Assignment A04: Catechin Content and Seed Plasticity of Cooked Cranberry Beans

As we begin to work on research-based assignments and to deal with nature's complexity, it is worthwhile to reflect on a quote from Melville's Moby Dick: "As for me, I am tormented with an everlasting itch for things remote. I love to sail forbidden seas, and land on barbarous coasts."

This assignment is based on two recent research articles. **Source 1:** *Physicochemical Properties and in Vitro Digestibility of Cooked Regular and Nondarkening Cranberry Beans (Phaseolus vulgaris L.) and Their Effects on Bioaccessibility, Phenolic Composition, and Antioxidant Activity*. Peter X. Chen, John H. Dupuis, Massimo F. Marcone, Peter K. Pauls, Ronghua Liu, Qiang Liu, Yao Tang, Bing Zhang, and Rong Tsao. (10.1021/acs.jafc.5b04005) *J. Agric. Food Chem.* **2015***, 63*, 10448−10458. **Source 2:** *A solution spectroscopy study of tea polyphenol and cellulose: effect of surfactants.* Deboleena Sarkar, Somnath Das, and Amitava Pramanik. (10.1039/c4ra04171b) *RSC Adv.* **2014**, *4*, 36196-36205.

Hyperlinks to the articles are provided in the assignment section of the course web site, and links to local copies of the articles (PDF) also are provided there. Cranberry beans (a.k.a., Romano beans, Barlotti beans, speckled sugar beans) are excellent salad beans; see, for example, http://www.realfoods.co.uk/article/the-real-foods-guide-to-pulses; http://www.pulsecanada.com/uploads/10/75/10755ec6c20325e09829ff95d032c31e/Cooking-with-Beans-Peas-Lentils-2014-No-Health-Check-Logo.pdf).

The goals of this assignment include (i) to learn about methods to study the bioaccessibility, phenolic composition, and antioxidant activity of phytochemicals of cranberry beans with focus on the flavanol catechin, (ii) to learn about the structure and the redox chemistry of catechin and to create Scheme 1 to describe this redox chemistry by improving Scheme 2 of Source 2, (iii) to re-create the UV-Vis spectra of catechin as a function of pH in your Figure 1 by simulation of Figure 1(b) of Source 2, (iv) to re-create the thermograms of Red Ridder and Non-Darkening Cranberry as Figure 2 by simulation of Figure 5A in Source 1, and (v) to write some text to summarize these items. The simulations require the generation and manipulation of large arrays

of data and you need to be well organized from the start. Use the posted "examples" and "samples" for guidance, and enjoy the challenge.

Read Source 1 and try to understand what the paper is about; this might take some time. Figure 1 in Source 1 shows the effects of cooking two varieties of cranberry beans on phenolic phytochemical content and antioxidant activity. The phenolic content is assessed by three kinds of measurements (TPC = Total Phenolic Content, TFC = Total Flavonoid Content, PAC = Total Proanthocyanidin Content) and the antioxidant activity is measured with the ORAC assay (Oxygen Radical Absorbance Capacity assay). Catechin is a "phenolic" and, more specifically, catechin is a "flavanol" and thus belongs to the class of "flavonoids". Proanthocyanidins are oligomeric flavonoids and many of these are oligomers of catechin and epicatechin and their gallic acid esters. Proanthocyanidins may liberate flavonoids upon enzymatic cleavage ("depolymerization") and/or ester hydrolysis. We will focus on catechin.

(a) Create Scheme 1 to Describe the Redox Chemistry of Catechin (improve on Scheme 2 of Source 2). Scheme 2 in Source 2 is supposed to explain the mechanism of catechin oxidation, i.e., the mechanism by which catechin reacts with oxidizing agents and thus acts as an antioxidant (a remover of oxidizing agents). The peer review for Source 2 was seriously lacking (even through *RSC Advances* is considered a good journal)! Take a look at this Scheme 2 and notice several major errors: The "quinone" moiety should contain two C=C double bonds. The "semiquinone" is shown as a neutral diradical when it should be a radical anion. Visit the link http://www.nature.com/articles/srep11033/figures/10, for a more accurate scheme to explain the oxidation of catechin. Each oxidation step removes one H atom by removal of one electron (by the oxidizing agent) and one proton (by a base), i.e., "$-e^-$, $-H^+$". Create your own Scheme 1 to describe the redox chemistry of catechin correctly. Show complete structures including all lone pairs, lone electrons and formal charges. Use correct arrows. Use color to enhance the message. Pay attention to alignment and spacing. Import the scheme into a Word file and add a Scheme legend.

(b) Create Figure 1 by Simulation of Figure 1(b) of Source 2 (UV/Vis Spectra of Catechin). Figure 1(b) of Source 2 shows the measured UV/Vis spectra of 0.2 mM solutions of catechin in water at different pH after 24 hours and the spectrum of a deaerated solution of catechin at pH = 7 after 24 hours. Your Figure 1 should be a complete re-creation of this Figure 1(b) in every way (same sizes, same labels, same tick marks, same colors, same everything; but omit "(b)") using the EXCEL software.

Each spectral curve $f(\lambda)$ can be approximated as the sum of a number of Gaussian functions $f_i(\lambda)$ which are determined by the positions of their maxima $\lambda_{max,i}$ and their extinction coefficients ε_i which determine the absorbance; $f(\lambda) = \Sigma\ f_i(\lambda)$. At least five Gaussians are needed in this simulation ($i = 5$). Start with reasonable initial guesses for $\lambda_{max,i}$ and ε_i and adjust the parameters to obtain a good fit. You may choose a fitting procedure of any sophistication; i.e., from visual inspection to mathematical regression. Refer to the posted examples to get an idea about the organization of your excel sheet to generate Figure 1.

Generation of Spectrum $f(\lambda)$: For each Gaussian, list the parameters for $\lambda_{max,i}$, ε_i and width on top of the sheet, i.e., in the posted example in rows 3, 4 and 5, respectively. List discrete values of λ in column B, i.e., 250 - 600 nm in steps of 5 nm (or better) starting in row 8 in the example. Then compute the values of the unnormed Gaussian function and place the values in a column to the right of the wavenumbers (i.e., column D in the example). Once you have the unnormed Gaussian values computed, determine the maximum value of your Gaussian (i.e., D5 in the example). You can now compute the value of the normed and weighted Gaussian and place it in a new column (i.e., Column E in the example) by dividing the value of the unnormed Gaussian by the maximum values of the Gaussian and multiplication by ε_i. Proceed in the same fashion for the other four Gaussians. Finally, compute the $f(\lambda)$ values in a new column and plot the spectrum as a unmarked XY scatter plot.

Generation of Spectra $f_m(\lambda)$: Apply the procedure for the spectrum simulation to each one of the six spectra (m = 1 – 6), i.e., simulate all of them as sums of Gaussian functions $f_{i,m}(\lambda)$ which are

determined by the positions of their maxima $\lambda_{max,i,m}$ and their extinction coefficients $\varepsilon_{i,m}$; $f_m(\lambda) = \Sigma f_{i,m}(\lambda)$. Plot all the simulated total functions $f_{i,m}(\lambda)$ in their respective colors and with the respective line style in one graph. (Do not plot the individual Gaussians $f_{i,m}(\lambda)$; only plot the spectra $f_m(\lambda)$.) Import the plot as Figure 1 into your Word file and add a Figure legend. Report the equations of the five Gaussians of the pH = 12 spectrum in the legend to this Figure 1.

(c) Create Figure 2 by Simulation of Figure 5A of Source 1 (Thermograms). Figure 5A of Source 1 shows the measured thermograms raw Red Rider (RR_R) and nondarkening (CND_R) cranberry beans as determined by differential scanning calorimetry. Your Figure 2 should be a complete re-creation of this Figure 5A in every way (same sizes, same labels, same tick marks, same line style, same everything; but omit "A") and with one additional feature (vide infra).

One could reproduce these curves as a sum of Gaussians as with the UV/Vis spectra. However, in this case it might be better to use a different approach: Each curve can be seen as the result of a line with negative slope and two inverted Gaussians. Read the text of Source 1 and note that a "peak" in the thermogram corresponds to a reduction of the heat flow. Hence, each thermogram can be simulated with a function $f(T) = aT - \Sigma f_i(T)$ ($a < 0$, $i = 1, 2$) where the Gaussians depend on $T_{max,i}$ (Temperature at which the heat flow reduction is at a maximum) and an associated heat transfer constant η_i (which controls the intensity of the heat flow reduction).

Plot both of the simulated thermograms $f_m(T)$ ($m = 1, 2$) in their respective line styles in one graph. Also show the linear baseline functions $f_{bl,m}(T) = a_m T$ as dotted lines. Import the plot as Figure 2 into your Word file and add a Figure legend. Report the equations for $f_m(T)$ ($m = 1, 2$) in the legend to this Figure 2.

(d) Write Text with Proper Bridges to Scheme 1 and Figures 1 and 2. Write a brief and concise description of the context (no more than 2 pages of text, double-spaced, Times New Roman, 12 pt, 1 inch margins, your names in the header, page numbers centered in the footer) at the beginning of a Word file. Cite the schemes and the figures in your text using appropriate bridges at the most suitable places. Scheme 1 and Figures 1 and 2 follow the text, each with its

own legend and on separate pages. Cite references in the text where needed and have them appear as numbered footnotes to the text.

Your text should include a brief introduction about the effects of thermal processing of Cranberry beans in water on catechin content and bean palatability, a description of the mechanism of the antioxidant functionality of catechin using Scheme 1 for illustration, a discussion of the UV/Vis spectra of catechin in the absence of oxidizing agents (Figure 1, dashed), a discussion of the UV/Vis spectra of catechin solutions after exposure to oxidizing agents (Figure 1, solid curves) and including an explanation of the pH dependence (considering Scheme 1), and, finally, a discussion of the information gained from the thermograms (Figure 2) that includes explanations of the terms "starch gelatinization" and "amylose-lipid complexation" and a reasonable attempt at an explanation of the two peaks in the thermograms. You might need to dig into and cite some literature as you attempt to understand this chemistry.

The assignment must be completed with MS WORD and MS EXCEL. Create one WORD file with the name "A04_'your_last_names'.docx" and one EXCEL file with the name "A04_'your_last_names'.xlsx". Organize the XL file as much as you can so that it will be accessible to the peer reviewers! Label your sheets; one sheet for part (b) and one for part (c). Perhaps show the graphs in separate sheets (you can "Move Chart" to a new sheet).

Deadlines: Submit both electronic files on Tuesday, 03/01/16 by midnight. Bring one hardcopy (stapled) of the Word file only to class on Wednesday, 03/02/16. Peer reviewers will receive the associated EXCEL file via email once peer review assignments will have been made.

Importing Graphs from Excel: (cf. A02 about importing ChemDraw schemes.)

There are many ways to import an Excel Graph into a WORD file. One way to import a graph from Excel into Word involves the following steps: select the graph in Excel, copy the graph to the clipboard (click "Copy" in the Edit menu, or type Command-C), go to the Word file, and paste the clipboard (click "Paste" in the Home menu, or type Command-V).

This import method inserts the graph as a Microsoft Excel Chart Object and such an object can be edited after it was inserted in the Word file. Double-clicking the chart in the Word file will open a Excel window where you can edit the graph and the respective XLSX file.

For other ways to import a graph from Excel into Word use "Paste Special". Click the small triangle under "Paste" in the Home menu to see the "Paste Options" and then select "Paste Special". A menu comes up and you can chose among various formats including Microsoft Excel Chart Object, Bitmap, several Picture formats (Enhanced Metafile, GIF, PNG, JPEG), and Microsoft Office Graphic Object.

Embedding a Microsoft Excel Chart Object can be advantageous and it is straightforward if the WORD file and the associated CDX files are in the same file system. However, in collaborations that involve several people working on several computer systems, there are advantages to using alternatives. As a general rule, we want to paste schemes and graphs as "Picture (Enhanced Metafile)". Even if the assignment only requests the submission of embedded figures, keep the associated XLSX files because you might be asked for their submission during review.