Iron-binding properties of plant phenolics and cranberry's bio-effects

Maolin Guo*, Carlos Perez, Yibin Wei, Elise Rapoza, Gregory Su

Department of Chemistry and Biochemistry, University of Massachusetts, Dartmouth, MA 02747

Fadi Bou-Abdallah and N. D. Chasteen

Department of Chemistry, University of New Hampshire Durham, NH 03824-3598, USA

Supporting information

S1. Apparent binding constant equations

Figs. S1-S8

S1. Apparent binding constants:

Estimation of the apparent binding constants for the formation of the flavonoid-Fe^{2+} complexes can be made as follows. [1]

a) For the ligand-substrate reaction with formation of 1:1 complex LS:

$$L + S \rightleftharpoons LS, \tag{4}$$

where L = flavonoid, $S = Fe^{2+}$, and LS = flavonoid-Fe^{2+} complex, the equilibrium constant (apparent binding constant) is given by:

$$K = \frac{[LS]_e}{[L]_e[S]_e} \tag{5}$$

The subscript e designates equilibrium concentrations. The ratios of the equilibrium LS complex concentration, $[LS]_e$, and the initial L concentration, $[L]_o$, can be derived from the absorbance of the solutions at a chosen wavelength both at equilibrium and far from equilibrium. The result of the derivation is as follows:

$$F_c = \frac{A_u - A_m}{A_u - A_c} = \frac{[LS]_e}{[L]_o} \tag{6}$$

where F_c is the fraction of L which formed a complex, the subscripts e and o stand for equilibrium and initial concentrations, respectively. A_u; A_m; and A_c are the absorbance (at a chosen wavelength) of solutions of L only (before Fe^{2+} was added); L and LS mixture (somewhere in the middle of titration); and LS only (at the end of titration) respectively. The concentration of free Fe^{2+} at equilibrium $[S]_e$ is derived from:

$$[S]_e = [S]_o - [LS]_e = [S]_o - F_c[L]_o \tag{7}$$

The apparent binding constant K can then be calculated from Eqs. (6), (7), and (8):

[1] E. Ojadi, Ph.D. Thesis, 1986, Brandeis University, Boston, MA, USA

$$K = \frac{F_c}{1 - F_c} \times \frac{1}{[S]_e} \quad (8)$$

b) For the ligand-substrate reaction with formation of 2:1 complex SL_2, the following equations apply:

$$S + 2L \rightleftharpoons SL_2 \quad (9)$$

$$K = \frac{[SL_2]_e}{[S]_e [L]_e^2} \quad (10)$$

$$t = 0,\ A_u = \varepsilon_L \ell\, [L]_o\ ,\ [S]_o = 0\ ,\ [SL_2]_o = 0 \quad (11)$$

$$t = \infty\ ,\ A_c = \varepsilon_c \ell\ [SL_2]_\infty = \frac{\varepsilon_c \ell [L]_o}{2} \quad (12)$$

$$t = t,\ A_m = \varepsilon_L \ell\ [L]_e + \varepsilon_c \ell\ [SL_2]_e \quad (13)$$

$$[L]_o = [L]_e + 2[SL_2]_e \quad (14)$$

$$[L]_e = [L]_o - 2[SL_2]_e \quad (15)$$

$$F_c = \frac{A_u - A_m}{A_u - A_c} \quad (16)$$

$$Fc = \frac{\varepsilon_L \ell [L]_o - \varepsilon_L \ell [L]_e - \varepsilon_c \ell [SL_2]_e}{\varepsilon_L \ell [L]_o - \frac{\varepsilon_c \ell [L]_o}{2}} \quad (17)$$

$$F_c = \frac{2[SL_2]_e}{[L]_o} \quad (18)$$

$$K = \frac{\frac{Fc}{[L]_o}}{2[S]_e \left(\frac{[L]_o - 2[SL_2]_e}{[L]_o} \right)^2} \quad (19)$$

$$[S]_e = [S]_o - [SL_2]_e \quad (20)$$

$$[S]_e = [S]_o - F_c[L]_o \tag{21}$$

$$K = \frac{F_C}{2[L]_o[S]_e(1-F_c)^2} \tag{22}$$

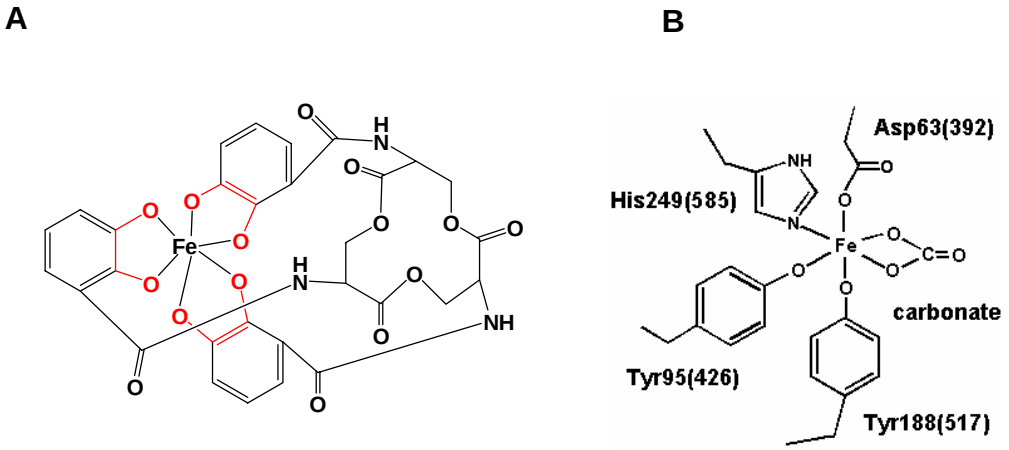

Fig. S1 The chemical structures of the "Fe-binding motif" in (A) enterobactin (a bacterial siderophore), and (B) Fe-binding site in transferrin.

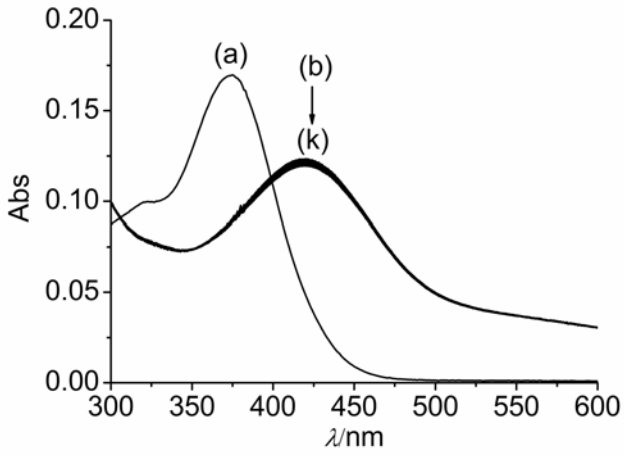

Fig. S2 UV/Vis spectra of quercetin and the kinetics after the addition of 1.0 mol eq. Fe^{2+} in 20 mM KPB buffer, pH 7.2. (a), quercetin (10 μM), (b) 30 s after the addition of 1 mol eq. Fe^{2+}, (c) – (k), 1 – 10 min after the addition of Fe^{2+}.

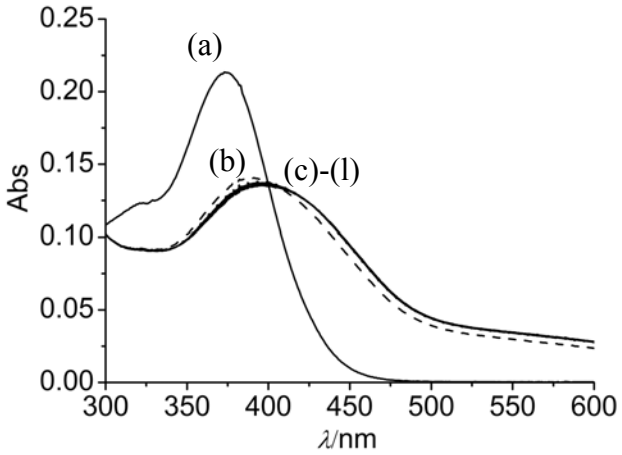

Fig. S3 UV/Vis spectra of quercetin and the kinetics after the addition of 0.5 mol eq. Fe^{2+} in 20 mM KPB buffer, pH 7.2. (a), quercetin (12 μM), (b) 30 s after the addition of Fe^{2+}, (c) – (l), 1 – 10 min after the addition of Fe^{2+}.

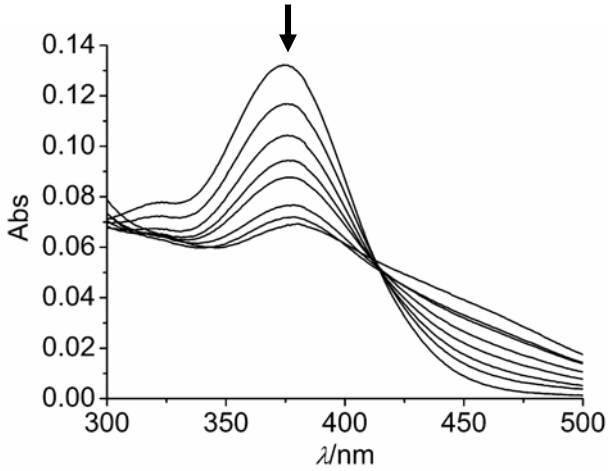

Fig. S4 Titration of quercetin by Ga^{3+} in 20 mM KPB buffer, pH 7.2. From top to bottom: 8 μM quercetin in the presence of 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3.0 mol eq. Ga^{3+}.

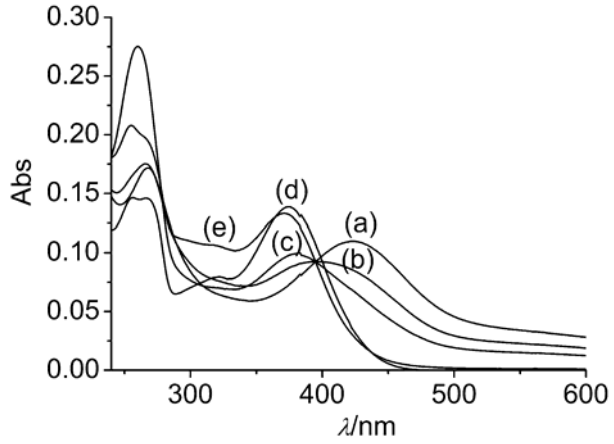

Fig. S5 Recovery of Fe^{2+} from ATP, citrate or EDTA by quercetin in 20 mM KPB, pH 7.2. (a) 10 μM quercetin-Fe^{2+}; (b) recovery from 10 μM citrate, (c) recovery from 10 μM ATP (d) recovery from 10 μM EDTA and (e) free 10 μM quercetin.

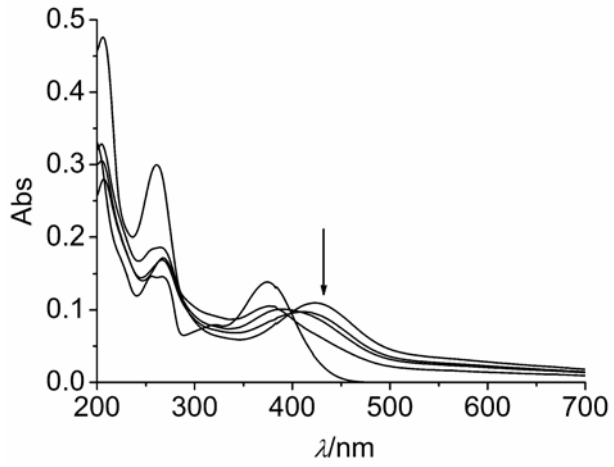

Fig. S6 UV/Vis spectroscopy of the recovery of Fe^{2+} from quercetin by ATP, citrate, and EDTA in KPB at pH 7.2. All the chelators were 10 μM. From top to bottom: quercetin-Fe^{2+}, with citrate, with ATP, with EDTA and quercetin alone, respectively.

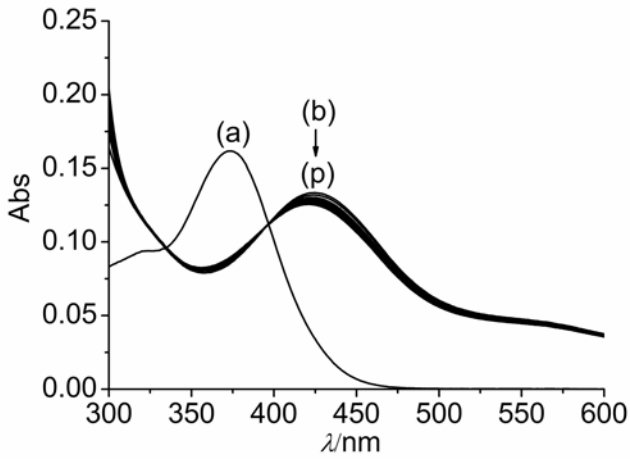

Fig. S7 UV/Vis spectra of quercetin and Fe^{2+} in the presence of H_2O_2 and ascorbate in 20 mM KPB, pH 7.2. (a) 10 μM quercetin only; (b) with the addition of 10 μM Fe^{2+}; and (c)-(p) 100 μM ascorbic acid and 200 μM H_2O_2 were added and the kinetics was monitored for 10 min (from top to bottom).

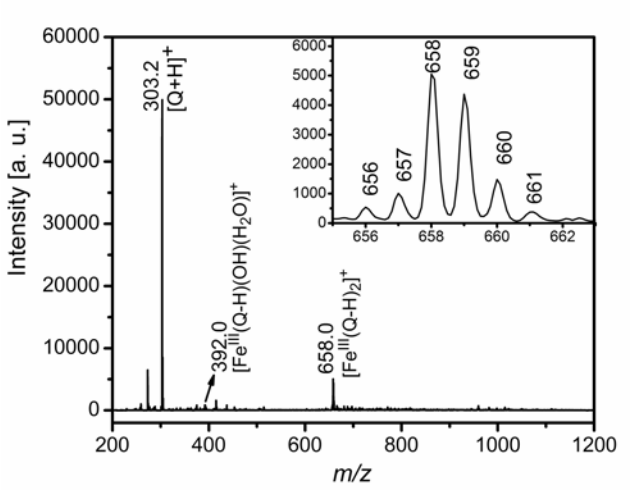

Fig. S8 Electrospray mass spectrum after addition of 20 μM of H_2O_2 to a solution of quercetin and Fe^{2+} (10 μM each, 1:1) in methanol/water (1/1, v/v). The inset is the isotopic pattern of the peak at *m/z* = 658.0.

After the addition of H_2O_2, the peaks for quercetin-Fe^{2+} complexes (*m/z* 375.1, 659.0, 712.9 and 1014.8) diminished, but peaks for quercetin-Fe^{3+} complexes (*m/z* 658.0 and 392.0) appeared. The overall ESI-Mass spectrum after the addition of H_2O_2 is almost identical to that of the mixture of quercetin (10 μM) and Fe^{3+} (10 μM) under similar conditions. This result suggests the conversion of Fe^{2+}-quercetin complexes to Fe^{3+}-quercetin complexes by H_2O_2 without Fenton chemistry.