JOURNAL OF CHEMICAL EDUCATION

pubs.acs.org/jchemeduc

Demonstration

From Agricultural Waste to a Powerful Antioxidant: Hydroxytyrosol as a Sustainable Model Substance for Understanding Antioxidant Capacity

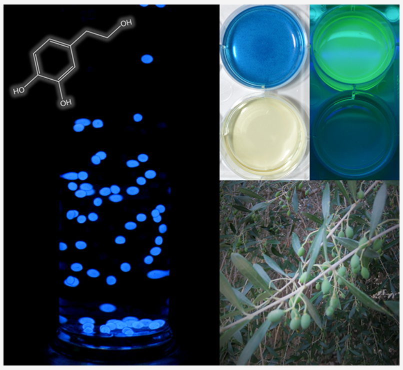
Dominik Diekemper, Benjamin Pölloth, and Stefan Schwarzer*

Cite This: *J. Chem. Educ.* 2021, 98, 2610−2617

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Students encounter antioxidants in many areas of their daily life. Antioxidants play a crucial role in cosmetics, in nutritional or dietary supplements, as additives for the preservation of food, and as a natural component in fruit and vegetables. Accordingly, antioxidants are believed to promote health through the prevention of oxidative stress by scavenging free radicals. The underlying oxidation and reduction processes of antioxidants are a central topic of chemistry classes. Additionally, antioxidants can be linked to aspects of sustainability. In this paper, the model substance chosen to demonstrate these aspects of antioxidants is hydroxytyrosol. Hydroxytyrosol is a natural, highly effective antioxidant, which is produced, for example, in olive trees and can be found in high concentrations in olive mill wastewater, which is potentially hazardous to the environment. In the first experiment, WELL-plate experiments are used to show the principal properties of antioxidants, both as reducing agents and radical scavengers, for the example of hydroxytyrosol. The experiments are based on the oxygen radical absorbance capacity (ORAC) test, which is widely applied in the food industry. In a second experiment, the properties of antioxidants are demonstrated using alginate balls as reaction vessels to produce a luminous bubble tea. Furthermore, the school-student-friendly extraction of hydroxytyrosol is presented.



KEYWORDS: *High School/Introductory Chemistry, Curriculum, Demonstrations, Laboratory Instruction, Environmental Chemistry, Green Chemistry, Phenols, Oxidation/Reduction, Nutrition*

Downloaded via HIMACHAL PRADESH UNIV on August 10, 2021 at 20:21:21 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

INTRODUCTION

Due to buzzwords such as "oxidative stress", "functional food", and "superfood", and an increasing awareness of the importance of a healthy diet, school students are confronted with the term "antioxidant" more and more in their daily lives. Trends in nutrition such as "naturally functional" and "sustainable" nutrition further underline the great importance of healthy eating. These trends can be used as a motivational context to explain the chemical background of antioxidants to students within the framework of redox processes. The subject of antioxidants has been addressed several times in different contexts of chemical education in past years, also with reference to olive oil.[1−3]

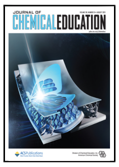
Thereby, hydroxytyrosol (4-(2-hydroxyethyl)-1,2-benzenediol), HTyr) could serve as a new, appropriate model substance to demonstrate the group of antioxidants in teaching units on the chemical properties of antioxidants in school chemistry. HTyr not only has a reference to everyday life, which can be motivating for students' learning, but also makes possible discussions of aspects of sustainability and popular scientific myths through the explicit discussion of scientific statements in advertisements.

HYDROXYTYROSOL, OLIVE OIL PRODUCTION, AND SUSTAINABILITY

HTyr is a very potent phenolic compound that can be obtained from olive trees, and it is also a byproduct of the manufacture of olive oil.[4] The typical climate of the Mediterranean basin, characterized by warm weather, dryness, and enduring sunlight irradiation, has allowed the development of endemic plants such as olive trees, which produce a high proportion of antioxidant molecules.[5] A very prominent representative of such an antioxidant is HTyr, which can be found in large quantities in the leaves and fruit of olive trees (*Olea europaea*). Worldwide, 1.8×10^6 tons of olive oil are produced every year; the majority is produced in the Mediterranean region, especially in Spain, Greece, and Italy.[6]

For extraction, only mechanical methods such as crushing and malaxation of the resulting paste are used in order to

Received: February 18, 2021
Revised: June 1, 2021
Published: July 9, 2021



© 2021 The Authors. Published by American Chemical Society and Division of Chemical Education, Inc.

https://doi.org/10.1021/acs.jchemed.1c00157
J. Chem. Educ. 2021, 98, 2610−2617

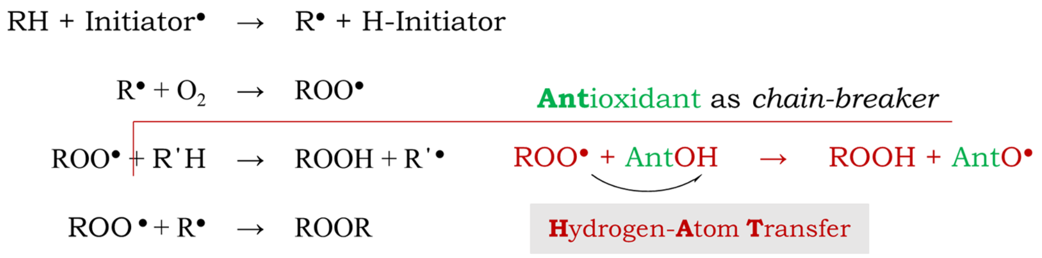


Figure 1. Antioxidants as "chain-breakers" in autoxidation processes, for example, in lipids.

maintain the natural organoleptic quality of the fruit. For the oil extraction phase, different processes are used.[7] Regardless of the process chosen, the oil extraction produces a large number of byproducts that have a high pollution potential for the environment because of the high content of phenols and polyphenols. At the end of each process, the main products remain: olive oil and olive mill wastewater (OMWW). OMWW is the main waste from the three-phase extraction processes, and the production of olive oil is estimated to yield about 10−30 × 10^6 cubic meters of OMWW per year.[7] Especially in the Mediterranean region, its highly polluting organic load and high resistance to biodegradation lead to several environmental and ecological problems.[8] The extent of the ecological problem was discussed in detail in an EU project conducted in 2009 called "Strategies to improve and protect soil quality from the disposal of olive mills' wastes in the Mediterranean region" (PROSODOL).[9] Besides organic substances such as sugar, pectin, and fats, and inorganic substances such as potassium, magnesium, calcium, and phosphorus, OMWW contains high concentrations of phenols and polyphenols. The high content of antioxidant compounds[10] makes OMWW highly resistant to biodegradation.[7] Due to the increasing costs of waste disposal and emerging ecological issues such as soil contamination or the inhibition of plants' growth, this wastewater has become a focus of current research. As outlined above, there is a growing interest in novel sources of natural antioxidants. Thus, instead of being seen as an environmental toxin, OMWW could be seen as a source of the antioxidants that are important additives in food, cosmetics, or measures taken to combat human disease. One of the main products in this context is HTyr. These outlined aspects make HTyr an excellent model substance for chemistry classes within the context of education for sustainable development (ESD).

As ESD plays an important role in the UN's Sustainable Development Goals (SDGs),[11] every educational domain and subject should contribute to ESD. However, chemistry and chemical research are particularly relevant in working toward global sustainable development, for example, by developing novel and modern materials (e.g., phosphors for energy-efficient LEDs[12]). Therefore, chemistry education plays a central role in fostering ESD.[13,14] Highlighting the role of chemistry in environmental and sustainability issues can also promote a positive perspective on chemistry learning and on chemistry itself.[15] Thus, the example of HTyr makes it possible not only to discuss the chemical concept of antioxidants but also to underline the links among geography, food technology, the environment, food manufacturing, and sustainable development. This in turn makes it possible to discuss different aspects of a real-world situation in chemistry class.[16] Further aspects of ESD can be discussed by referring to the principles of green chemistry: The use of OMWW could minimize negative environmental aspects and could prevent the production of waste in olive oil production.[17]

■ USE OF HYDROXYTYROSOL AS AN ANTIOXIDANT

A further interesting aspect of HTyr that could be readily discussed is its use as a food supplement. While the role of HTyr and antioxidants is generally described as life-prolonging in advertisements and media, the scientific base for these hypotheses is complex. On one hand, several studies have implied that HTyr has cardioprotective, anti-inflammatory, and even anticancerogenic effects.[18−20] Due to its antioxidative properties, it has been reported to protect against low-density lipoprotein (LDL) oxidation,[21] as well as to have a preventive function against type 2 diabetes.[22] However, on the other hand, recent studies did not find antioxidants to have any effects on the lifespan.[23−25] In contrast, it was even shown that too high concentration of antioxidants such as α-tocopherol or β-carotene can be harmful, while an appropriate number of free radicals was found to be beneficial for living processes.[26,27] The use of HTyr in education thus makes it possible to discuss the interconnection of scientific discourse, popular science, and economics with the example of the multibillion USD market of antioxidants as food supplements. The widely spread but scientifically controversial theory that free radicals are the main reason for aging and for many diseases could also be critically discussed, while also using easily comprehensible scientific papers in high-end scientific journals.[25]

■ CHEMISTRY OF ANTIOXIDANTS

In general, antioxidants are molecules that slow down or prevent the oxidation of another molecule.[28] Well-known and naturally occurring antioxidants are ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids, and the group of polyphenols. Other artificial antioxidants that are used in food and cosmetics are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).[29] In popular literature, but also in several scientific publications, the terms antioxidants and radical scavengers are often used synonymously. To avoid confusion, these two terms should be clearly distinguished for educational purposes. From a chemical point of view, not every oxidation process is a free-radical reaction. However, many oxidation processes, especially those under physiological conditions[27] or those with the diradical oxygen, are likely to involve free radicals. In food products, especially in fats, autoxidation is an important mechanism (Figure 1) that leads to the spoilage of food. Autoxidation reactions are subdivided into initiation, propagation, and termination reactions. Only recently, it was shown that technically important autoxidation reactions can also be induced in the absence of a radical initiator by molecule-induced radical formation pathways.[30,31]

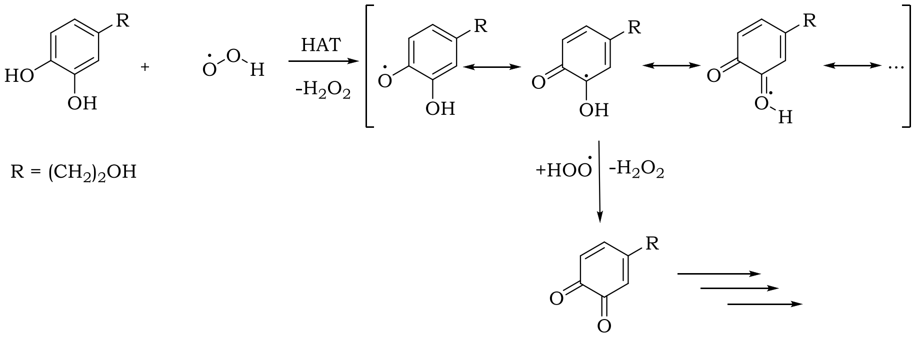


Figure 2. HAT reaction of HTyr as a free-radical scavenger with the hydroperoxyl radical.

Figure 3. Preparation of the olive solution for the experiments with the Soxhlet extractor and with simple infusion.

The most potent antioxidants are thus capable of scavenging free radicals and are oxidized themselves during this process. Olive phenols, such as HTyr, have been found to mainly scavenge radicals by hydrogen-atom-transfer (HAT).[32] In this mechanism, a hydrogen atom (proton and electron) is transferred to a free-radical species (such as superoxide-anion, hydroxyl radicals, or peroxyl radicals). Radical scavengers are thus characterized by their ability to form relatively stable radicals. The radical product of HTyr is resonance-stabilized due to conjugation (Figure 2).[28] Furthermore, a hydrogen bond between the radical electron and the ortho-standing hydroxyl group has an additional stabilizing effect.[33] Due to these effects, the reaction of radical scavengers with free radicals is energetically favorable compared to the unwanted reactions of free radicals such as, for example, autoxidation or reactions with lipids. The produced HTyr radical is thermodynamically stabilized and, thus, is unlikely to propagate further radical chains.[28] In contrast, it can recombine with another free radical to yield a metastable diketone. (Note: Recent research indicates that the reaction could also proceed by a concerted double proton-transfer electron-transfer rather than by a double HAT.[34] However, for educational processes, the HAT reaction seems to be more easily comprehensible.) In radical chain reactions, antioxidants can thus "break the chain" and end the process. It is this property that has given antioxidants the name "chain-breaking antioxidants"[35] or "antagonists of autoxidation".[36]

To quantify the antioxidative capacity and to compare antioxidants, several antioxidant capacity assays have been developed in the food industry. The ORAC value is particularly well-known for its "health claim" on foods and food supplements. The principle of the measurement is based on the reaction of the radical generator AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride) with fluorescein. The generated radicals oxidize the fluorescein, which inhibits the fluorescence of the fluorophore. This reaction is suppressed or

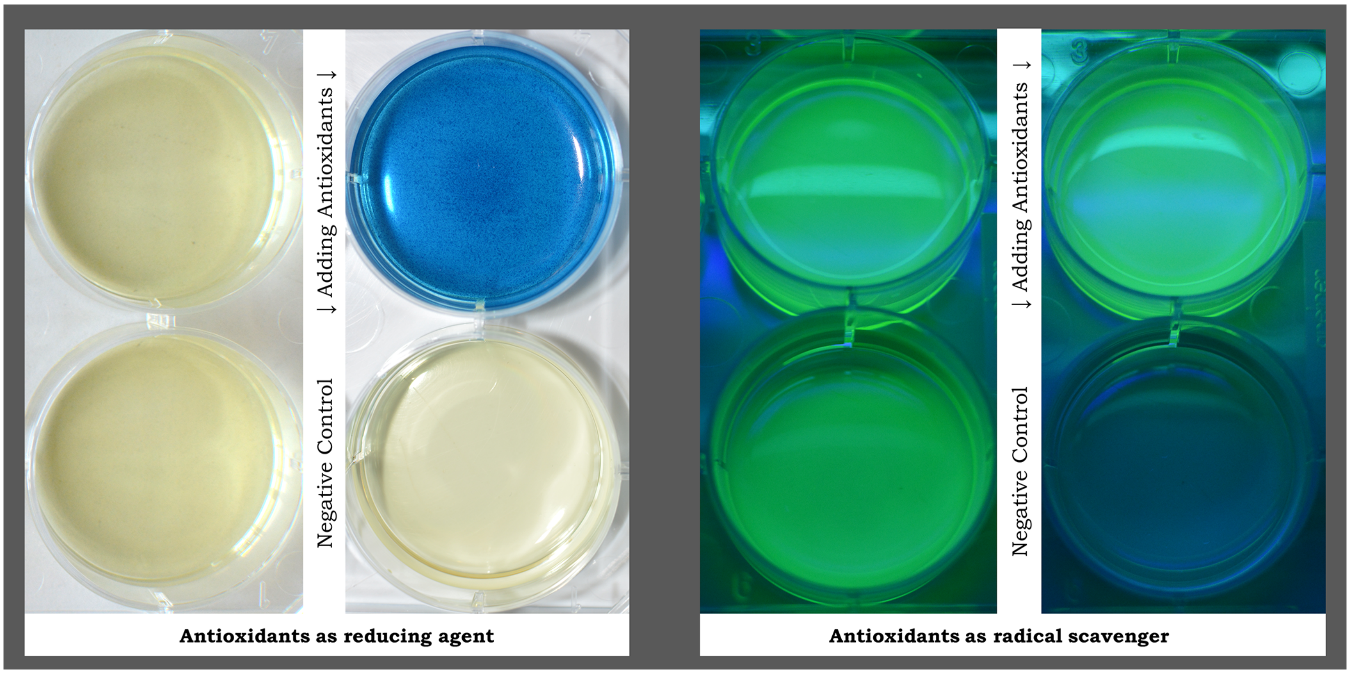


Figure 4. Experimental results of the experiment in the WELL-plate.

slowed down if an antioxidant is present. The generated radicals react first with the antioxidant and not with the fluorescein.[35] The delay of fluorescence quenching then indicates the antioxidative capacity.

Other antioxidant capacity assays, such as, for example, the TRAP (total radical-trapping antioxidant parameter),[37] TEAC (trolox equivalent antioxidant capacity),[38,39] or FRAP (ferric reducing ability of plasma)[40] assay, are based on complex and expensive analytics. The version of the ORAC assay developed in this study can be used with minimal equipment even in high schools.

■ HYDROXYTYROSOL AS A MODEL SUBSTANCE

As outlined above, HTyr can be easily used to explain the chemical background of antioxidants. The manufacturing as well as the handling of the simple and inexpensive substance is suitable for experiments for school students. The capability of HTyr to scavenge free radicals can be easily explained by its structural properties, such as its vicinal hydroxyl groups. Furthermore, the accompanying reducing properties can be readily demonstrated. The combination of these two properties, the structural simplicity of the molecule, and its use in daily life products make HTyr a suitable substance for the discussion of the chemistry of antioxidants in education. The basic principles of antioxidants can be introduced for the model substance HTyr and can subsequently be transferred to other substances such as vitamin C or vitamin E. Commercially, HTyr is currently found particularly in cosmetics and in several food supplements. However, due to the cost-intensive extraction process, chemically pure HTyr is too expensive for a school experiment. Thus, two methods are presented with which the starting substance can be very easily and inexpensively obtained for the following experiments in schools.

Soxhlet Extraction and Infusion

To produce an extract containing HTyr, 5 g of fresh olive leaves (*Olea europaea*) are shredded with scissors and placed in a Soxhlet tube. The extraction thimble is placed in the extraction space of the Soxhlet extractor. A 50 mL portion of distilled water is used as the solvent. The Soxhlet extractor is operated for about 6 h. A pale, dark green extract is generated. Alternatively, HTyr can be obtained even more simply through infusion. For this purpose, 5 g of leaves from an olive tree are shredded with scissors and put into a tea filter, and 600 mL of boiling water is poured over them. The mixture is left to cool and extract overnight (Figure 3).[41] A high-performance liquid chromatography (HPLC) analysis revealed that both extracts contained approximately 4−8 mg/L HTyr (for details and spectra, see the Supporting Information). The identity of HTyr was confirmed by comparison with a commercial reference sample and by gas chromatography with mass spectrometry (GC−MS). Most likely, the extract also contains other organic antioxidants (e.g., tyrosol). Nonetheless, control experiments with a solution containing 4 mg/L commercial HTyr confirmed that the extracted amount of HTyr alone is already sufficient as an antioxidant for a visible effect in the described experiments.

■ EXPERIMENT 1: INVESTIGATING THE PRINCIPAL PROPERTIES OF ANTIOXIDANTS

Media and food packaging make a multitude of promises concerning the effects of antioxidants. The terms antioxidant and radical scavenger are thereby often used synonymously, as described above. For this reason, in this simple experiment series, the two properties are presented using visible, simple reactions to help students to gain an understanding of the chemical abilities of antioxidants. On one hand, a general antioxidant effect is demonstrated, which is related to the reduction ability of the substances. On the other hand, their function as radical scavengers is shown with a version of the above-presented ORAC assay.

Materials

The necessary materials include the following: 6-WELL cell culture plate, UV lamp or flashlight, iron(III) chloride solution

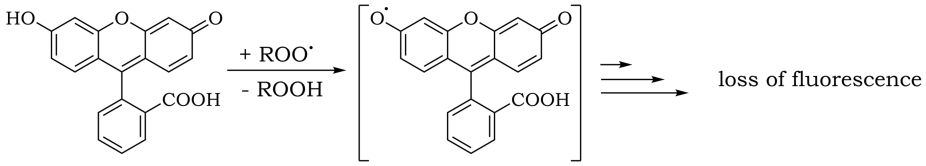


Figure 5. Fluorescein's loss of fluorescence in ORAC reaction (HAT mechanism).[35]

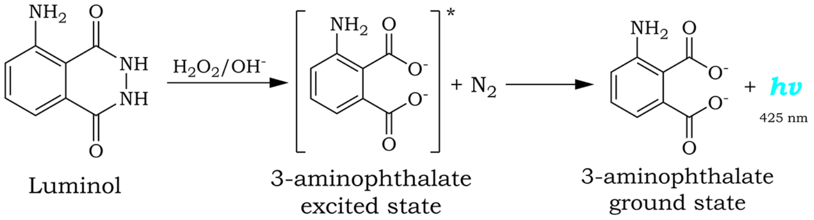


Figure 6. Luminol reaction.

(w = 0.5%), potassium ferricyanide solution (w = 0.5%), uranine solution (fluorescein disodium salt, w = 0.0015%), hydrogen peroxide (3%), and antioxidant solution.

Experimental Procedure

A 2 mL portion of the $FeCl_3$ solution and 2 mL of the $K_3[Fe(CN)]_6$ solution are placed in two chambers of the WELL-plate. A drop of the prepared antioxidant solution is added to one of the two chambers. In another pair of wells, 4 mL of uranine solution and 0.5 mL of hydrogen peroxide are mixed in each of the two wells. One drop of the antioxidant solution is added to one well. The reaction is observed under UV excitation with a UV lamp or a simple UV flashlight (Figure 4).

Results and Discussion

When the antioxidant is added to the mixture of iron solutions, a blue solid complex, $K[Fe^{II}Fe^{III}(CN)_6]$ (Prussian blue), precipitates, which is a common detection method for Fe^{2+} ions. Accordingly, some of the Fe^{3+} ions are reduced to Fe^{2+} by the polyphenols in the solution. Thus, the reducing capability of the polyphenols is tested.

The function of antioxidants as free-radical scavengers is demonstrated by quenching the fluorescence of fluorescein, as it is also done in the ORAC value test. Due to the homolytic decomposition of the hydrogen peroxide under UV radiation, two hydroxyl radicals are formed, which further form peroxide radicals by reacting with oxygen.

Fluorescein loses its ability to fluoresce due to the radical attack (see Figure 5). In the presence of an antioxidant, this mechanism is prevented, the peroxide radical is intercepted by the antioxidant (HAT mechanism, see Figure 2), and the fluorescence is retained for a certain time. Only if all the antioxidants are consumed through this mechanism fluorescein starts to decompose. A student worksheet can be found in the Supporting Information (Supporting Information).

For a further discussion, it may be important to be aware of the fact that the two properties of HTyr depend on each other: While the reduction of Fe^{3+} may include free radicals (Fenton's reaction), the radical reactions of fluorescein and HTyr are formally oxidations. Thus, the separate demonstration of both properties allows a discussion of the aspect that these two categories are neither exclusive nor identical.

EXPERIMENT 2: LUMINOUS BUBBLE TEA

Bubble tea, which became particularly popular in the 2010s, uses alginate balls as "bubbles". These alginate balls have already been employed for demonstration purposes in various contexts of chemistry education.[42] In the experiment presented here, the classic luminol reaction (see Figure 6) is combined with the protective effect of the antioxidants. It is shown that antioxidants are themselves oxidized during protection; they are consumed. Depending on the concentration of the antioxidant, another substance can be protected against oxidative influences for a different length of time. This can be clearly demonstrated using different concentrations and a different duration of the lighting reaction.

Materials

Ammonium chloride, sodium carbonate, luminol, hydrogen peroxide (w = 10%), copper sulfate solution ($c(CuSO_4)$ = 1 mol/L), sodium alginate, calcium chloride, glucose, magnetic hot plate stirrer, three 100 mL beakers, 250 mL beaker, a spatula, micro spoon, pipets, sieve, plain cylinder or test tube, Erlenmeyer flask, and antioxidant solution.

Experimental Procedure

For the preparation of the bubble tea solution, 10 drops of the copper sulfate solution are diluted with 25 mL of distilled water in a small Erlenmeyer flask. In a 100 mL beaker, 0.25 g of ammonium chloride, 0.1 g of sodium carbonate, and a spatula tip of luminol are dissolved in 25 mL of water while being stirred on the magnetic hot plate stirrer. A 1 mL portion of the prepared copper sulfate solution is added. Then, 5 g of glucose and 0.5 g of sodium alginate are added to the solution. After the solution has been stirred and has swelled for about 5 min, any lumps are removed.

For the preparation of the bubbles, 0.75 g of calcium chloride is dissolved in 50 mL of distilled water, and the bubble tea solution is dropped with a pipet. Small balls are formed in both solutions, which can be removed with a sieve. The procedure is repeated using a separate beaker with a solution of 0.75 g of calcium chloride dissolved in 50 mL of the already prepared antioxidant solution.

For the demonstration of the glowing bubbles, a plain cylinder is filled with hydrogen peroxide (10%). The balls are consecutively added to the solution with a micro spoon.

Results and Discussion

Immediately, the balls without the antioxidant begin to glow in the hydrogen peroxide solution (see Figure 7). The balls that have been treated with the antioxidant solution first sink to the bottom and only light up with a delay of several seconds. As soon as all the antioxidant is consumed, they start to glow and to rise due to the formation of N_2 in the vessel. The observed delay indicates how long antioxidants protect the luminol against oxidation, that is, until they are fully consumed themselves. Because the density of the balls was set precisely by means of the added glucose, the balls first sink to the bottom and then rise again due to the formation of N_2 in the luminol reaction. This ensures a dynamic and impressive movement in the solution (for a video, see the Supporting Information). By varying the amount of antioxidant used, different delays can be observed. This demonstrates the different antioxidative capacities.

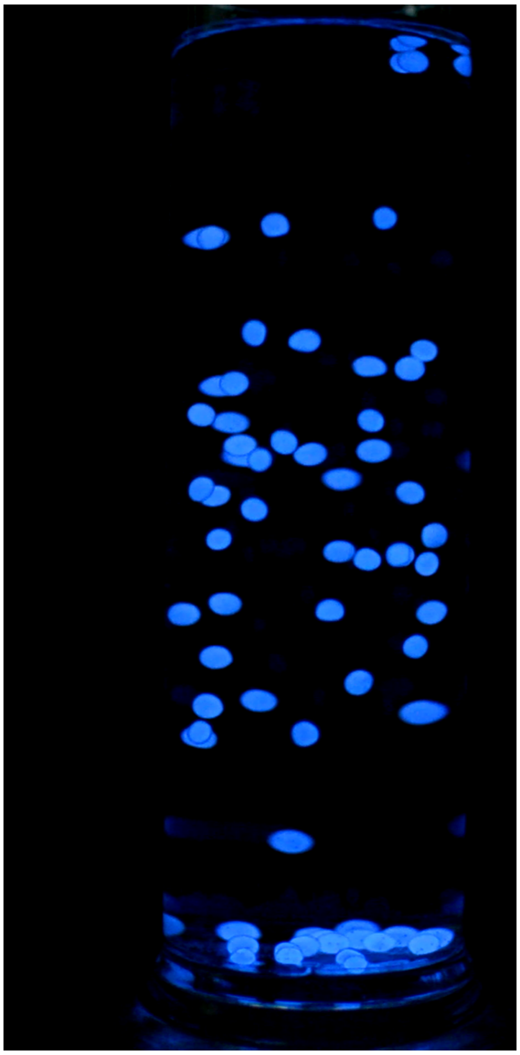


Figure 7. Luminous luminol bubbles in the dark.

HAZARDS

For safety reasons, safety goggles and a laboratory coat should be worn at all times during the experimental procedure. Contact between the chemical substances and skin must be strictly avoided.

Experiment 1

UV light excitation is harmful to the retina, so anti-UV coated goggles should be worn for safety reasons. Leftover product solutions of iron(III) should be transferred into a container designated for metal salts.

Experiment 2

Hydrogen peroxide should be considered corrosive and should not be exposed to acidic solutions that contain oxidizable solvents such as acetone. Leftover solutions of NH_4Cl, Na_2CO_3, $CaCl_2$, and $CuSO_4$ should be transferred into a container designated for metal salts. Leftover hydrogen peroxide solution should be diluted and then disposed of in a sink.

DISCUSSION

If the connection to the sustainable substance Htyr is not desired, other antioxidants can be used. However, it should be noted here that, for example, the pH value can influence the course of the reaction when ascorbic acid is used. If necessary, the pH value must be set to a neutral level with a buffer.

CONCLUSION AND OUTLOOK

In this paper, we describe two newly developed experiments that are suitable for school students. By conducting these experiments, the chemical properties of antioxidants can be discussed with the students in detail. The experiments make it possible to clearly demonstrate that antioxidants are oxidized themselves while protecting other substances. Furthermore, the experiments underline the chemical roles of antioxidants as reducing agents and radical scavengers. As the subject of antioxidants and functional food is likely to be further promoted in media and advertisements, this class of substances remains part of the everyday world of the students. Especially due to recent scientific discussions regarding the health-promoting effect of antioxidants,[25] a basic understanding of the chemical background of antioxidants, their abilities, and their limits could promote students' critical evaluation skills. The antioxidant hydroxytyrosol will continue to be the subject of specialist research due to the large amounts that are released during the production of olive oil and that are hazardous to the environment. This example illustrates the importance of chemistry education and knowledge in the evaluation of local and global systems with regard to sustainable development.[43] Thus, the combination of hydroxytyrosol with the school-relevant topic of redox processes, antioxidants, and radical scavengers demonstrates the relevance of chemical contents for a sustainable living environment for the students.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00157.

Spectroscopy details and student worksheet (PDF, DOCX)

Video of experiment 2 (MP4)

AUTHOR INFORMATION

Corresponding Author

Stefan Schwarzer – *Department of Chemistry Education, Eberhard Karls Universität Tübingen, 72076 Tübingen,*

Germany; orcid.org/0000-0002-4134-5684;
Email: Stefan.schwarzer@uni-tuebingen.de

Authors

Dominik Diekemper – *Department of Chemistry Education, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany;* orcid.org/0000-0003-0916-475X
Benjamin Pölloth – *Department of Chemistry Education, Eberhard Karls Universität Tübingen, 72076 Tübingen, Germany;* orcid.org/0000-0001-7777-9903

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jchemed.1c00157

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors wish to thank all the participating students who carried out the experiment during the developmental process for their precious feedback, Andreas Steiger who dealt with this topic in his thesis, student research assistant Lena Baumgartner, and chemical-technical assistant Gudrun Baltjan.

■ REFERENCES

(1) Donnelly, T. H. The Origins of the Use of Antioxidants in Foods. *J. Chem. Educ.* **1996**, *73* (2), 158.

(2) Coleman, W. F. Molecular Models of Antioxidants and Radicals. *J. Chem. Educ.* **2008**, *85* (3), 464.

(3) Blatchly, R. A.; Delen, Z.; O'Hara, P. B. Making Sense of Olive Oil: Simple Experiments to Connect Sensory Observations with the Underlying Chemistry. *J. Chem. Educ.* **2014**, *91* (10), 1623–1630.

(4) Martínez, L.; Ros, G.; Nieto, G. Hydroxytyrosol: Health Benefits and Use as Functional Ingredient in Meat. *Medicines* **2018**, *5* (1), 13.

(5) Visioli, F.; Bellomo, G.; Galli, C. Free Radical-Scavenging Properties of Olive Oil Polyphenols. *Biochem. Biophys. Res. Commun.* **1998**, *247* (1), 60–64.

(6) Haddad, K.; Jeguirim, M.; Jerbi, B.; Chouchene, A.; Dutournié, P.; Thevenin, N.; Ruidavets, L.; Jellali, S.; Limousy, L. Olive Mill Wastewater: From a Pollutant to Green Fuels, Agricultural Water Source and Biofertilizer. *ACS Sustainable Chem. Eng.* **2017**, *5* (10), 8988–8996.

(7) *Olive Mill Waste: Recent Advances for the Sustainable Management*; Galanakis, C. M., Ed.; Academic Press, 2017.

(8) Bernini, R.; Merendino, N.; Romani, A.; Velotti, F. Naturally Occurring Hydroxytyrosol: Synthesis and Anticancer Potential. *Curr. Med. Chem.* **2013**, *20* (5), 655–670.

(9) PROSODOL—Strategies to Improve and Protect Soil Quality from the Disposal of Olive Mills' Wastes in the Mediterranean Region. https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=3297&docType=pdf (accessed 2021-04-30).

(10) Visioli, F.; Romani, A.; Mulinacci, N.; Zarini, S.; Conte, D.; Vincieri, F. F.; Galli, C. Antioxidant and Other Biological Activities of Olive Mill Waste Waters. *J. Agric. Food Chem.* **1999**, *47* (8), 3397–3401.

(11) United Nations. 17 Goals. https://sdgs.un.org/goals (accessed 2021-04-30).

(12) Diekemper, D.; Schnick, W.; Schwarzer, S. Microwave Synthesis of a Prominent LED Phosphor for School Students: Chemistry's Contribution to Sustainable Lighting. *J. Chem. Educ.* **2019**, *96* (12), 3018–3024.

(13) Burmeister, M.; Eilks, I. An example of learning about plastics and their evaluation as a contribution to Education for Sustainable Development in secondary school chemistry teaching. *Chem. Educ. Res. Pract.* **2012**, *13* (2), 93–102.

(14) Burmeister, M.; Schmidt-Jacob, S.; Eilks, I. German chemistry teachers' understanding of sustainability and education for sustainable development—An interview case study. *Chem. Educ. Res. Pract.* **2013**, *14* (2), 169–176.

(15) Garner, N.; Siol, A.; Huwer, J.; Rolf Hempelmann, R. H.; Eilks, I. Implementing innovations in chemistry learning and sustainability education in a non-formal student laboratory context. *LUMAT* **2015**, *3* (4), 449–461.

(16) Iyere, P. A. Chemistry in Sustainable Development and Global Environment. *J. Chem. Educ.* **2008**, *85* (12), 1604.

(17) Zuin, V. G.; Eilks, I.; Elschami, M.; Kümmerer, K. Education in green chemistry and in sustainable chemistry: perspectives towards sustainability. *Green Chem.* **2021**, *23* (4), 1594–1608.

(18) D'Angelo, S.; Ingrosso, D.; Migliardi, V.; Sorrentino, A.; Donnarumma, G.; Baroni, A.; Masella, L.; Antonietta Tufano, M.; Zappia, M.; Galletti, P. Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. *Free Radical Biol. Med.* **2005**, *38* (7), 908–919.

(19) González Arbeláez, L. F.; Ciocci Pardo, A.; Fantinelli, J. C.; Schinella, G. R.; Mosca, S. M.; Ríos, J.-L. Cardioprotection and natural polyphenols: an update of clinical and experimental studies. *Food Funct.* **2018**, *9* (12), 6129–6145.

(20) Echeverría, F.; Ortiz, M.; Valenzuela, R.; Videla, L. A. Hydroxytyrosol and Cytoprotection: A Projection for Clinical Interventions. *Int. J. Mol. Sci.* **2017**, *18* (5), 930.

(21) Rietjens, S. J.; Bast, A.; Haenen, G. R. M. M. New Insights into Controversies of the Antioxidant Potential of the Olive Oil Antioxidant Hydroxytyrosol. *J. Agric. Food Chem.* **2007**, *55* (18), 7609–7614.

(22) Umeno, A.; Horie, M.; Murotomi, K.; Nakajima, Y.; Yoshida, Y. Antioxidative and Antidiabetic Effects of Natural Polyphenols and Isoflavones. *Molecules* **2016**, *21* (6), 708.

(23) Keaney, M.; Gems, D. No Increase in Lifespan in Caenorhabditis Elegans Upon Treatment with the Superoxide Dismutase Mimetic EUK-8. *Free Radical Biol. Med.* **2003**, *34* (2), 277–282.

(24) Bjelakovic, G.; Nikolova, D.; Gluud, C. Antioxidant supplements to prevent mortality. *JAMA* **2013**, *310* (11), 1178–1179.

(25) Scudellari, M. The science myths that will not die. *Nature* **2015**, *528* (7582), 322–325.

(26) Doonan, R.; McElwee, J. J.; Matthijssens, F.; Walker, G. A.; Houthoofd, K.; Back, P.; Matscheski, A.; Vanfleteren, J. R.; Gems, D. Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in Caenorhabditis elegans. *Genes Dev.* **2008**, *22* (23), 3236–3241.

(27) Azzi, A. Antioxidants: Wonder drugs or quackery? *Biofactors* **2017**, *43* (6), 785–788.

(28) Craft, B. D.; Kerrihard, A. L.; Amarowicz, R.; Pegg, R. B. Phenol-Based Antioxidants and the In Vitro Methods Used for Their Assessment. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11* (2), 148–173.

(29) Vaclavik, V. A.; Christian, E. W. Food Additives. In *Essentials of Food Science*; Springer: New York, 2008; pp 447–469. DOI: 10.1007/978-0-387-69940-0_18.

(30) Sandhiya, L.; Jangra, H.; Zipse, H. Molecule-Induced Radical Formation (MIRF) Reactions-A Reappraisal. *Angew. Chem., Int. Ed.* **2020**, *59* (16), 6318–6329.

(31) Sandhiya, L.; Zipse, H. Radical-Pair Formation in Hydrocarbon (Aut)Oxidation. *Chem. - Eur. J.* **2019**, *25* (36), 8604–8611.

(32) Galano, A.; Alvarez-Idaboy, J. R.; Francisco-Márquez, M.; Medina, M. E. A quantum chemical study on the free radical scavenging activity of tyrosol and hydroxytyrosol. *Theor. Chem. Acc.* **2012**, *131* (3), 1173.

(33) Wright, J. S.; Johnson, E. R.; DiLabio, G. A. Predicting the Activity of Phenolic Antioxidants: Theoretical Method, Analysis of Substituent Effects, and Application to Major Families of Antioxidants. *J. Am. Chem. Soc.* **2001**, *123* (6), 1173–1183.

(34) Quintero-Saumeth, J.; Rincón, D. A.; Doerr, M.; Daza, M. C. Concerted double proton-transfer electron-transfer between catechol

and superoxide radical anion. *Phys. Chem. Chem. Phys.* **2017**, *19* (38), 26179−26190.

(35) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *J. Agric. Food Chem.* **2001**, *49* (10), 4619−4626.

(36) Simic, M. G. Free radical mechanisms in autoxidation processes. *J. Chem. Educ.* **1981**, *58* (2), 125.

(37) Wayner, D. D. M.; Burton, G. W.; Ingold, K. U.; Locke, S. Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. *FEBS Lett.* **1985**, *187* (1), 33−37.

(38) Miller, N. J.; Rice-Evans, C.; Davies, M. J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, *84* (4), 407−412.

(39) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26* (9−10), 1231−1237.

(40) Benzie, I. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239* (1), 70.

(41) Steiger, A. *Development of New Experiments on the Modern Material Hydroxytyrosol for Science Lab for School Students*. Degree Thesis, Ludwig-Maximilians-University, Munich.

(42) Ducci, M. Redox Reactions in Sodium Alginate Beads. *WJCE* **2019**, *7* (2), 40−44.

(43) Matlin, S. A.; Mehta, G.; Hopf, H.; Krief, A. One-World Chemistry and Systems Thinking. *Nat. Chem.* **2016**, *8*, 393−398.