Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Ultrafast spectroscopy of the hydrophilic carotenoid crocin at various pH

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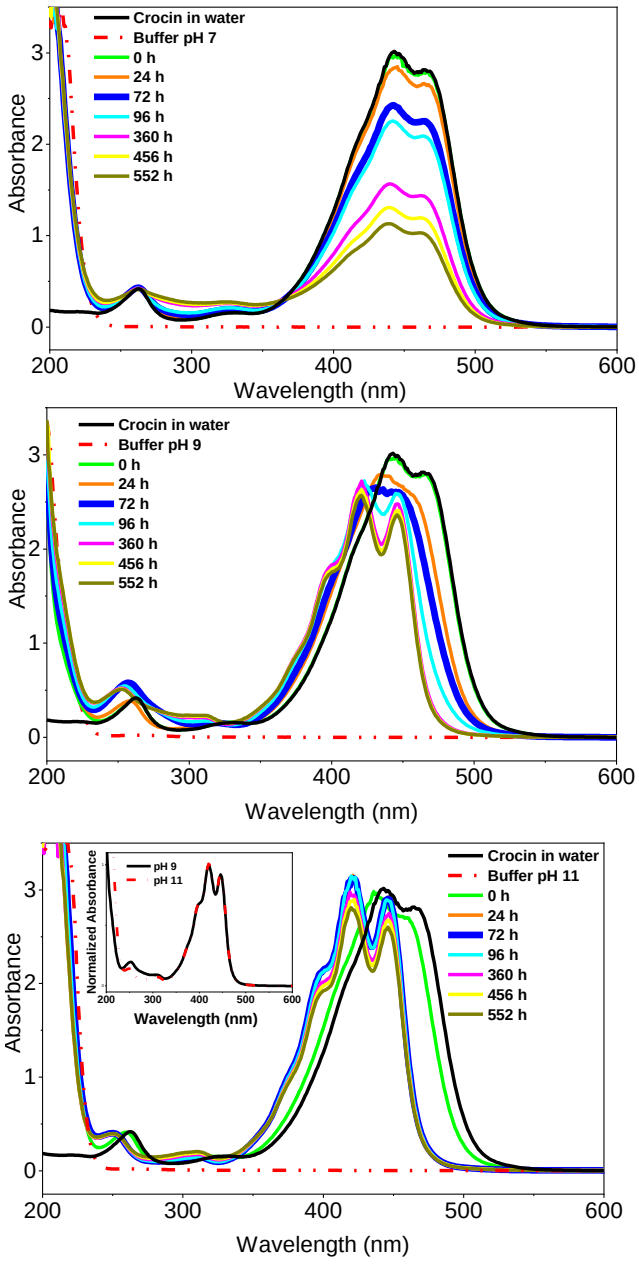

Figure S1. Absorption spectra and their time evolution over an extended period of time. The thick lines represent the samples used for transient absorption measurements. The time dependence of the absorption spectra for 25 μM Crocin in water (pH ~ 6.5) and 0.1 M pH 7, 9, and 11 buffer solutions was measured in the dark at room temperature. The zero times correspond to Crocin's initial absorption spectra in water and at pH 7, 9, and 11. The red dotted line illustrates the absorption of 0.1 M buffer solutions. In the pH 11 inset, normalized absorption spectra of Crocin in pH 9 (black line) and pH 11 (red dotted line) are presented at 552 hours.

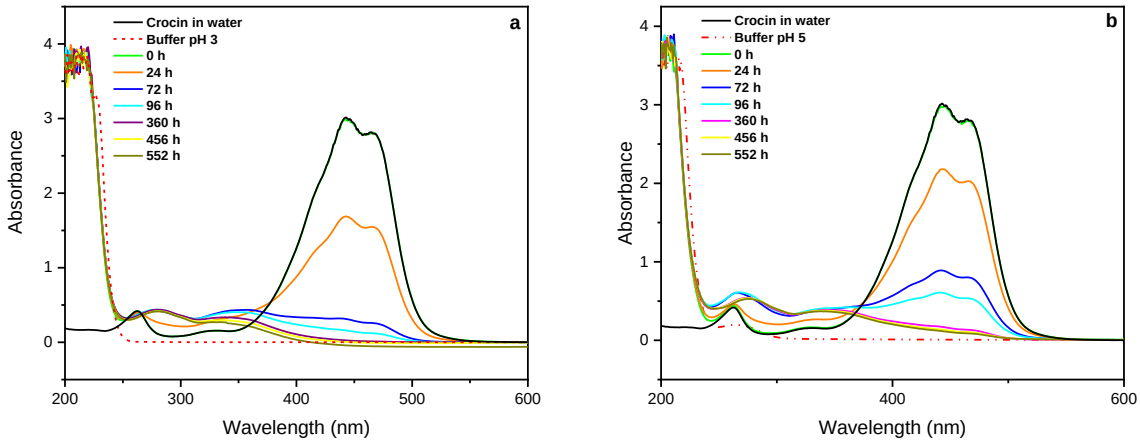

Figure S2. The stability of crocin under acidic conditions. The graph illustrates absorption spectra and their temporal evolution over an extended period of time. The time-dependent absorption spectra for 25 μM Crocin in water (pH ~ 6.5) and 0.1 M a) pH 3 and b) pH 5 buffer solutions were measured in the dark at room temperature. The zero times align with Crocin's initial absorption spectra in water and at pH 3 and 5. The red dotted line on the graph represents the absorption of 0.1 M buffer solutions.

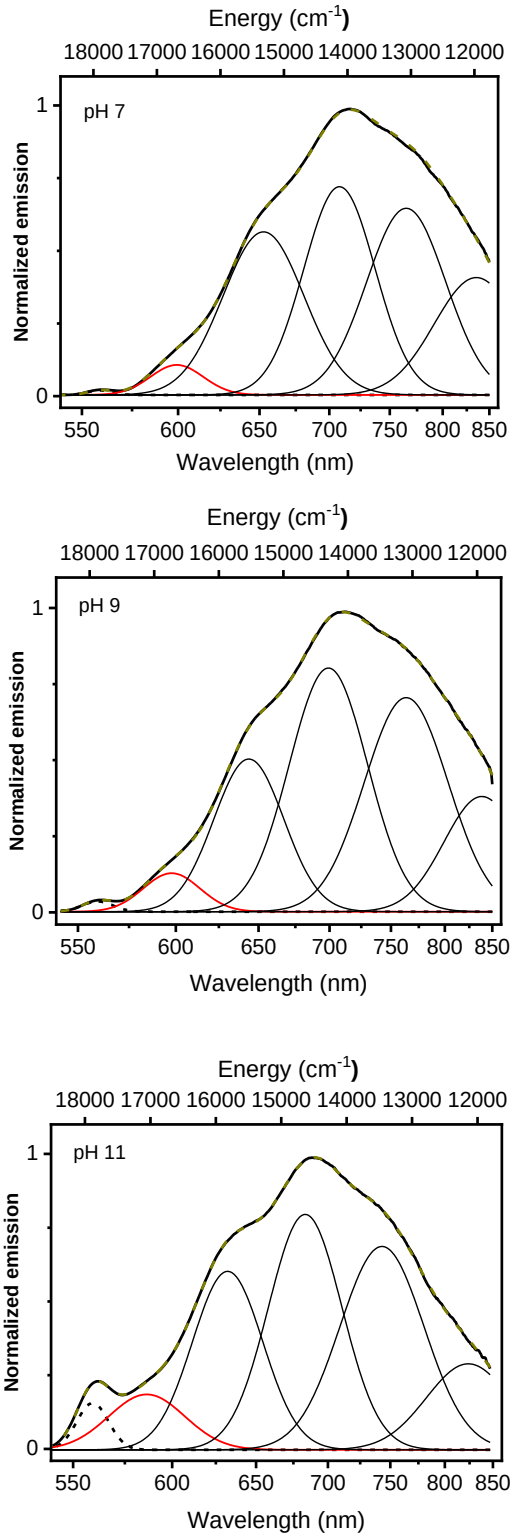

Figure S3. The Gaussian fits of emission spectra of crocin in pH 7, 9, and 11 (Ex: 470 nm). The red Gaussian corresponds to the 0-0 band.

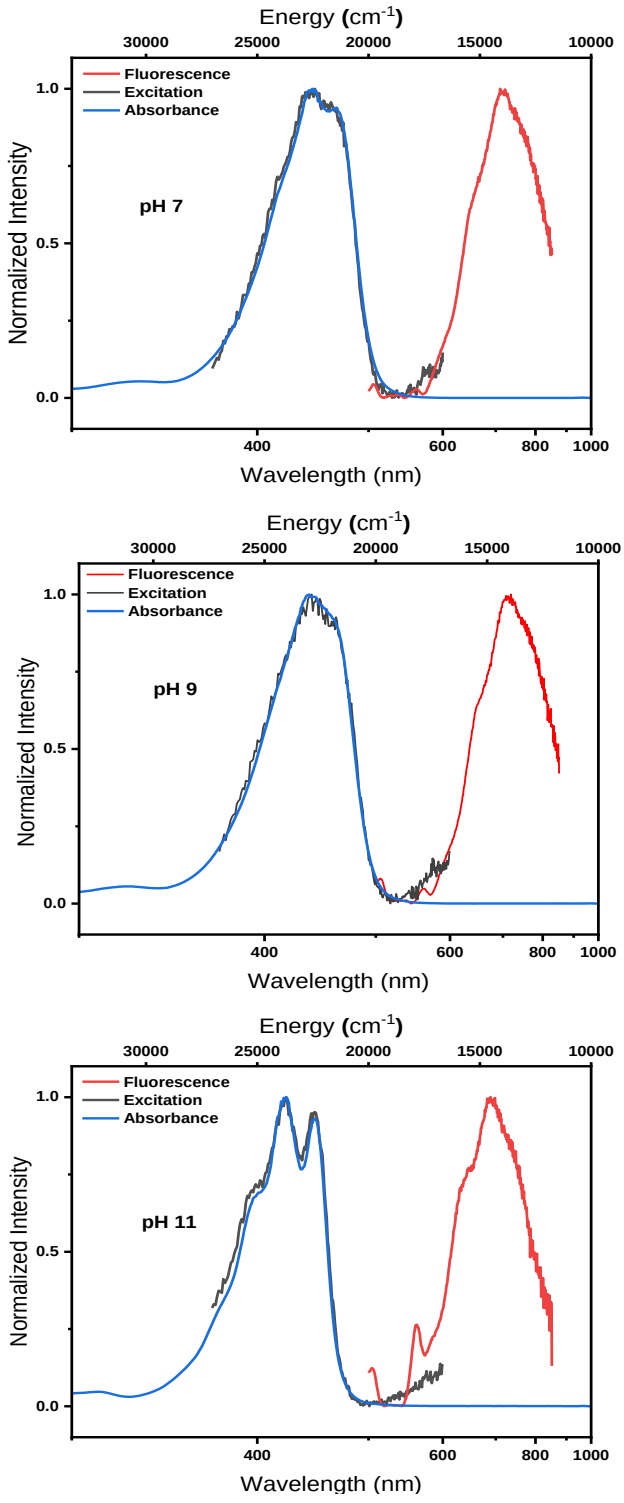

Figure S4. Absorption, emission, and excitation spectrum of crocin in pH 7, 9, and 11. All spectra were normalized and the excitation wavelength for emission spectra is 470 nm. Excitation spectra were recorded at the emission maximum.

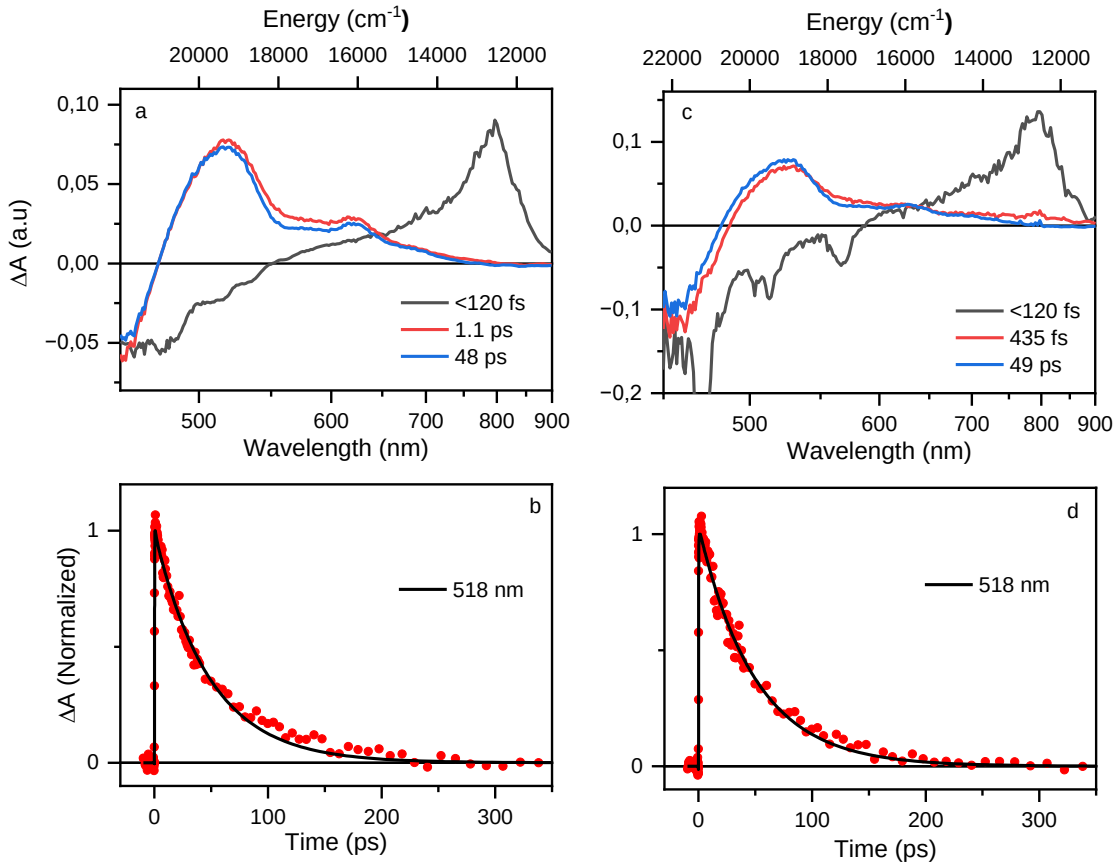

Figure S5. Left: EADS and kinetics of crocin at pH 9 excited at 450 nm fitted to three components (not enough). Right: EADS and kinetics of crocin at pH 9 excited at 470 nm – three components are enough.

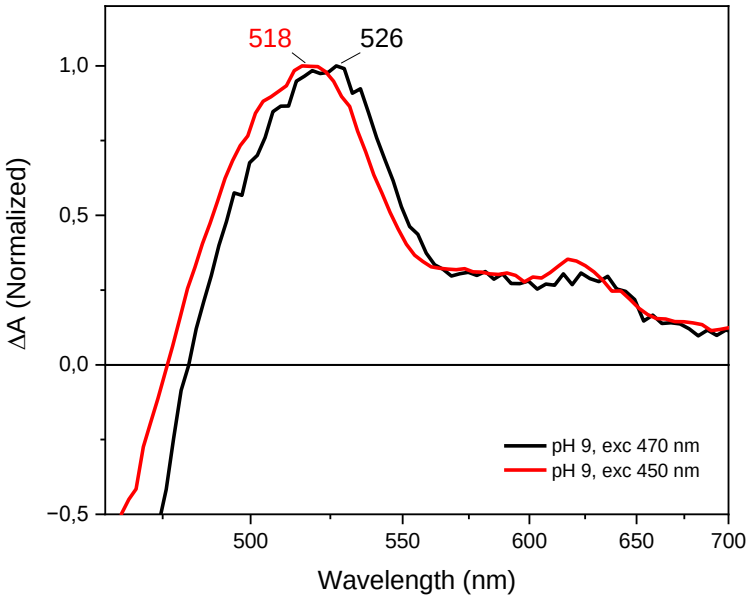

Figure S6. Comparison of transient absorption spectra for three fit components at pH 9, measured after 470 nm (black) and 450 nm (red) excitations at 5 ps.

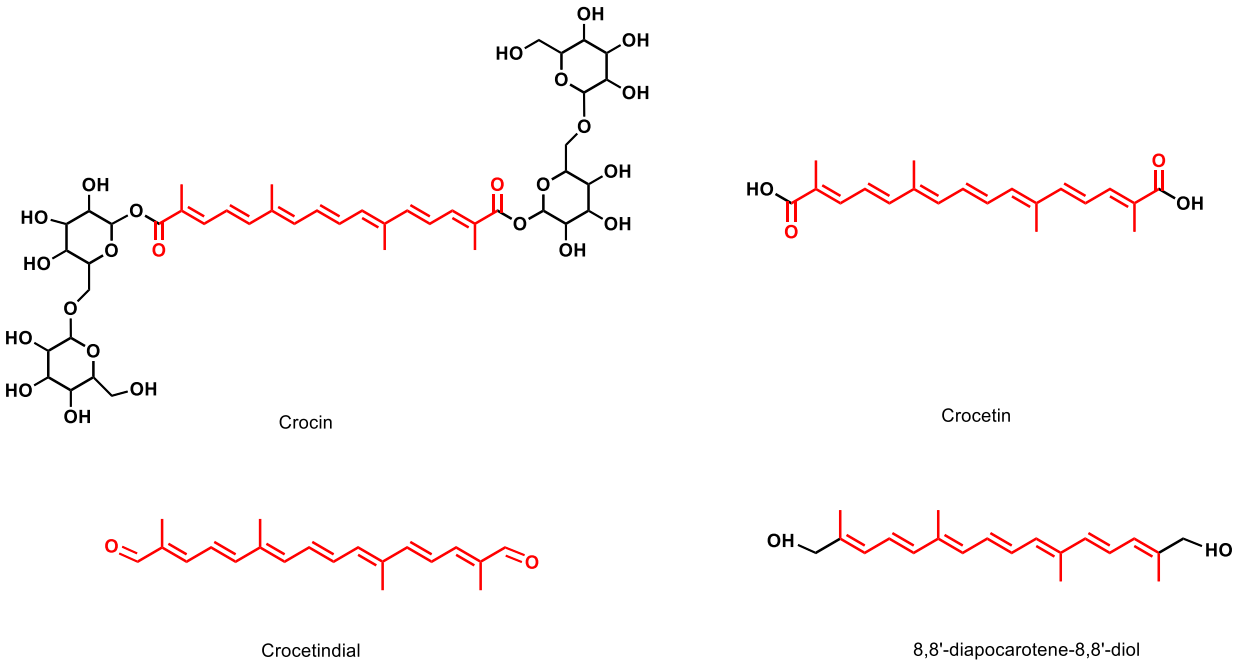

Figure S7. The molecular structure of crocin and similar molecules, which are discussed in the main text. The conjugation length of the molecules is represented in red.

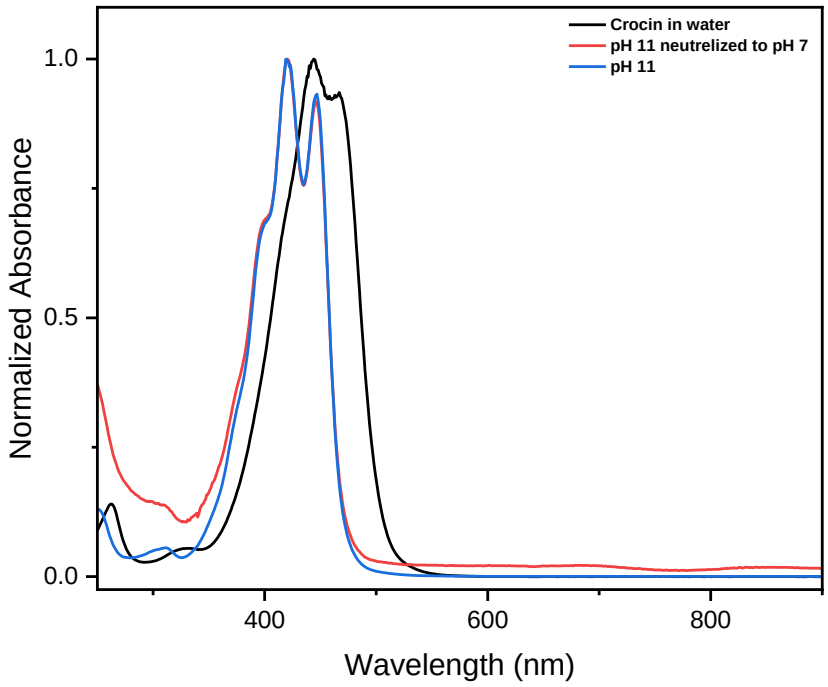

Figure S8. Normalized absorption spectra of crocin in water (black), crocin in pH 11 buffer solution (blue), and crocin neutralized from pH 11 to pH 7 using buffers.

High-performance liquid chromatography (HPLC) analysis of crocin at different pH

Sample heterogeneity was assessed by reverse-phase HPLC, using a Waters Alliance HPLC system with PDA 2998 detector (Waters, USA). Pigments were separated on a reverse phase Nova-Pak C18 column (3.9 × 300 mm, 4 μm, silica-based, end-capped; Waters, USA) using a linear gradient elution. A tertiary solvent system used was as follows: solvent A (80:20 methanol: 0.5 M ammonium acetate (aq., pH 7.2), v/v), solvent B (90:10 acetonitrile: water, v/v), solvent C (100% ethyl acetate). The gradient consists of injection into 100 % solvent A, followed by a ramp to 100 % solvent B within 4 minutes. The gradient follows by a transition to 20 % solvent B and 80 % solvent C in 14 minutes.[1] The flow rate was 1 ml min^{-1}. The method was used because it is suitable for many carotenoids and well established in our laboratory. Samples were injected in a water-based buffer as indicated elsewhere in the text. Samples from high pH were neutralized prior to injection by dilution with buffer with pH 7 due to the limits of the used HPLC column. Samples from pH 9 and pH 11 were neutralized after two days of reaction to be comparable to the conditions during the optical spectroscopy experiments.

The HPLC chromatogram of crocin (Fig. S9) shows one broad peak immediately at the front of the elution, at ~ 2.1 min. No other components were detected in the sample. Sample treated by pH 11 consisted of one major component at ~ 2.7 min (the void volume peak at 2.1 min is prominent in these data due to the low concentration of the injected sample). The spectrum of this component is virtually identical to bulk spectra of crocin at pH 11 (Fig. S10). The chromatogram of crocin treated at pH 9 contains two major peaks. The first one, at 2.25 min, forms 77 % of the sample and has a spectrum similar to crocin but blue-shifted by 11 nm to 429 nm. The second peak in pH 9-treated sample is identical with that from the pH 11 sample. Based on these data it can be concluded that crocin treated by pH 11 for two days is converted to another, less polar, species. Saponification of the glycoside side-chains of crocin is one of the possible origins of this species. Identity of the intermediate species in the sample at pH 9 is not clear. Since it is less polar than crocin at pH 7 and more polar that the species identified at pH 11, it is feasible that at pH 9 due to very slow saponification reaction there is a substantial fraction of monoesters. Such asymmetric molecule could explain some spectroscopic features of crocin at pH 9, but we note that its absorption spectrum does not indicate a presence of an asymmetric keto-group thus the picture is more complicated.

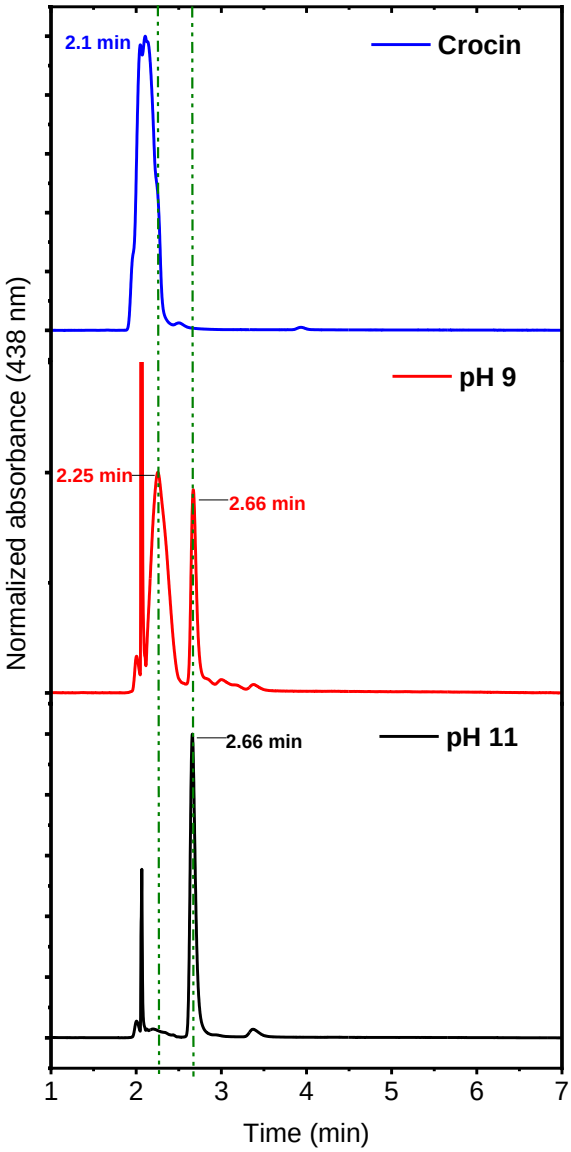

Figure S9. HPLC chromatograms of crocin (blue), crocin at pH 9 neutralized to pH 7 (red), and crocin at pH 11 neutralized to pH 7 (black). Elution times of identified peaks are shown in the graph.

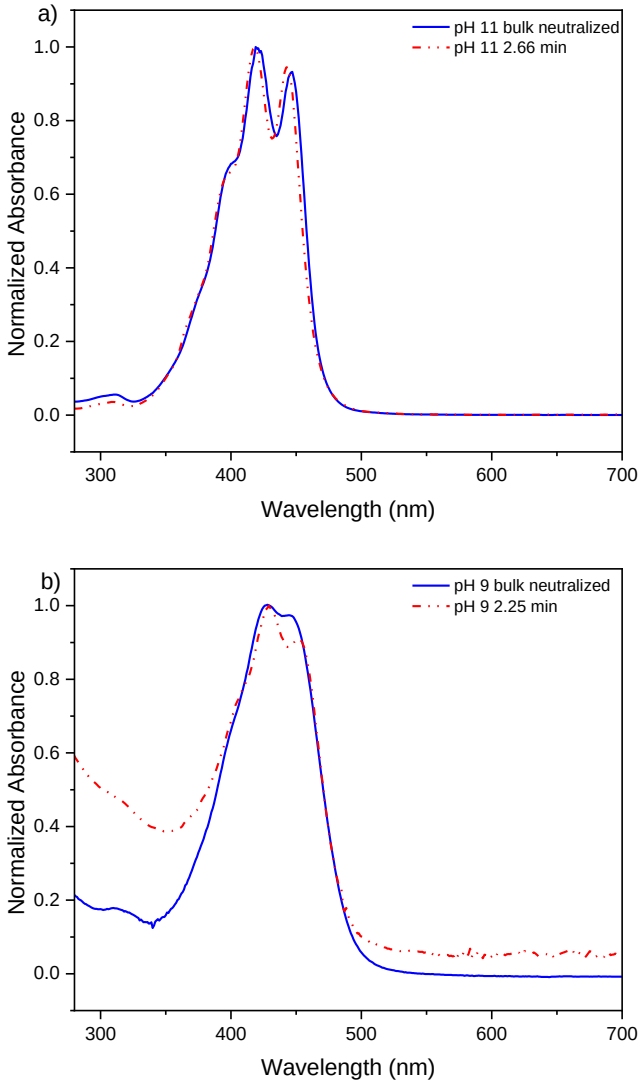

Figure S10: Comparison of normalized absorption spectra of crocin at a) pH 11 (neutralized to pH 7, shown in blue), and HPLC peak at 2.66 min of the pH 11 sample (show in red dotted line). b) pH 9 (neutralized to pH 7, shown in blue), and HPLC peak at 2.65 min of the pH 9 sample (show in red dotted line). The red spectra are extracted from HPLC data.

References

1. R. Litvín, D. Bína, M. Herbstová and Z. Gardian, *Photosynth. Res.*, 2016, **130**, 137-150.