Assessment of Antioxidant Capacities in Foods: A Research Experience for General Chemistry Students

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It is well established and well advertised that foods rich in antioxidant compounds promote good health. However, there is little known regarding the effects of various preparation, processing, or storage methods on antioxidant activity (1). The laboratory curriculum, or module, described here provides practical experience in scientific research through the investigation of student-formed hypotheses relating to the antioxidant capacities of foods.

The module is six weeks long and uses the curricular approach developed by the Center for Authentic Science Practice in Education (CASPie) (2). The first three weeks focus on providing students with skills for analyzing antioxidant capacity in foods. The final three weeks allow the students to develop and conduct an experiment using the knowledge and skills they learned from the earlier laboratory periods. Students write a final paper in the style of a scientific journal article and complete an experiment summary for the author of the module (J.R.B.) who reports their findings and experimental design.

The laboratory procedures for the first three weeks of the module focus on three assays commonly used in the field of food science. In the first activity, the trolox equivalent antioxidant capacity (TEAC) assay, spectrophotometric methods are used to compare the antioxidant capacities of solutions with that of trolox, an accepted antioxidant standard (3–6). Antioxidant capacity is investigated by determining the reactivity of the antioxidant with a radical ion. Next, students use high-performance liquid chromatography (HPLC) to investigate concentrations of ascorbic acid, a known nutrient antioxidant, in food products (4, 7). Finally, the students determine the total polyphenolic content in foods with Folin–Ciocalteau reagent using epicatechin as a polyphenolic standard (3–6, 8). Polyphenolic compounds are known to contribute to the antioxidant capabilities of the foods. Standard curves are used in the analyses of both polyphenolic and ascorbic acid content.

In weeks four through six, students investigate a hypothesis that they develop. The hypothesis is specifically related to the overall research question of the module. The students employ the assays from the first three weeks to develop a procedure with guidance from their literature review and their teaching assistants (TAs) or instructors. An overview of the experimental activities is provided in the module calendar shown in Table 1.

**Experimental**

**Comparison of Known Antioxidants by the TEAC Assay**

A 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS**+) solution is prepared from ABTS and K$_2$S$_2$O$_8$ (6), and the absorbance peak is determined by UV–vis spectrophotometry. This radical solution is combined with sample antioxidants, in varying concentrations, and allowed to react for six minutes. The total absorbance at the peak for ABTS**+ is then measured again and compared to that for the radical solution with no antioxidant (the control). Percent inhibition is calculated as

$$\text{% inhibition} = \frac{\Delta A_{6 \text{ min}}}{A_{\text{control}}} \times 100\%$$

where $A_{\text{control}}$ is the absorbance of the control solution and $\Delta A_{6 \text{ min}} = A_{\text{control}} - A_{\text{sample}}$ is the difference in absorbance between the sample after six minutes and the control. The antioxidant trolox is used as a standard while quercetin and epicatechin are used as sample antioxidants. Graphs relating the percent inhibition of the reaction solution to the concentration of the antioxidant are prepared. TEAC values, standardized values of antioxidant capacity, are determined by comparing the slopes of the lines from the samples to that of trolox as follows:

$$\text{TEAC value} = \frac{\text{slope}_{\text{sample}}}{\text{slope}_{\text{trolox}}}$$

**Determination of Ascorbic Acid Concentration by HPLC**

The ascorbic acid concentration in juice samples is determined by HPLC. Juice samples are mixed 1:1 with a solution of 0.35 mM tris(2-carboxyethyl)phosphine hydrochloride preservative in 5% acetic acid (TCEP solution) (7). The mixture is centrifuged and filtered. Dilutions of the filtered solutions in TCEP solution are analyzed by HPLC. An external standard curve of ascorbic acid in TCEP solution is used to quantify ascorbic acid in juice samples.

**Phenolics Quantification with Folin–Ciocalteau Reagent**

The total phenolic contents of fruit juices are determined by quantitative comparison to the visible absorbance peak of epicatechin (8). Water, fruit juice samples, and epicatechin standards of various concentrations are combined with Folin–Ciocalteau reagent and allowed to sit for seven minutes. Na$_2$CO$_3$ is added to each vessel and stirred at room temperature for 30 minutes.

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**Table 1. Module Calendar**

<table>
<thead>
<tr>
<th>Lab Period</th>
<th>Activities</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Making solutions and spectral scanning of radical ion</td>
</tr>
<tr>
<td>2</td>
<td>TEAC assay</td>
</tr>
<tr>
<td>3</td>
<td>HPLC analysis of ascorbate and total phenolics assay</td>
</tr>
<tr>
<td>4–6</td>
<td>Research design and experimentation</td>
</tr>
</tbody>
</table>
In the Laboratory

The absorbance is determined for each sample at 750 nm. A standard curve of the epicatechin absorbance values is created and used to assess the polyphenolic content of each of the fruit juice samples.

Hazards

TCEP and the Folin–Ciocalteau reagent can both cause burns. ABTS, trolox, and epicatechin are all irritating to the eyes, respiratory system, and skin. Quercetin is a suspected carcinogen. K$_2$S$_2$O$_8$ is an irritant and is combustible.

Original Research Project

In the final three laboratory periods, an experiment investigating the antioxidant capacities of foods is designed and conducted. Students carry out a literature review to investigate what research has been done. They then develop a hypothesis to investigate different factors that may affect the antioxidant capacities of foods, such as preparation, storage, and source. A food product is then chosen for analysis, and an extraction procedure for the chosen food product is designed with the aid of an extraction guide and a list of available supplies.

Students carry out the experiments they designed and analyze their data. Findings are reported in two ways. First, students submit an interactive, online form describing their experiment and results. This form provides the data directly to the researcher who authored the module. Second, students prepare a paper in the style of a journal article that they submit to their instructors.

Example Research Project

The following experiment was designed by three students carrying out the antioxidants module in a second-semester general chemistry course. Their goal was to examine the effect of cooking on the antioxidant capacity and phenolic content of cumin. The research question and hypothesis were based on scientific literature that the students reviewed themselves (9, 10). The students hypothesized that cooking would result in a degradation of polyphenolics and, subsequently, a decrease in antioxidant capacity.

The students designed and carried out their extraction procedure with guidance from their TA and the laboratory module. They performed the polyphenolics and TEAC assays on fresh and boiled cumin samples. Their results are summarized in Figures 1 and 2. The students concluded that boiling cumin in water led to an 86% reduction in the concentration of polyphenolic compounds and a 47% reduction in total antioxidant capacity of the recovered spice. Upon the module author’s examination of the data, the students’ results were surprising, as polyphenolic antioxidants are relatively heat stable compared to other common antioxidants. An alternative explanation may involve extraction of the polyphenolic compounds into the water used for boiling, instead of compound degradation.

Discussion

Because the goal of this module is authentic research, it is important that the students do not repeat the same experiments year after year. Although verification through multiple groups of students is helpful, eventually nothing new will be learned and the experiments will become trite. It is also imperative that data obtained from the students’ projects are new as the data are valuable to the author of the module. Those who implement the module should maintain contact with the module author and exchange ideas before and throughout the course implementation. The author may express interests in certain food products or in certain processes that may alter antioxidant capacity. For example, the author most recently requested information in fruit blended teas and the human digestion process. With this in mind, the course instructor can make the students aware of this interest. However, it is important that the students have the freedom to design their own experiments.

Conclusions

This six week experience provides students with an opportunity to carry out authentic research as part of their first-year chemistry laboratory course. Through practice with hypothesis development, experimental design, literature review, and writing scientific papers, general chemistry students gain valuable experience in scientific research. In many cases, such as the example described above, students’ analysis of their data leads
to further questions that could lead to further experiments if they had more time to devote to the project. This, too, is a lesson about the way that research often proceeds. Students also gain knowledge of and practice with techniques such as making solutions, spectral measurements, liquid chromatography, and the making and use of standard curves. In addition to these skill-based gains, students learn about food chemistry and the ways in which scientists investigate the presence and potency of antioxidants in foods.

Acknowledgments

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Literature Cited


Supporting JCE Online Material

Abstract and keywords
Full text (PDF)
Supplement
Instructions for the students
Notes for the instructor