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Touring the Tomato: A Suite of Chemistry Laboratory Experiments

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Abstract

An eight-session interdisciplinary laboratory curriculum has been designed using a suite of analytical chemistry techniques to study biomaterials derived from an inexpensive source such as the tomato fruit. A logical progression of research-inspired laboratory modules serves to “tour” the macroscopic characteristics of the fruit and the submicroscopic properties of its constituent cuticular biopolymers by atomic force microscopy (AFM), UV–visible, and nuclear magnetic resonance (NMR) methods at increasingly detailed molecular levels. The modular curriculum can be tailored for specialty undergraduate courses or summer high school workshops. By applying analytical tools to investigate biopolymers, making connections between molecular and microscale structure, and linking both structural regimes to the functional properties of natural polymers, groundwork is established for further student investigations at the interface of chemistry with biology or chemical engineering.

Keywords

High School/Introductory Chemistry; Upper-Division Undergraduate; Curriculum; Laboratory Instruction; Hands-On Learning/Manipulatives; Biophysical Chemistry; Bioanalytical Chemistry; NMR Spectroscopy; UV-Vis Spectroscopy; HPLC; Pedagogy

The tomato is a familiar ingredient of our everyday meals, yet its significance extends beyond nutrition to both agriculture and bioengineering. Chemically, a tomato fruit contains water, lipid waxes, lycopene pigments, and distinctive polysaccharide and polyester biopolymers. Starting from a simple analysis of the mass percent of water, a detailed molecular picture of these constituents and their interactions can be developed through the use of complementary analytical tools. This article describes a readily adaptable modular laboratory course based on the application of a panel of experimental techniques including UV–vis spectroscopy, high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy, and atomic force microscopy (AFM) to examine various chemical constituents of the tomato fruit. Drawing on previously published instructional exercises related to tomato juice and the lycopene pigment^{1–3} and research investigations of the structural properties of the tomato fruit cuticles,^{4–6} we have designed a comprehensive set of laboratory modules that may be tailored to specialty courses for undergraduates or

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R.E.S. and N.M. designed the laboratory modules that were developed for student use, tested in the laboratory, and subjected to student feedback by S.S. and S.C. S.S., S.C., and R.E.S. co-wrote the article.

ASSOCIATED CONTENT

Supporting Information Instructions for the students; guidance for the instructor. This material may be available free of charge via the Internet at <http://pubs.acs.org>

adapted for campus-based summer programs that introduce high school students to scientific research. Our design is also suitable for the determination of composition and structure in biomaterials from other vegetables and fruits (e.g., grapefruit, carrot, beet) or for engineered polymers (e.g., textiles, plastics).⁷

Rationale

The eight-session laboratory curriculum presented in Table 1 applies a battery of analytical techniques to an inexpensive but versatile class of biomaterials. The use of analytical tools to investigate biopolymers derived from a fruit can build the foundation for training at the interface of chemistry with biology or chemical engineering, making essential connections between molecular and microscale structure and linking both structural regimes to the regulatory functions of natural and designed polymer coverings. The first five experiments comprise a logical series that focuses on the macroscopic properties of the fruit. Following these five modules, submicroscopic studies of fruit cuticular biopolymers by NMR and AFM constitute a molecular-level “tour” of the tomato. Finally, the curriculum provides opportunities for student-designed mini-projects that extend the measurements in time, tweak the experimental protocols, or apply the experiments to other fruit biopolymers or textile materials.

Notably, modules 6–8 can be presented as demonstrations if the requisite laboratory infrastructure is unavailable or the students have insufficient background in spectroscopy. For students at undergraduate institutions, the complete laboratory course can be laid out in eight sessions with an approximate duration of 2–3 hours each. NMR and AFM modules can also be assigned as a group project or activity. The order of the modules can be adjusted depending on both logistical considerations and the learning objectives of different disciplinary curricula. For instance, if a solid-state NMR facility is not available, (Fourier transform) infrared spectroscopy (FT-IR) could be used to characterize the functional groups of dewaxed tomato cuticle (see the Supporting Information).

Equipment

To conduct this series of biophysical and analytical experiments, a chemistry laboratory should be equipped with an analytical balance, drying oven, incubator–shaker, UV–vis spectrophotometer, and HPLC. An NMR spectrometer and an atomic force microscope are also required for modules 6–8 (details included in the Supporting Information).

Experiments

The overall scheme of the experimental modules is presented in Figure 1. The laboratory course begins with a macroscopic investigation of the tomato, and then delves into the constituents of the fruit with increasing spatial detail.

Estimation of the percent water in the fruit serves to compare the total relative quantities of liquid and solid components (biopolymeric materials), revealing that water is the major component by mass. This experiment also demonstrates the effect of water loss on the overall morphology of the fruit, a significant factor in maintaining the freshness and marketability of edible produce. Next, students focus on the fruit cuticle, a hydrophobic protective covering of the tomato fruit that acts as a strong defensive barrier to biotic and abiotic stresses and regulates water loss. The cutin biopolyester, lycopene pigment, and waxes form the cuticle’s primary structural framework, which is supported by complex polysaccharides (cellulose and pectin) present in the plant cell wall.⁸ To remove cell-wall materials from the cuticle, tomato skins peeled from a fresh fruit are treated with an aqueous mixture of cellulase and pectinase enzymes to remove associated cellulose and pectin.⁸ The

thickness of the native cuticle is measured with calipers, testing for reproducibility of the measurement and variation among nominally identical cuticular samples. The lycopene pigment extracted from enzymatically treated cuticle is characterized by UV–vis absorption spectroscopy or HPLC. Additionally, solvent extraction (dewaxing) separates the wax constituents, allowing students to compare the membrane thickness of dewaxed cuticles (cutin) and permitting molecular characterization of the wax mixture by solution-state NMR.⁹ Analogously, the microscale surface topology and molecular composition of fruit cuticles are investigated by AFM^{6,10} and solid-state ¹³C-NMR,^{8,11} respectively. A detailed description of the experimental procedures is found in the Supporting Information.

Hazards

Chloroform and methanol, though used in modest volumes for the lycopene characterization and dewaxing procedure, are highly flammable, toxic, and irritating. Acetonitrile exhibits modest toxicity in small doses, but neither poisoning in humans by inhalation nor skin absorption is clearly documented. Students should work in a fume hood, taking care to avoid inhalation and exposure to the skin. Lycopene should be kept in a dark container and protected from light; it is oxidized readily owing to multiple double bonds. Gloves and goggles should be used while handling toxic sodium azide added to the enzyme cocktail solution. People with medical implants must not enter within the fringe field of the superconducting NMR magnet. Spinning NMR rotors may act as projectiles, so wear safety glasses at all times.

Representative Results

The suite of experiments was piloted as a summer course for college-bound high school students, who had completed a year each of biology and chemistry instruction, at The College of Staten Island (CSI) and at The City College of New York (CCNY). Typically, a group of 12 students worked in pairs during three, 3-hour sessions per week for four consecutive weeks.

Module 1

Four groups of high school students measured water percent in tomatoes. Their acquired data (Supplemental Table S1) showed that tomato fruit has a substantially higher content of water (above 90%) than biopolymers on a mass-per-mass basis. The rate of water loss was also measured: about 75% of the water was lost in 3 hours for a piece of halved cherry tomato, whereas only 55% was lost for whole fruit during the same time. These observations may be developed into mini-projects that compare the rate of water loss as a function of exposed surface area or between different types of fruits.

Modules 2 and 3

After enzymatic removal of cell walls, students used calipers to measure the thickness of the natural waxy and dewaxed cuticles, respectively. With few exceptions, they found that the waxy cuticle is thicker compared to the dewaxed sample (cutin), implying that the wax constituent contributes to the thickness of the tomato membrane (Supplemental Figure S1).

Module 4

The lycopene pigment responsible for the red color of the fruit^{1,12,13} showed a characteristic UV–vis absorption band centered at 471 nm that was compared with authentic lycopene (Supplemental Figure S2). A comparative analysis of HPLC retention times between the extracted pigment and commercial lycopene was also used to verify the chemical identity of the extracted pigment (Supplemental Figure S5).

Module 5

The fruit cuticles were dewaxed exhaustively by solvent extraction⁸ to obtain tomato cutins, which retained a similar macroscopic appearance (Figure 1) but exhibited changes in microscale surface structure and molecular composition revealed in Modules 7 and 8.

Module 6

A structural fingerprint of the waxes obtained by organic solvent extraction was deduced by solution-state NMR, using either a one-dimensional ^1H spectrum to tentatively identify the functional groups or a two-dimensional (^{13}C heteronuclear multiple quantum coherence, HMQC) experiment⁹ (Figure 1 and Supplemental Figure S4) to yield a chemical shift correlation map between directly bonded ^1H and ^{13}C nuclei that fingerprinted the alkane, alkanol, alkene, alkanolic acid, sterol, and triterpenoid molecular groupings more definitively.

Module 7

Surface features of the native and dewaxed cuticles were examined by AFM⁸ (Figure 1 and Supplemental Figure S5), demonstrating changes in topography and surface roughness.

Module 8

After removing waxes from the tomato cuticle by solvent extraction, ^{13}C cross-polarization magic-angle-spinning (CPMAS) solid-state NMR^{6,8} of the insoluble cutin biopolymer was used in conjunction with sodium acetate and “plastic bag” standards to identify the major functional groups of the hydroxyfatty acid building blocks (Figure 1 and Supplemental Figure S6); an FT-IR alternative that provides similar chemical information is outlined in the Supporting Information. At CCNY, each student team designed and made a PowerPoint presentation of a mini-research project to follow up on questions raised by the laboratory exercises or in their background readings; in an alternative implementation at CSI, students made measurements of, for example, variations in water content or cuticle thickness for different fruits and vegetables.

Discussion

The laboratory modules described herein offer students a multifaceted research-inspired “tour” of the tomato. Starting with basic measurements of the mass percent of water, cuticle thickness, and pigment properties, the program proceeds in the fashion of Disneyland’s “Adventure Thru Inner Space”¹⁴ to examine the underlying structure of the cuticle: the overall molecular composition of its wax and cutin constituents (by NMR) and the microscale surface topology (by AFM). The flexible design of the eight-session suite allows the instructor to tailor choices to high school or college students, emphasizing disciplines including nutrition, food processing chemistry, or chemical engineering. If solid-state NMR facilities are not available, functional groups in the solid cuticles may be characterized by FT-IR.^{15,16}

In addition, these interdisciplinary laboratory exercises provide a starting point for student-designed mini-projects that compare water content in common fruits or vegetables, monitor water loss through fruit cuticles or engineered polymer films, and compare the surface microstructure of natural and commercial waterproofing materials. Thus beyond sparking interest in biological materials, this curriculum has the potential to advance broader educational goals related to critical thinking and creative innovation in the physical sciences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overall scheme for the experimental modules, showing physical and chemical procedures that permit macroscopic, microscale, and molecular characterization of tomato fruit constituents. Representative results (indicated by dark red dashed arrows) include a UV-vis absorption spectrum of the extracted lycopene pigment, a two-dimension ^1H - ^{13}C solution-state correlation NMR spectrum of waxes and pigments, and both a solid-state CPMAS ^{13}C NMR spectrum and an AFM image of dewaxed tomato cuticle (cutin). Detailed procedures appear as Supporting Information.

Table 1

Summary of Laboratory Modules

Module	Experiment ^a	Discipline(s) ^b
1	Mass percent of water in tomato	G, A
2	Enzymatic removal of cell walls from tomato cuticle	BC
3	Thickness of tomato cuticle	G, A, BP
4	Lycopene characterization by UV-vis absorption spectroscopy and HPLC	A, BC
5	Dewaxing of fruit cuticles by solvent extraction	A, O
6	<i>Structural fingerprint of cuticular waxes by solution-state NMR</i>	<i>BC, O</i>
7	<i>Surface analysis of tomato cuticles by AFM</i>	<i>A, BP</i>
8	<i>Molecular profile of dewaxed cuticles (cutin) by solid-state NMR</i>	<i>BP</i>

^aOptional modules are noted in italics.

^bDisciplines are general (G), analytical (A), biophysics (BP), biochemistry (BC), 70 and organic (O) chemistry. The duration of each experiment is 2–3 hours.