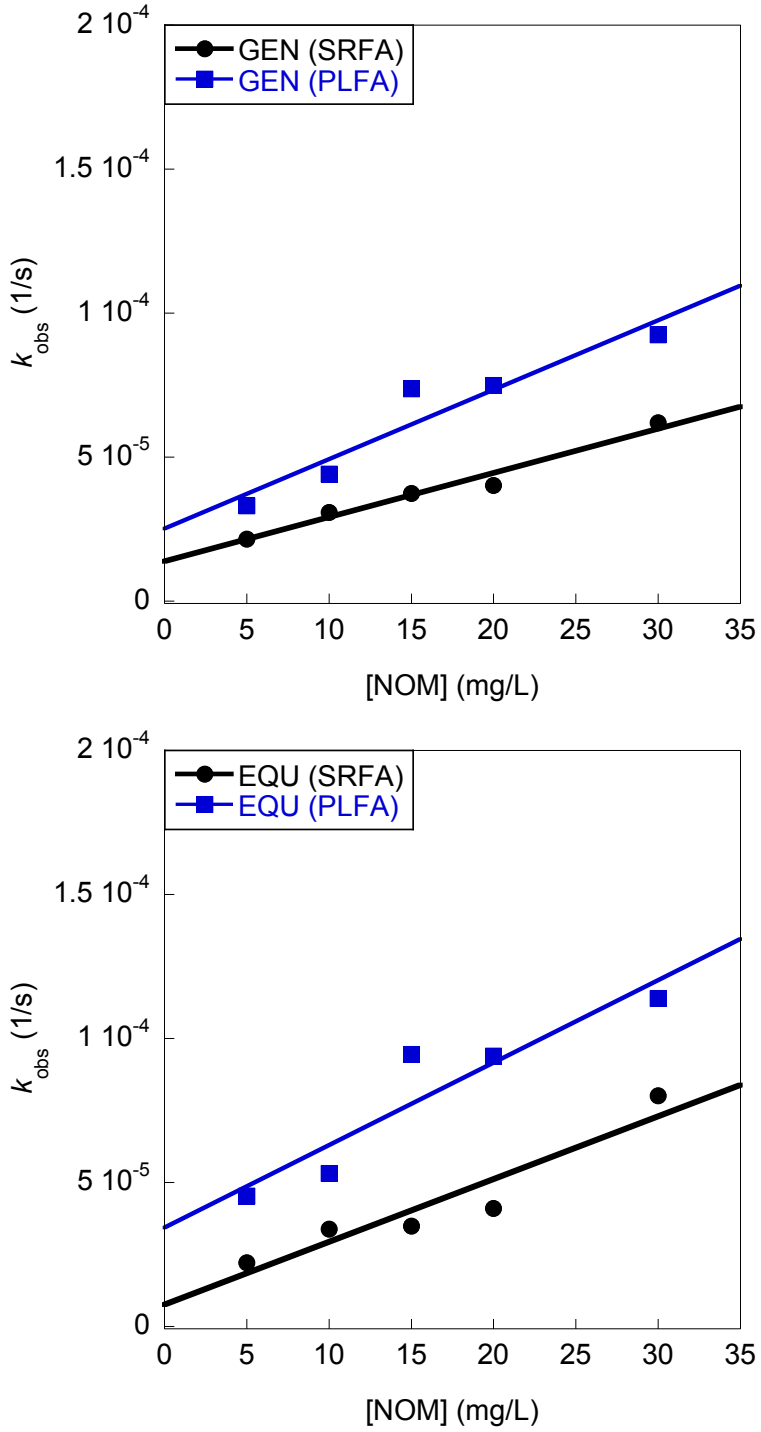

Figure S7. Comparison of observed indirect photolysis rate constants (k_{obs}) measured at different concentrations of added SRFA (black circles) or PLFA (blue squares) sensitizers. In addition to the NOM, the solutions also contained 2 μM genistein (top panel) or equol (bottom panel) at pH 8. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2. Note that these data are plotted using the same scale as was used for biochanin A in Figure 2 in the main text, thus showing the relative indirect photochemical reactivity of biochanin A, genistein, and equol.

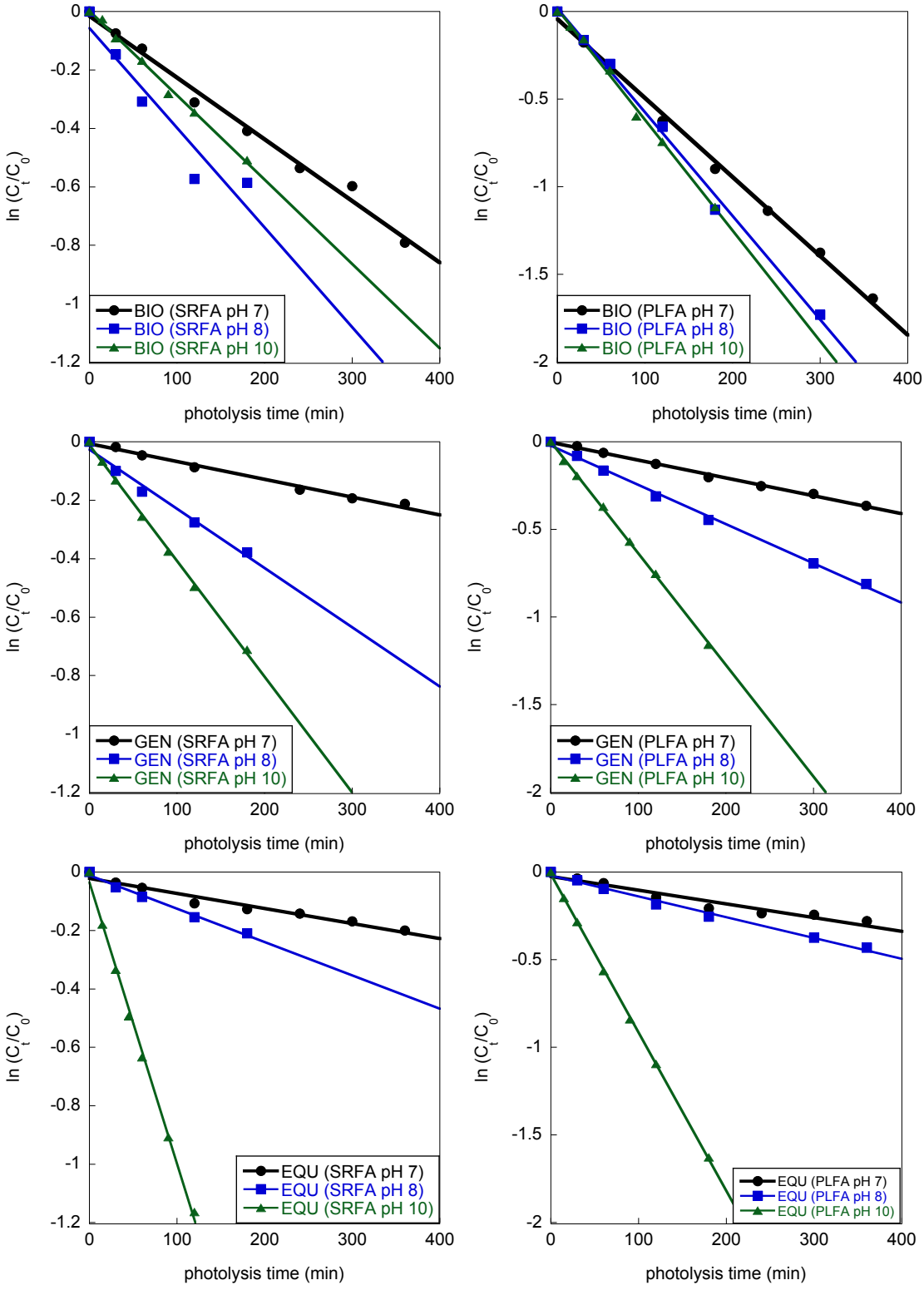

Figure S8. Effect of pH on the indirect photodegradation of 2 μM biochanin A (top panels), genistein (middle panels), and equol (bottom panels) with 10 mg/L added SRFA (left panels) or PLFA (right panels). Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2. Symbols are as follow: pH 7 (black circles); pH 8 (blue squares); and pH 10 (green triangles).

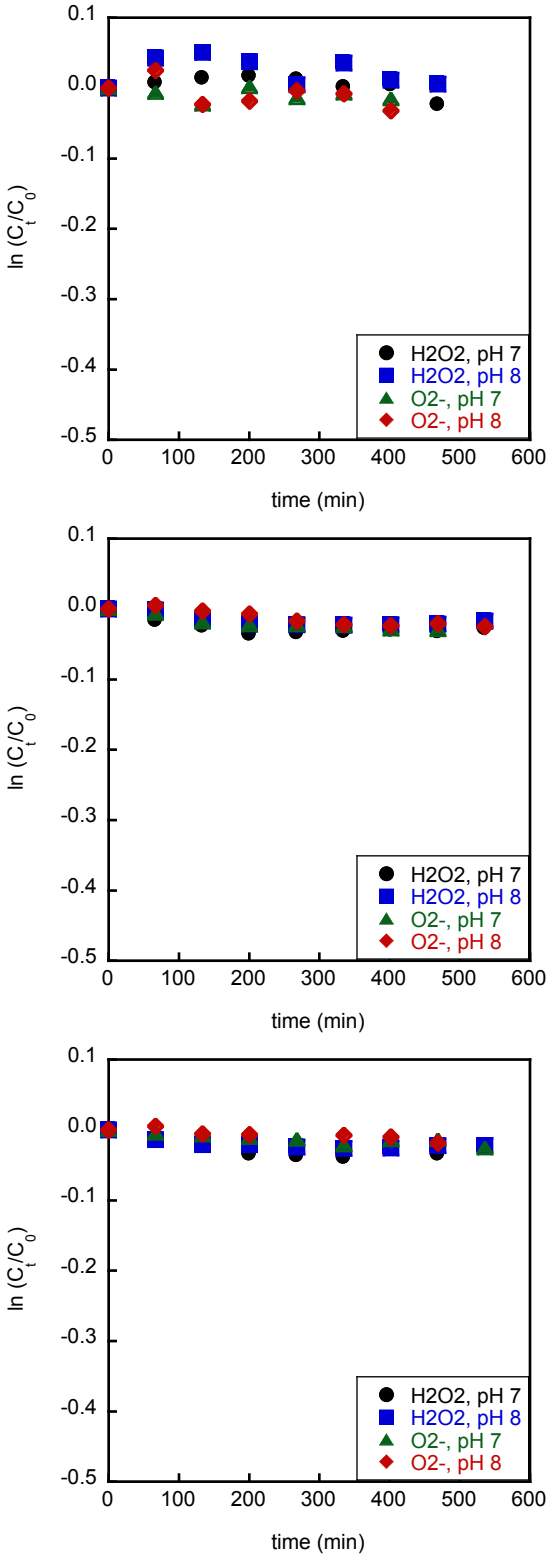

Figure S9. Effect of hydrogen peroxide (1 mM; H_2O_2) and superoxide (100 nM; $O2^-$) radical anion on the degradation of biochanin A (top panel), genistein (middle panel), and equol (bottom panel) in pH 7- and pH 8-buffered DI water.

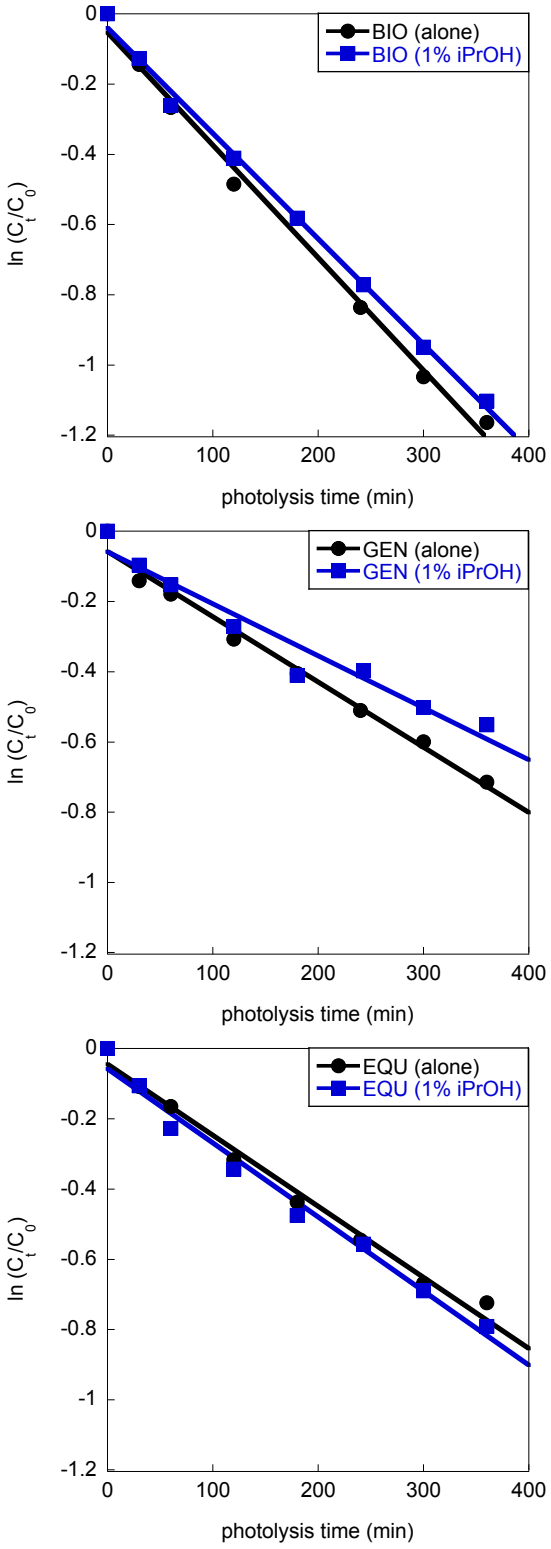

Figure S10. Effect of added radical quencher isopropanol on the photodegradation of 2 μM solutions of biochanin A (top panel), genistein (middle panel), and equol (bottom panel) with 10 mg/L PLFA in pH 8 buffered solutions. Data from experiments with no added isopropanol are shown as black circles and data with 1% v/v isopropanol added are shown as blue squares. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2.

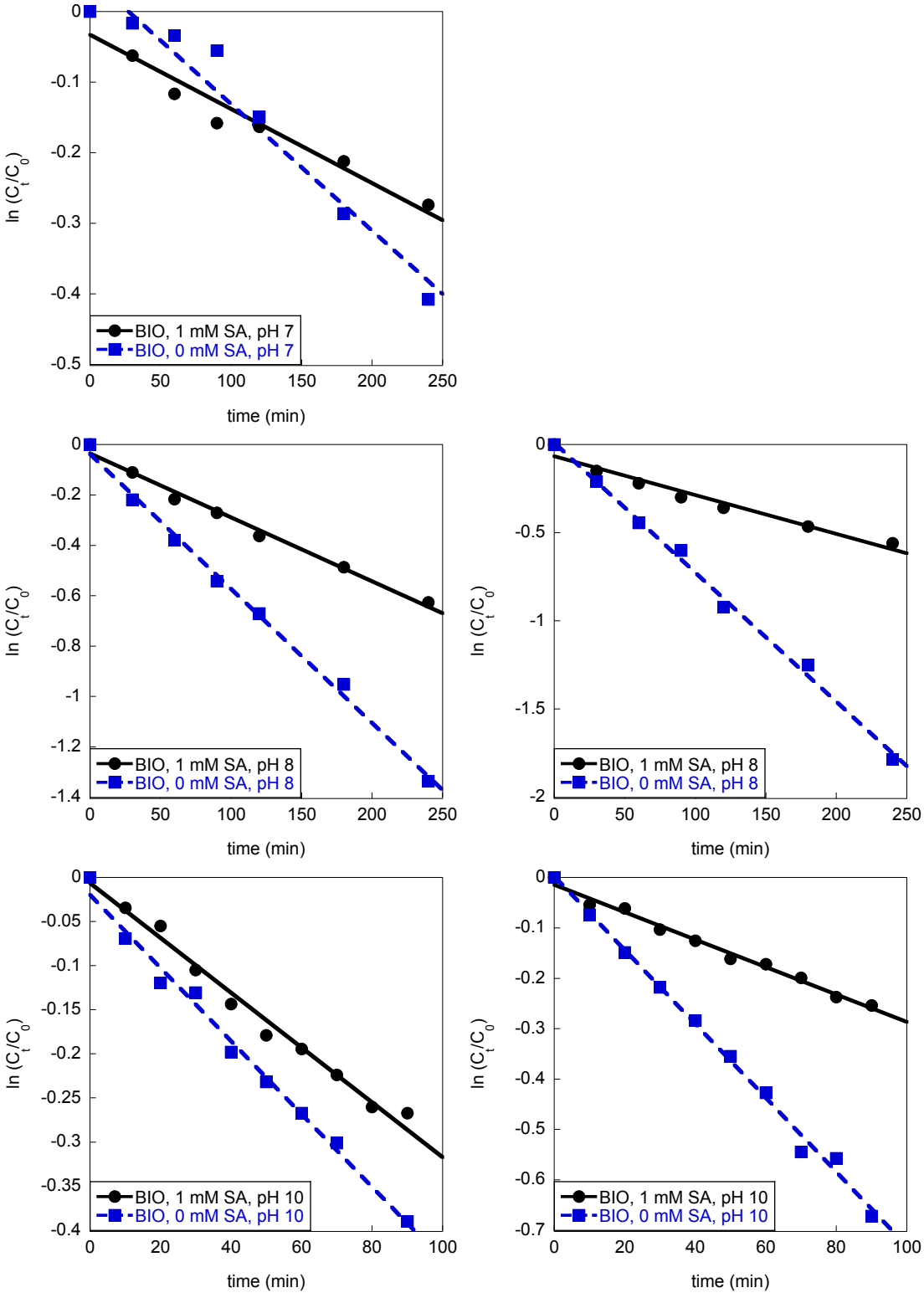

Figure S11. Effect of added triplet quencher sorbic acid on the photodegradation of 2 μM of biochanin A in 10 mg/L solutions of SRFA (left panels) or PLFA (right panels) at pH 7 (top panel), pH 8 (middle panels), and pH 10 (bottom panels). Data from experiments with no added sorbic acid are shown as blue squares and data with 1 mM sorbic acid added are shown as black circles. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2. Note that Figure 4 in the main text shows the effect of sorbic acid on biochanin A photodegradation in PLFA solutions.

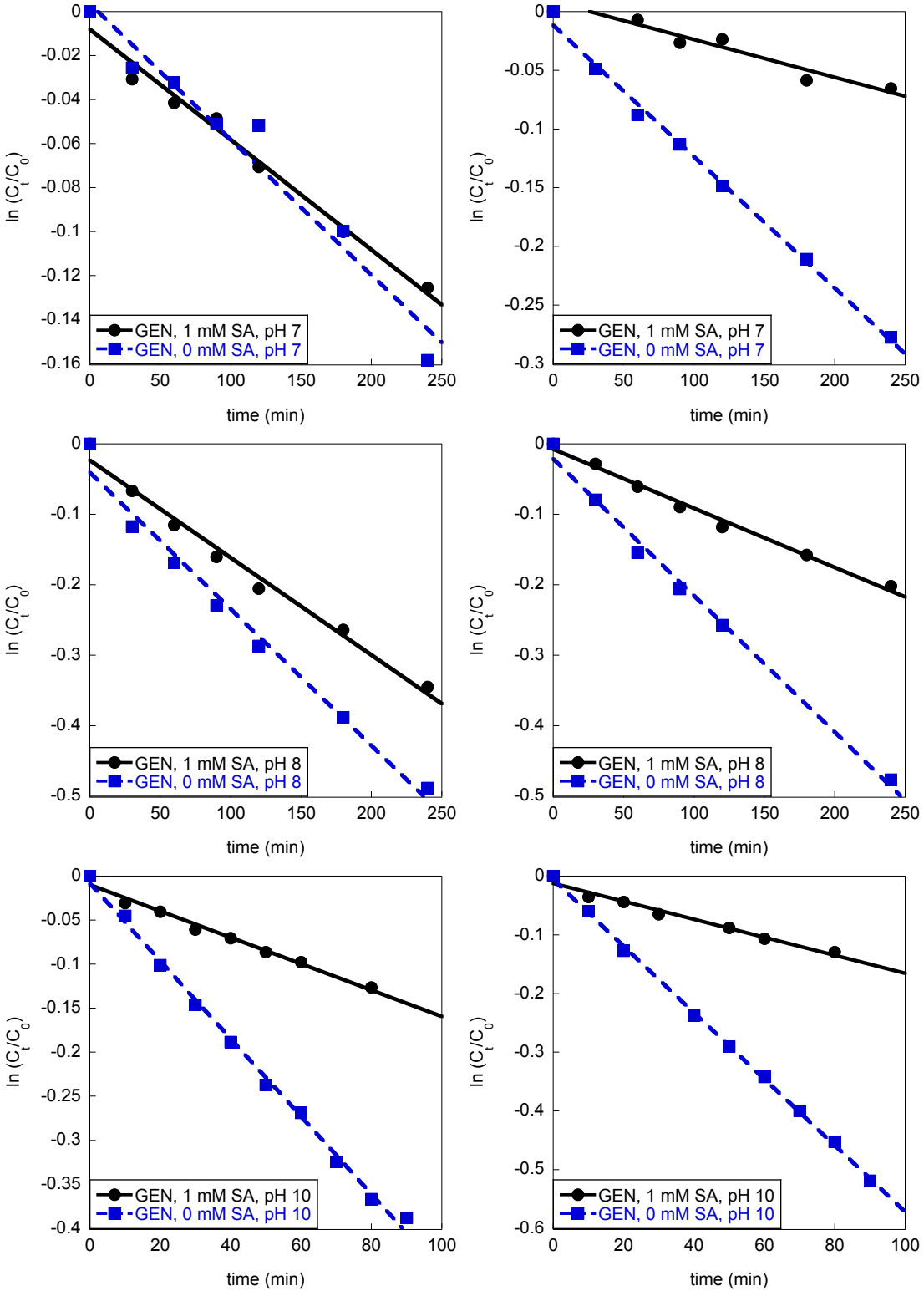

Figure S12. Effect of added triplet quencher sorbic acid on the photodegradation of 2 μM of genistein in 10 mg/L solutions of SRFA (left panels) and PLFA (right panels) at pH 7 (top panel), pH 8 (middle panels), and pH 10 (bottom panels). Data from experiments with no added sorbic acid are shown as blue squares and data with 1 mM sorbic acid added are shown as black circles. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2.

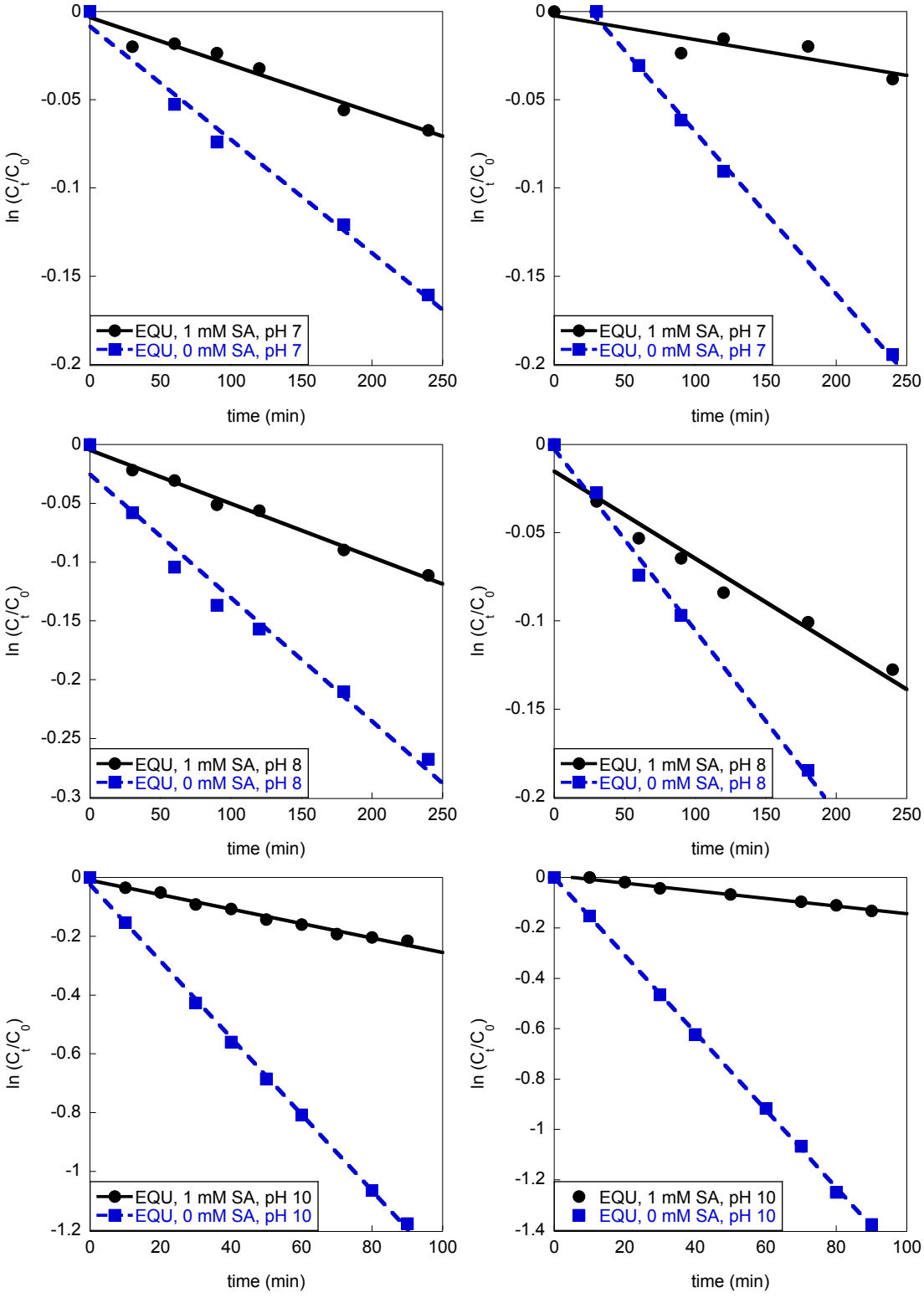

Figure S13. Effect of added triplet quencher sorbic acid (SA) on the photodegradation of 2 μM of equol in 10 mg/L solutions of SRFA (left panels) and PLFA (right panels) at pH 7 (top panel), pH 8 (middle panels), and pH 10 (bottom panels). Data from experiments with no added sorbic acid are shown as blue squares and data with 1 mM sorbic acid added are shown as black circles. Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2.

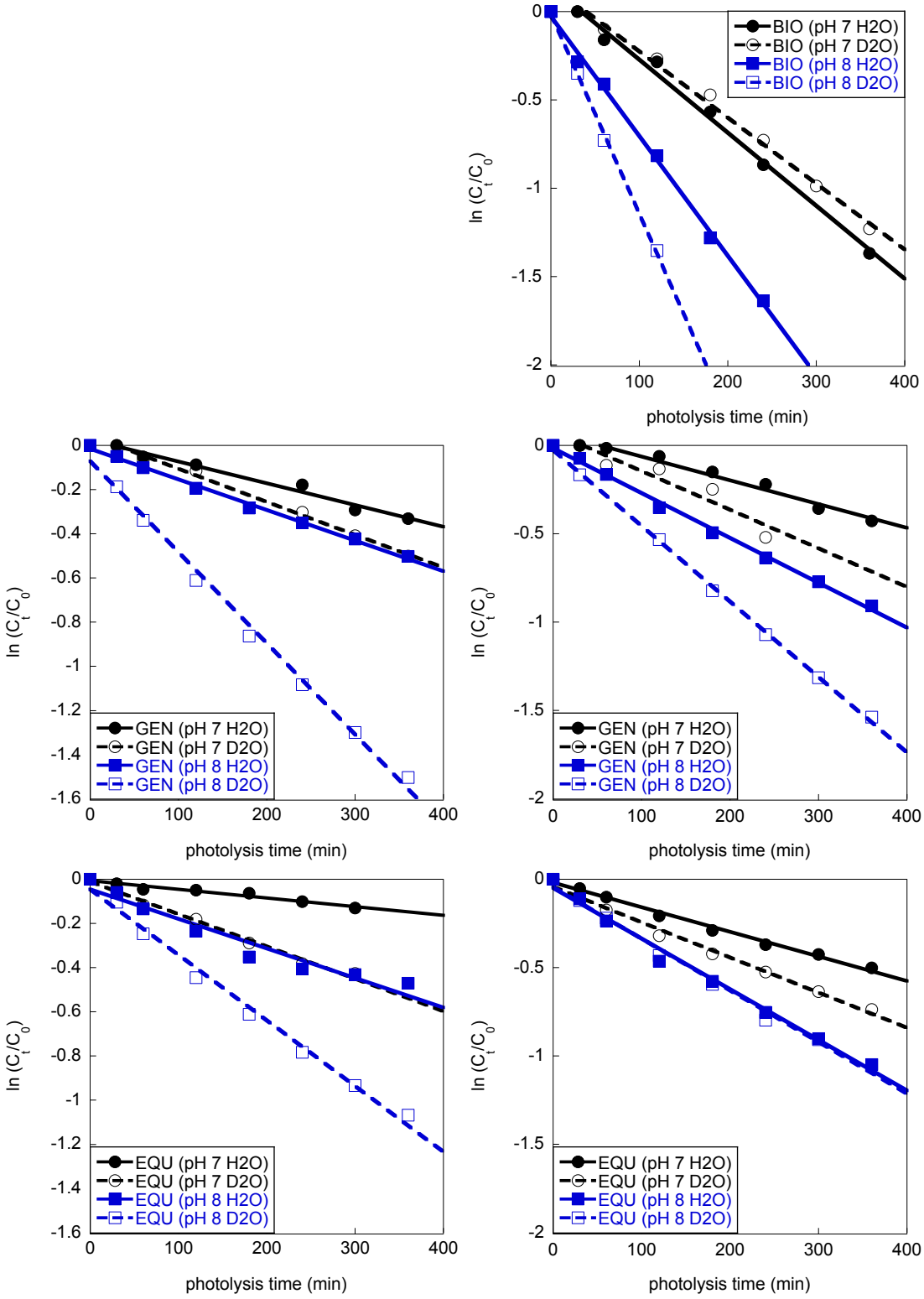

Figure S14. Effect of solvent composition on the photodegradation of 2 μM of biochanin A (top panel), genistein (middle panels), and equol (bottom panels) in 10 mg/L solutions of SRFA (left panels) or PLFA (right panels). Experiments at pH/pD 7 are shown as black circles and those at pH/pD 8 are shown as blue squares. Closed symbols and solid trendlines are for 100% H_2O and open symbols and dashed trendlines are for 90% D_2O. Note that Figure 3 in the main text shows biochanin A photodegradation in SRFA solutions of different solvent compositions.

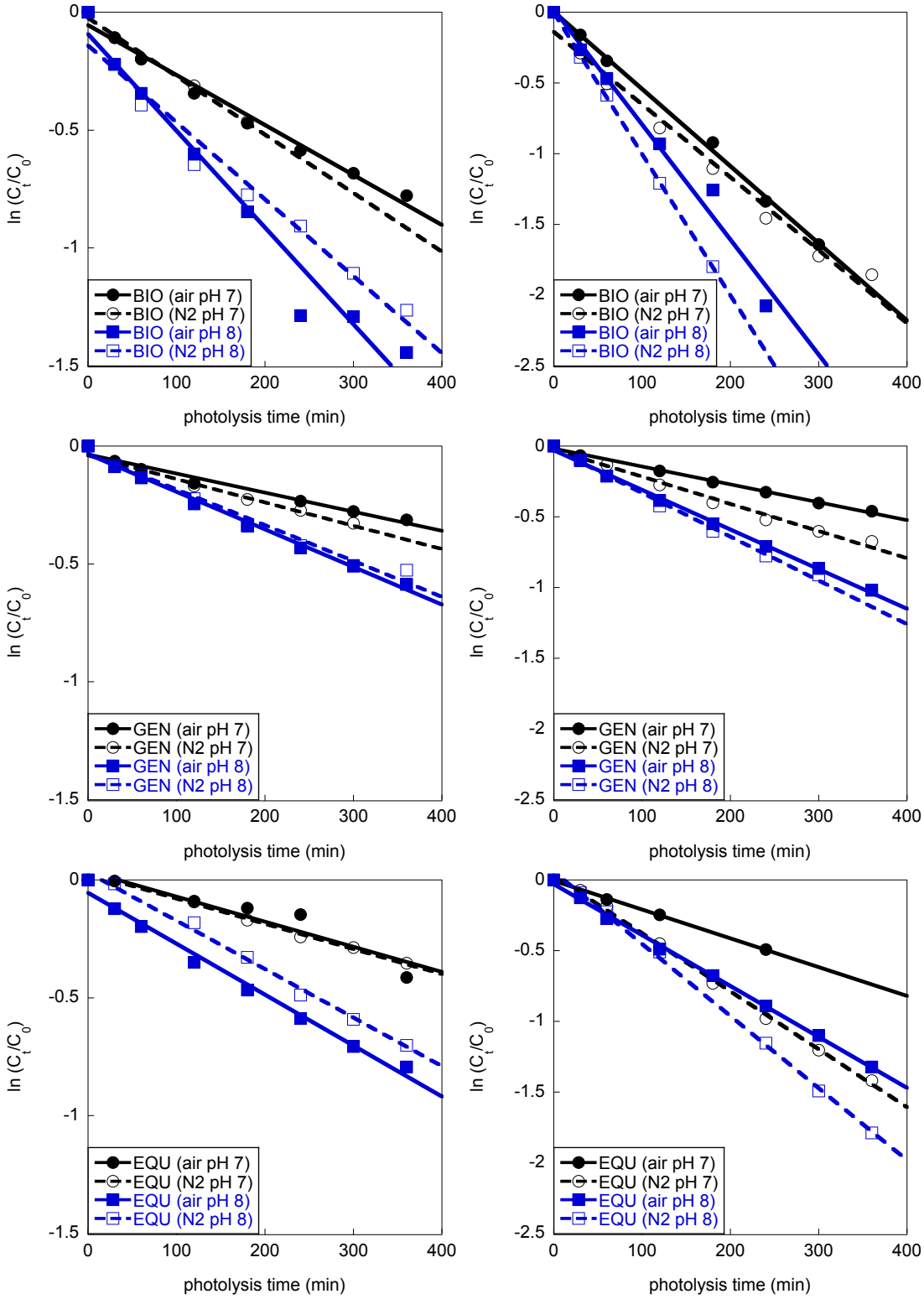

Figure S15. Effect of dissolved gases on the photodegradation of 2 μM biochanin A (top panels), genistein (middle panels), and equol (bottom panels) with 10 mg/L SRFA (left panels) or PLFA (right panels). Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2. Experiments at pH 7 are shown as black circles and those at pH 8 are shown as blue squares. Closed symbols and solid trendlines are for air-saturated solutions and open symbols and dashed trendlines are for nitrogen-saturated solutions.

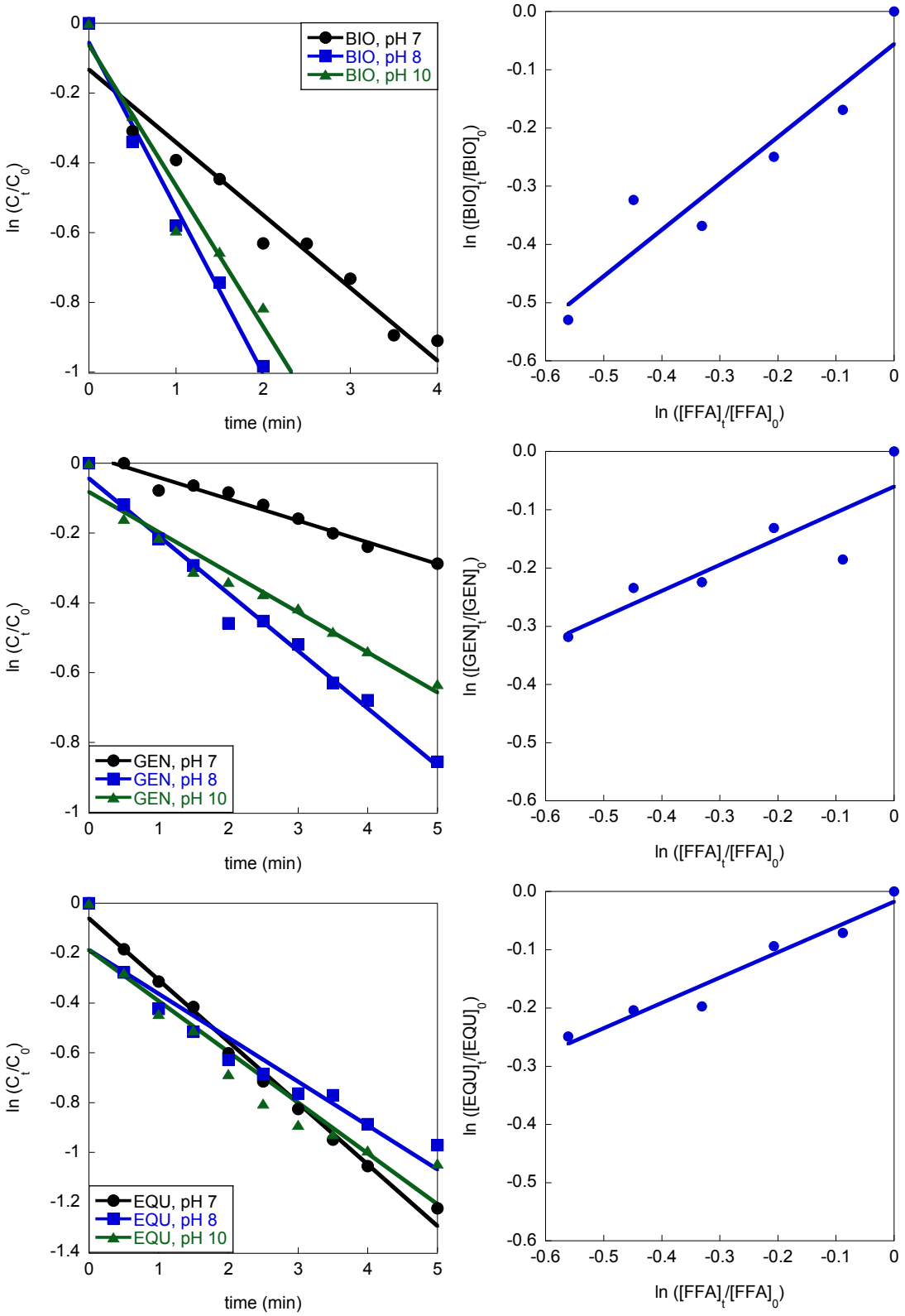

Figure S16. Singlet oxygen experiments. Top panels are for biochanin A, middle panels are for genistein, and bottom panels are for equol. Left panels show degradation in perinaphthenone (5 μM; 250 W/m^2) sensitized photolysis experiments at pH 7 (black circles), pH 8 (blue squares), and pH 10 (green triangles). Right panels show relative loss rates for phytoestrogens and the singlet oxygen probe molecule FFA in experiments with thermally generated singlet oxygen formed through the reaction of MoO_4^{2-} (1 mM) and hydrogen peroxide (400 mM) at pH 8.

Table S1. Indirect Photochemical Half-lives for Biochanin A, Genistein, and Equol Measured Under a Variety of Conditions [a]

		biochanin A	genistein	equol
NOM	condition	$t_{1/2}$ (hr)	$t_{1/2}$ (hr)	$t_{1/2}$ (hr)
SRFA	10 mg/L SRFA, pH 7	5.5	19	22
	5 mg/L SRFA, pH 8	5.6	8.9	8.7
	10 mg/L SRFA, pH 8	3.6	6.2	5.7
	15 mg/L SRFA, pH 8	3.2	5.1	5.5
	20 mg/L SRFA, pH 8	2.6	4.8	4.7
	30 mg/L SRFA, pH 8	1.9	3.1	2.4
	10 mg/L SRFA, pH 10	4.0	2.9	1.2
PLFA	10 mg/L PLFA, pH 7	2.6	11	15
	5 mg/L PLFA, pH 8	2.8	5.8	4.3
	10 mg/L PLFA, pH 8	1.6	4.4	3.6
	15 mg/L PLFA, pH 8	1.2	2.6	2.0
	20 mg/L PLFA, pH 8	1.0	2.6	2.0
	30 mg/L PLFA, pH 8	NM	2.1	1.7
	10 mg/L PLFA, pH 10	1.8	2.7	1.3

[a] Photolysis experiments were conducted in a solar simulator employing a filtered Xe lamp operated at 765 W/m^2