LINKING CHEMICAL CONCEPTS OF ENZYMATIC FUNCTION IN THE INTRODUCTORY BIOLOGY LAB

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Introduction

- Indicators are commonly used as a signifier of enzyme activity
- But in our experience, students often conflate the reaction signified by the indicator with the reaction catalyzed by the enzyme

$$x \xrightarrow{enzyme} y + z \xrightarrow{indicator+boiling} \text{color change}$$

- What's more, they can confuse an indicator reaction with experimental manipulations in the study of enzyme activity

$$x \xrightarrow{enzyme+\Delta\ temp} y + z \xrightarrow{indicator+boiling} \text{color change}$$

- We have chosen catalase as a commonly available easily detectable enzyme to show enzyme activity in our introductory biology course

$$H_2O_2 \xrightarrow{catalase} H_2O + O_2$$

- Catalase is also advantageous, because students can delve deeper into catalase activity using a colorimetric indicator along with Beer's law to quantify activity

$$2\ H_2O_2 \xrightarrow{catalase} 2\ H_2O + O_2 \xrightarrow{indicator} \text{color change}$$

- At our institution, science courses run in 8-week blocks and we've begun to notice an effect on the feeling of "community" amongst our science students
- We are also always looking for new opportunities to introduce inquiry and research into the laboratory class

- In this series, students in two courses in the biology major, introductory biology II and organic chemistry II, will work together to propose a source of dietary flavonoid from which to test on bacterial catalase activity

$$H_2O_2 \xrightarrow[\uparrow\ flavonoid]{catalase} H_2O + O_2 \xrightarrow{indicator} \text{color change}$$

- In addition, students build their lab technical skills including micropipetting, aseptic technique, microbiological culture, extraction, infrared spectroscopy interpretation, and searching and interpreting the primary literature

- This lab series is intended for introductory biology and organic chemistry students, but could be adapted for a single course in biochemistry

Class Activities

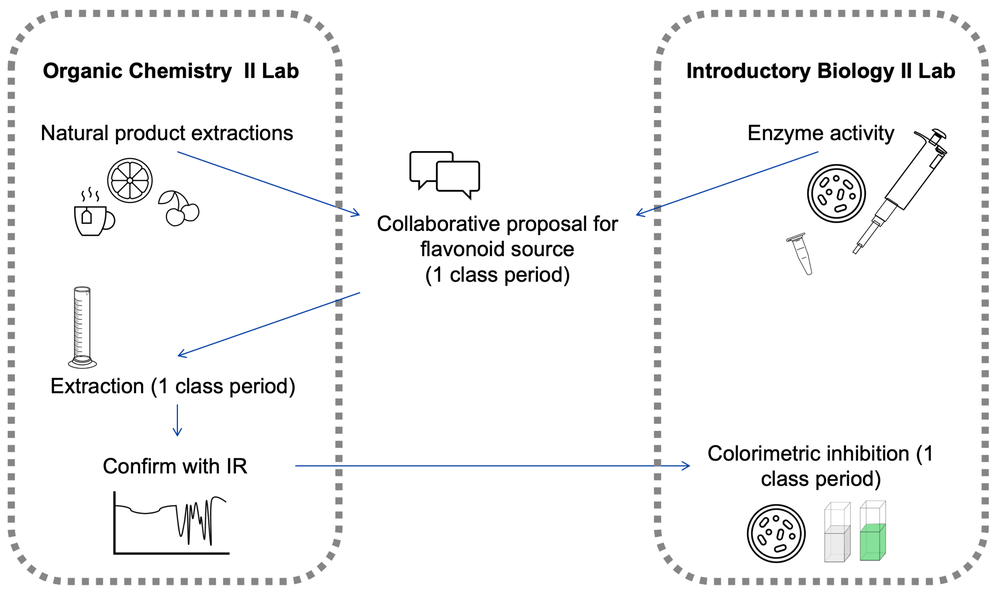

Figure 1. Class activities for parallel organic chemistry II and introductory biology II lab courses. These activities include background on extractions from natural products in organic chemistry and enzyme activity in biology. Students from these classes will then collaborate on proposing a flavonoid source. Students in organic chemistry will extract their flavonoid and confirm its identity using infrared spectroscopy (IR). Students in biology will receive this product and use it as an inhibitor in their own enzyme activity experiments.

	Organic Chemistry II	Introductory Biology II
Week 1	Introduction and lab safety Background	Introduction and lab safety Water lab
Week 2	Extraction of caffeine Analysis of caffeine Isolation of chlorophyll	Indicators lab Characterizing carbohydrates lab Growing bacteria
Week 3	Proposal of phthalate intervention CURE	Measuring enzyme activity (sucrase) Presenting your results Using a micropipette
Week 4	Set up phthalate intervention CURE Isolation of cinnamaldehyde	Denaturing enzymes (sucrase) **Measuring enzyme activity (catalase)**
Week 5	**What are flavonoids?** **Collaborate with Introductory Biology II to propose source** **Isolation of flavonoids**	**What are flavonoids?** **Collaborate with Organic Chemistry II to propose source** Lab report comparison activity
Week 6	Extract phthalates Continue phthalate CURE	**Inhibition of catalase activity with flavonoids** Presenting your results
Week 7	Finish phthalate CURE	Improving your lab report activity Outlining the introduction and conclusion
Week 8	Final exam	Final exam Lab report due

Figure 2. Proposed class schedules for organic chemistry II and introductory biology II lab courses with implementation of this new lab series. Activities pertaining directly to this lab series are indicated in bold.

Preliminary Results

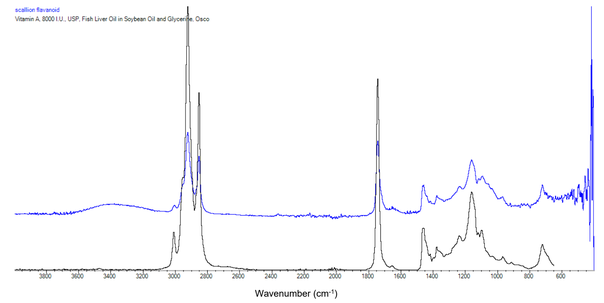

Figure 3. FTIR spectra of scallion extract in comparison to vitamin A spectra. Students selected the following plant materials as possible sources of flavonoids: broccoli, parsley, red grape, lemon, orange, black tea, and scallions. They had an average mass percent of extract ranging from 12.9 % (orange peel) to 33.7 % (black tea).

Cobalt Indicator Assay Principle

$$2\ H_2O_2 \xrightarrow{catalase} 2\ H_2O + O_2$$

$$H_2O_2 + 2\ [Co(II)(H_2O)_6]^{2+} + 6\ HCO_3^- \xrightarrow{catalase} 2\ [Co(III)(CO_3)_3]^{3-} + 2\ H_2O + 4H^+$$

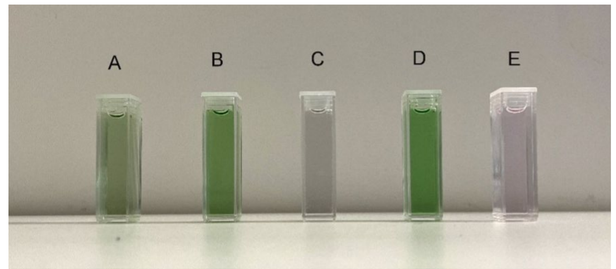

Figure 3. Color intensity of different solutions tested. (A) Catalase + green tea extract + cobalt indicator, (B) green tea extract + cobalt indicator, (C) catalase alone, (D) cobalt indicator alone, (E) blank.

Future Work

- To date, we've begun implementation of natural product extraction in the organic II course, and will pilot bacterial-derived catalase enzyme activity in biology II lab classes next spring
- We're looking for feedback on:
  - Best practices to coordinate parallel lab courses
  - Assessment instruments for sense of community development amongst the courses within the major
  - Advice on how to decide which lab sections can serve as a control (as a small institution, we will have instructor effects)

References

Elamin, M.M. (2020). Solvent extraction and spectroscopy identification of bioactive compounds from medicinal shrub *Tamarix gallica*. *Function Foods in Health and Disease*. *10*(11), 456-464. https://www.doi.org/10.31989/ffhd.v10i11.744

Hadwan, M. H. (2018). Simple spectrophotometric assay for measuring catalase activity in biological tissues. *BMC Biochemistry*, *19*(1), 7. https://doi.org/10.1186/s12858-018-0097-5

Liu, X., Liu, Y., Shan, C., Yang, X., Zhang, Q., Xu, N., Xu, L., & Song, W. (2022) Effects of five extraction methods on total content, composition, and stability of flavonoids in jujube. *Food Chemistry*:X, 14. https://doi.org/10.1016/j.fochx.2022.100287