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Laboratory Experiment

Establishing the Effect of Solvent Polarity on Carotenoid Extraction: A Small-Scale Solid–Liquid Extraction and Alkene Identification Experiment for Senior High School Students

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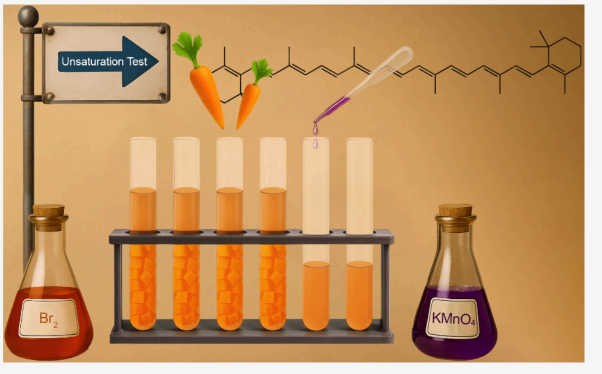
ABSTRACT: We present a small-scale, inquiry-based experiment for senior high school students that explores the effect of solvent polarity on the extraction of carotenoids and the identification of unsaturation through qualitative chemical tests. Using 3 g of diced carrot and three solvents—heptane, ethanol, or water—students conducted solid–liquid extractions, followed by bromine water and potassium permanganate testing. These visual tests detect the presence of unsaturated carotenoids based on color changes associated with electrophilic addition or oxidation reactions. Heptane, a nonpolar solvent, proved most effective in extracting carotenoids, while ethanol showed moderate effectiveness and water was ineffective due to its high polarity. This visually engaging and pedagogically robust experiment reinforces key organic chemistry concepts such as solubility, molecular polarity, and functional group reactivity within a 2 h lab session designed for classroom implementation.



KEYWORDS: *High School/Introductory Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Addition Reactions, Alkenes, Plant Chemistry*

INTRODUCTION

Carotenoids are natural pigments responsible for the orange, yellow, and red hues found in many fruits and vegetables, including carrots, pumpkins, and sweet potatoes. Structurally, they are tetraterpenoids composed of 40 carbon atoms arranged in a long polyene chain with nine conjugated double bonds and terminal functional groups. This extended conjugation enables carotenoids to absorb visible light, giving them their distinctive colors. Among them, *β*-carotene (Figure 1) is the most well-known due to its role as a precursor to vitamin A, an essential nutrient for vision, immune function, and skin health. In addition to its nutritional value, *β*-carotene also serves as a potent antioxidant, protecting cells from oxidative damage caused by free radicals—agents associated with cancer, cardiovascular diseases, and other degenerative conditions.

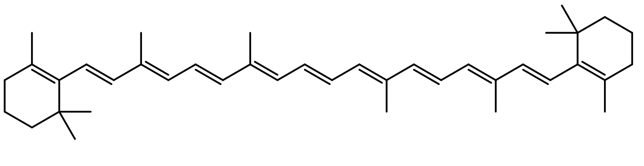


Figure 1. Representative structure of *β*-carotene, a common carotenoid.

Laboratory experiments that explore the relationship between solvent polarity and solubility provide students with a valuable foundation for understanding key principles in organic chemistry. At the university level, such experiments often involve multistep syntheses, chromatographic separations, or spectroscopic analyses—activities that are pedagogically robust but typically require extended time and specialized instrumentation. While highly effective in advanced settings, these methods are often impractical for high school classrooms or outreach programs due to time constraints and limited resources.

To bridge this gap, we developed a simplified, inquiry-based carotenoid extraction experiment[1−6] specifically designed for senior high school students. The activity introduces core concepts such as solvent–solute interactions, functional group identification,[7−12] and solid–liquid extraction[13−22] using familiar materials within a 2 h lab format. Our approach builds on a growing body of literature demonstrating the effectiveness

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of simplified pigment extraction experiments in educational settings. For example, spinach-based experiments[23,24] have been successfully used to isolate and analyze both chlorophylls and carotenoids while reinforcing key ideas in solubility and spectroscopic behavior.

Building on this framework, our carotenoid extraction experiment emphasizes practical skills and conceptual understanding using a minimal set of accessible reagents and equipment. By replacing complex instrumentation with colorimetric chemical tests and leveraging observable changes to assess extraction efficiency, the activity provides an engaging, hands-on learning experience aligned with precollege learning objectives.

Solid−liquid extraction is a widely used technique in both academic and industrial settings for isolating target compounds from plant material using an appropriate solvent.[13,18] Since carotenoids are lipid-soluble, their extraction efficiency depends heavily on solvent polarity.[16] Nonpolar solvents like *n*-hexane, heptane, petroleum ether, and cyclohexane are generally preferred,[17] although moderately polar solvents such as acetone and ethanol can also extract carotenoids along with other solutes.[19] In contrast, water, being highly polar, is expected to be ineffective.[16]

In this experiment, students extract carotenoids from diced carrot samples using three different solvents—heptane, ethanol, and water (Figure 2)—and then evaluate the extracts using two qualitative tests for unsaturation: bromine water and potassium permanganate. The observed reactions involve the fading of bromine and potassium permanganate colors when carotenoids are present. The pathway shown in parentheses (labeled "not observed") represents the basic form of the Baeyer test and is included for reference only (Figure 3). The bromine water test involves decolorization of a reddish-brown bromine solution via electrophilic addition to double bonds.[4,25,26] The potassium permanganate test (Baeyer's Test)[27−30] involves oxidation of double bonds, resulting in decolorization and formation of a brown MnO_2 precipitate. These observable changes offer clear confirmation of unsaturation and reinforce theoretical concepts through direct visual evidence.

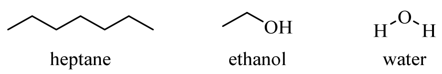

Figure 2. Molecular structures of heptane, ethanol, and water.

Designed as a collaborative, hands-on activity, this experiment encourages students to compare the effectiveness of different solvents, share and analyze group data, and draw conclusions about the role of solvent polarity in organic extractions. The simplicity of the procedure—requiring only 3 g of diced carrots and readily available solvents—makes it highly suitable for classrooms with limited equipment. Furthermore, the incorporation of basic chemical tests deepens students' understanding of molecular structure and reactivity.

Developed by university lecturers and adapted for precollege learners, this experiment promotes critical thinking, teamwork, and scientific literacy, while providing a solid foundation for further study in organic and analytical chemistry.

■ LEARNING OBJECTIVES AND ASSESSMENT

The primary goal of this experiment was to introduce high school students to the role of solvent polarity in organic extractions and the qualitative analysis of chemical reactions. Students were first asked to observe the extracts immediately after heating and note any visible differences in color intensity among the three solvents. This initial observation provided insight into the relative efficiency of each solvent for carotenoid extraction. Student analysis then proceeded on two levels: (1) assessing the presence and intensity of the orange color in the extracts and (2) evaluating the reactivity of the extracts with bromine water and potassium permanganate as evidence of unsaturation. Together, these observations allowed students to connect solvent polarity with both extraction efficiency and qualitative functional group testing.

Students were divided into groups, with each group performing extractions using the three solvents. They then analyzed their extracts through qualitative chemical tests, recording observations on color changes. Each group photographed their test results and compiled their data with findings from other groups to draw conclusions about solvent effectiveness and the importance of solubility principles in organic chemistry. This collaborative approach allowed students to experience the benefits of data sharing and systematic experimentation while working within the constraints of a 2 h lab session.

Through this experiment, students achieved the following learning objectives:

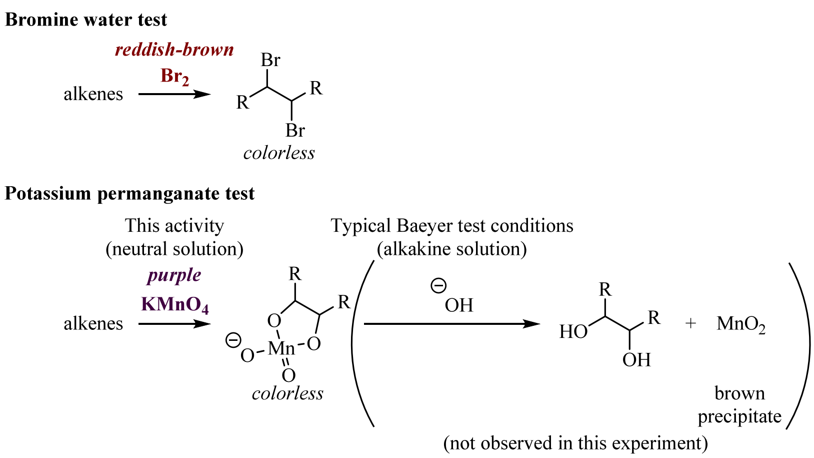

Figure 3. Reactions used in unsaturation tests.

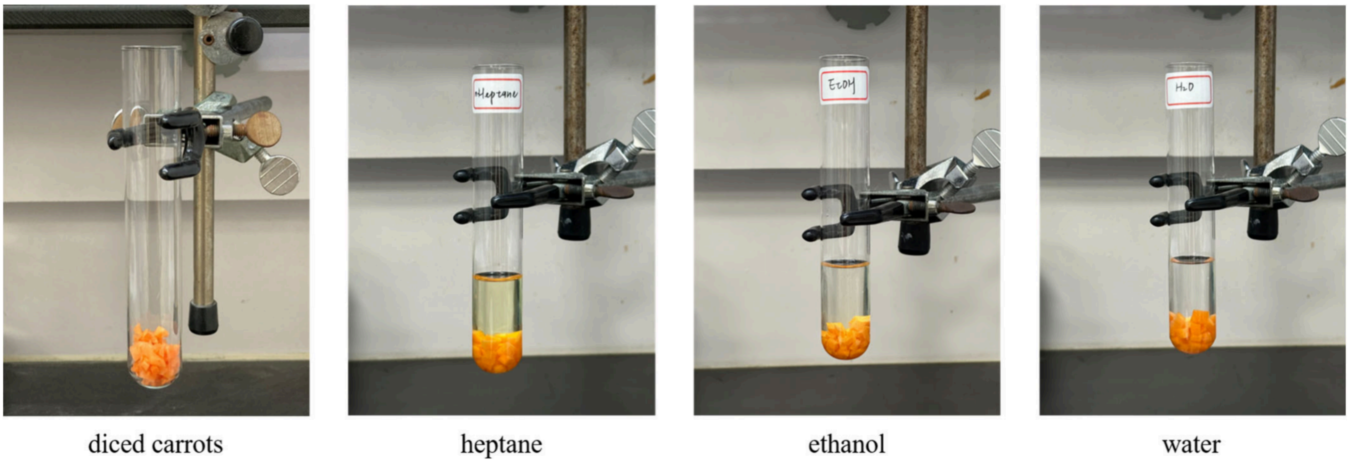

Figure 4. Simple solid–liquid extraction. Mixtures of carrot and solvent.

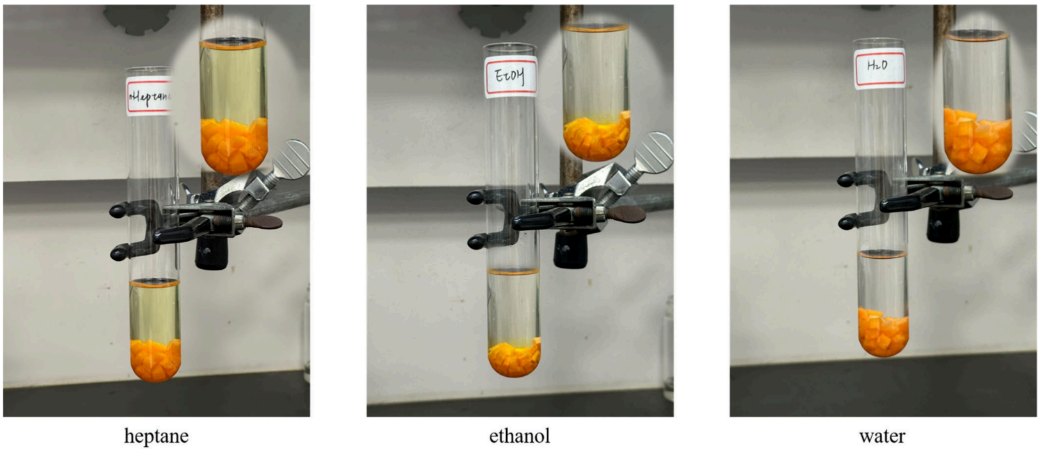

Figure 5. Simple solid–liquid extraction, after heating (1 min).

- Successfully extracted carotenoids from carrots using three different solvents (heptane, ethanol, and water).
- Observed and interpreted color changes in the bromine water and potassium permanganate tests to identify the presence of unsaturated carotenoids.
- Evaluated the impact of solvent polarity on carotenoid extraction efficiency.
- Applied the principle of “like dissolves like”, recognizing why lipid-soluble compounds dissolve best in nonpolar solvents.
- Collaborated with peers by comparing extraction results across groups to formulate well-supported conclusions.

Assessment of Student Performance

Student outcomes for this experiment were assessed through postlab reports and in-lab observations, where students demonstrated their ability to

- accurately perform the carotenoid extraction procedure using three solvents and record detailed experimental observations;
- identify the most effective solvent for carotenoid extraction and explain their reasoning based on experimental results;
- analyze the chemical test reactions and justify their conclusions from the bromine water and potassium permanganate tests;
- compare their results with those of other student groups to collaboratively determine the best solvent for carotenoid extraction and the role of solubility in the extraction process.

Through this structured experiment and assessment, students developed a foundational understanding of solubility principles, organic extraction techniques, and the importance of selecting appropriate solvents for isolating lipid-soluble compounds.

■ EXPERIMENT OVERVIEW

Small-Scale Solid–Liquid Extraction of Carotenoids

Students performed a solid–liquid extraction (Figures 4 and 5) by submerging 3 g of diced carrot cubes (0.5×0.5 cm^2) in 15 mL of solvent (heptane, ethanol, or water) in a 2.5×14.5 cm^2 test tube. The samples were then heated in a 50 °C water bath for 1 min.

After heating, the test tubes were allowed to cool, and the carotenoid-rich solutions were carefully separated from the solid carrot residue using a pipet. The extracts were then tested with bromine water and potassium permanganate to detect the presence of unsaturated compounds.

Detection of Carotenoids via Bromine and Potassium Permanganate Testing

Each group analyzed their assigned carotenoid extract by performing qualitative chemical tests to detect unsaturated

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compounds in their samples. The experimental design included separate bromine water and potassium permanganate tests for each solvent extract.

Bromine Water Test for Unsaturated Compounds (Figures 6–9): Students transferred 2 mL of their extract into a clean test

Figure 6. Appearance of bromine water (2%).

tube (1 × 10 cm^2) and added 4 drops of bromine solution (Br_2, 2% in water). Since Br_2 is an aqueous solution (2%), a key procedural variation was implemented: only the heptane extracts were mixed with 1 mL of ethanol prior to Br_2 addition. This step was necessary because heptane is nonpolar and immiscible with water, making it difficult for the aqueous Br_2 to effectively interact with the extract. The addition of ethanol, a polar organic solvent, helped to homogenize the mixture by increasing the miscibility of the heptane extract with the aqueous Br_2. They observed whether the orange-brown color of bromine (Figure 6) faded upon mixing, which would indicate a reaction with unsaturated carotenoids. The following results were recorded:

- Heptane extract → Color faded, indicating the presence of carotenoids (Figure 7). Although the indicator color diminished, the extract retained visible coloration, suggesting that heptane has a greater extraction efficiency than ethanol.
- Ethanol extract → Color faded, confirming carotenoid extraction (Figure 8).
- Water extract → No color change, suggesting that carotenoids were not extracted (Figure 9).

Potassium Permanganate Test for Oxidation Reactions (Figures 10–13): The potassium permanganate test was used to evaluate the oxidation potential of carotenoid-containing extracts and to help determine the presence of unsaturated compounds. Students tested extracts obtained using heptane, ethanol, and water. Because $KMnO_4$ is prepared as a 1% aqueous solution (Figure 10), the heptane extracts were first combined with 1 mL of isopropanol before $KMnO_4$ was added. This modification ensured miscibility, allowing the aqueous $KMnO_4$ solution to effectively contact the nonpolar heptane extract. The test produced distinct results depending on the solvent used:

- Heptane extract: A clear fading of the purple $KMnO_4$ color was observed, indicating a positive oxidation reaction with the unsaturated carotenoids (Figure 11). This result confirms that carotenoids were effectively extracted using heptane and were reactive under oxidative conditions.
- Ethanol extract: The ethanol extract tested with $KMnO_4$ showed a noticeable fading of the purple color, indicating a positive reaction for unsaturation (Figure 12).
- Water extract: No observable reaction occurred, indicating that carotenoids were not extracted with water. This supports the understanding that carotenoids, being lipid-soluble, are insoluble in polar solvents like water (Figure 13).

Experimental Analysis and Student Observations

Each group compared their test results and visually analyzed their extracted solutions, using collaborative data from all groups to determine which solvent was the most effective for extracting carotenoids from carrots. The results were compiled into a summary table (Table 1) and discussed in relation to solvent polarity and carotenoid solubility. Complete experimental instructions, safety precautions, and waste disposal procedures are provided in the Supporting Information.

■ LABORATORY SAFETY

Laboratory coats, safety goggles, and disposable nitrile gloves must be worn throughout the experiment, as all reagents used and all products formed in this experiment are potential skin and eye irritants or harmful if inhaled or swallowed. All manipulations should be carried out in a well-ventilated fume hood, particularly when handling heptane, ethanol, bromine

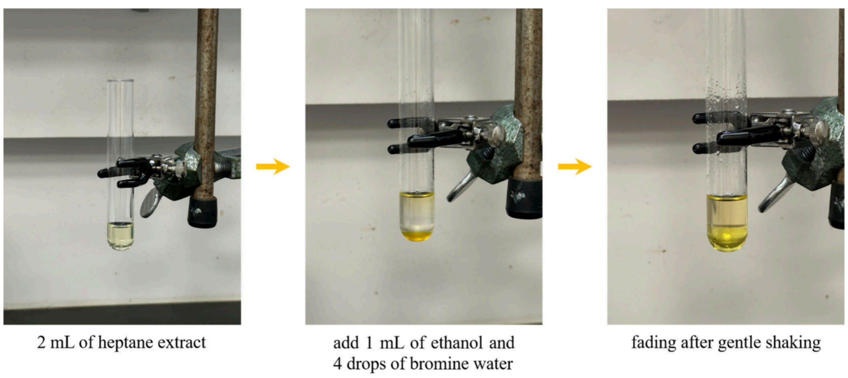

Figure 7. Carotene detection: Heptane extraction using Br_2.

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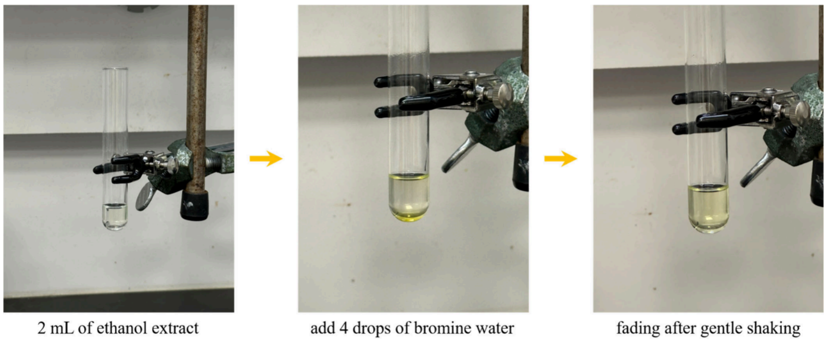

Figure 8. Carotene detection: Ethanol extraction using Br_2.

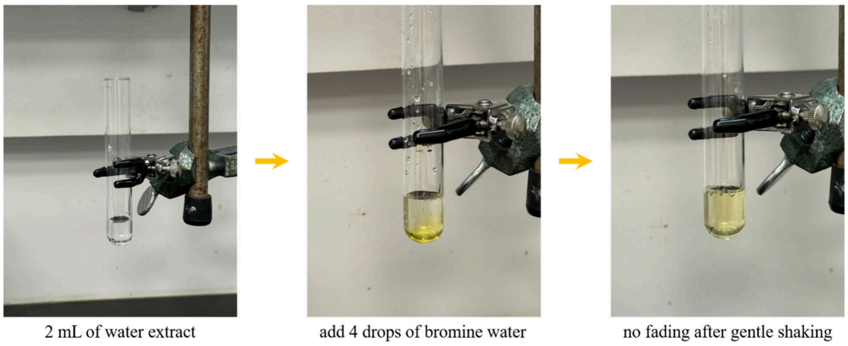

Figure 9. Attempted detection of carotenoids using Br_2.

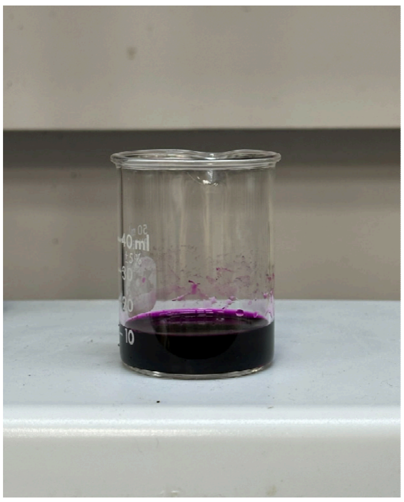

Figure 10. Appearance of $KMnO_4$ solution (1% in water).

solution, and potassium permanganate, which pose significant inhalation and toxicity risks.

Specific chemical risks:

- Bromine solution causes severe burns and eye damage and should be handled with extreme care.
- Potassium permanganate is a strong oxidizer that can react violently with organic materials if not handled properly.
- Ethanol, isopropanol, and heptane are all highly flammable liquids that must be kept away from open flames and ignition sources.
- Carotenoid extracts containing solvents should not be inhaled directly and must be disposed of according to proper laboratory waste procedures.

A detailed list of chemical hazards and waste disposal guidelines is included in the student procedure (Supporting Information). Proper chemical disposal is critical, and all organic

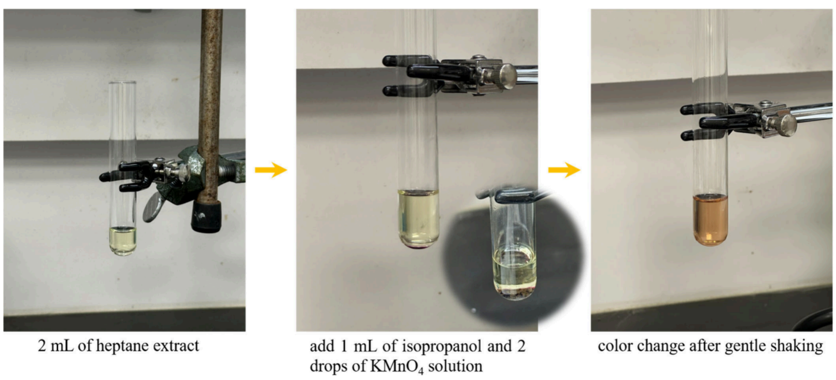

Figure 11. Carotene detection: Heptane extraction using $KMnO_4$.

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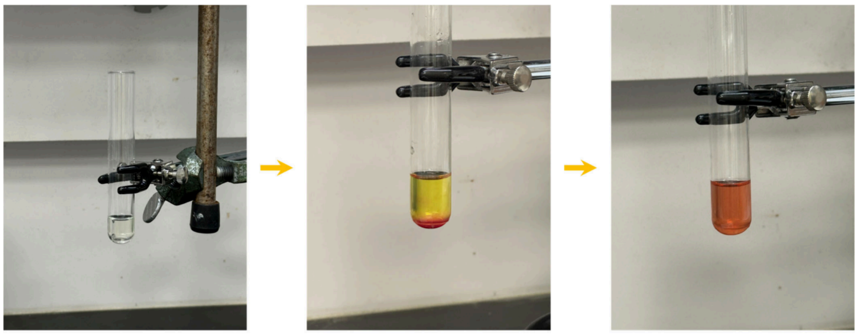

Figure 12. Carotene detection: Ethanol extraction using $KMnO_4$.

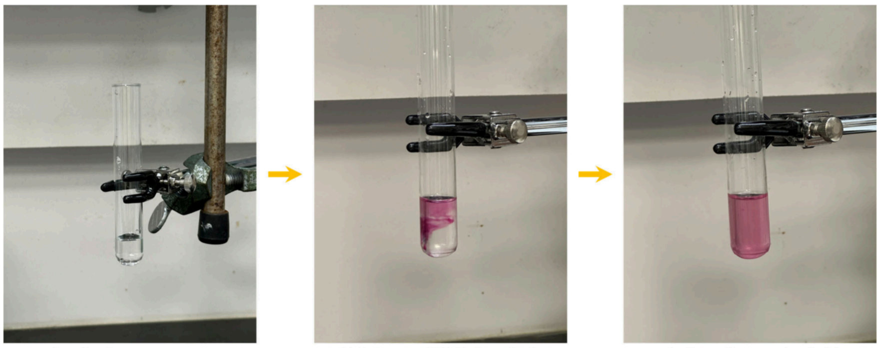

Figure 13. Attempted detection of carotenoids using $KMnO_4$.

Table 1. Representative Student Observations of Qualitative Test Outcomes for Carotenoid Extraction Using Different Solvents

Entry	Solvent	Color of Extract before Testing	Br_2 Test	$KMnO_4$ Test	Interpretation
1	Heptane	Orange	Color faded	Purple faded	Strong extraction
2	Ethanol	Light orange	Color faded	Purple faded	Moderate extraction
3	Water	Colorless	No change	No change	Ineffective extraction due to high polarity

solvent waste must be collected in designated containers for proper handling.

■ RESULTS AND ASSESSMENT

This experiment was conducted with 30−40 visiting high school students during a 2 h laboratory session as part of an introductory chemistry outreach program. Students worked in pairs, with each group performing a solid−liquid extraction using three solvents: heptane, ethanol, or water. After heating and extracting carotenoids from 3 g of diced carrot, students carried out bromine water and potassium permanganate tests to detect the presence of unsaturated compounds in their extracts.

Prior to the experiment, students participated in a prelab lecture that introduced essential concepts, including solubility principles, the influence of solvent polarity on organic extractions, and the chemical reactivity of carotenoids. A short instructional video was also provided to demonstrate proper qualitative testing techniques. These preparatory activities ensured that students could independently complete the extraction and testing procedures with minimal guidance from instructors.

Observations and Solvent Efficiency

This experiment highlighted the impact of solvent polarity on the efficiency of carotenoid extraction and the qualitative detection of unsaturation using bromine and potassium permanganate tests. Heptane, a nonpolar solvent, produced deeply orange-colored extracts, consistent with the expected solubility of nonpolar carotenoids like β-carotene. Upon the addition of bromine water or $KMnO_4$ solution, the heptane extracts exhibited clear color fading—confirming the presence of unsaturated compounds. A small volume of ethanol was added to improve miscibility before bromine testing, ensuring effective contact between the extract and aqueous reagent.

Ethanol, as a moderately polar solvent, may also solubilize other polar compounds from the carrot matrix. This coextraction could lower the relative concentration of carotenoids in the solution, leading to a less intense visible color compared to the heptane extract.

Water extracts, in contrast, remained colorless and gave no observable reaction in either test. This clearly demonstrated that water, being highly polar, is ineffective at extracting nonpolar carotenoids. The absence of both coloration and chemical reactivity in water extracts reinforced the concept of "like dissolves like", offering a strong visual foundation for understanding solubility and extraction principles.

Student Performance and Data Interpretation

Across the participating groups, students successfully completed the extraction and testing procedures within the allotted 2 h session. Each group handled all three solvent systems, providing a comparative basis for understanding how solvent polarity affects carotenoid extraction.

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Students were able to correctly identify which solvents were more effective based on visual changes in the qualitative tests. Most noted that heptane gave the most intense orange extract and clearest fading in both bromine and $KMnO_4$ tests. Ethanol was seen as partially effective, while water was consistently identified as ineffective.

Instructors observed that students engaged in meaningful group discussions during the postlab period, using shared data to support conclusions. These peer comparisons encouraged collaboration and deeper conceptual understanding. The experiment thus met its pedagogical goals: reinforcing solubility rules, introducing functional group identification, and strengthening data analysis skills in a guided, hands-on setting.

POST-LAB ASSESSMENT AND STUDENT ENGAGEMENT

To reinforce conceptual understanding and foster critical thinking, the experiment concluded with structured postlab activities. Following the hands-on portion of the lab, students participated in a guided class discussion facilitated by the instructor. During this discussion, students were encouraged to share their group's observations, compare qualitative results across different solvents, and collaboratively determine which solvent most effectively extracted carotenoids. This peer comparison approach promoted scientific dialogue and deepened their understanding of the underlying chemistry concepts, such as the principle of "like dissolves like" and the role of solvent polarity.

In addition to the discussion, each student completed a postlab report designed to assess their comprehension and ability to synthesize experimental outcomes. The report included the following components:

- A summary of experimental procedures and group observations for all three solvents tested (heptane, ethanol, and water).
- Analysis of qualitative test outcomes, particularly color changes in bromine and potassium permanganate tests.
- Evaluation of solvent effectiveness based on polarity and solubility considerations.
- Reflection questions prompting students to articulate the reasoning behind their conclusions, supported by both their own data and comparisons with peer groups.

In addition to individual written reports, postlab assessment emphasized peer discussion facilitated by the instructor. A typical example involved one group noting that their ethanol extract appeared lighter orange than the heptane extract, while another group pointed out that both extracts still showed fading when tested with bromine water. Guided by prompts such as "What does this suggest about ethanol's ability to extract carotenoids compared to heptane?", students refined their reasoning and connected their observations to solvent polarity and solubility principles. These facilitator-led exchanges demonstrated how collaborative discussion helped students interpret experimental variability, clarify misconceptions, and reinforce conceptual understanding.

SUMMARY

This experiment introduces high school students to foundational principles of organic chemistry through the context of carotenoid extraction and qualitative testing. By comparing the extraction efficiency of heptane, ethanol, and water, students gain a practical understanding of the role of solvent polarity in solid–liquid extractions and the concept of "like dissolves like". The use of bromine water and potassium permanganate tests provides straightforward, observable confirmation of unsaturation in carotenoid extracts, linking molecular structure to reactivity. The experiment's simplicity, minimal equipment requirements, and strong visual outcomes make it suitable for classroom settings with limited resources. Moreover, the collaborative structure fosters scientific reasoning, critical thinking, and peer discussion. As a whole, the activity offers a safe, accessible, and educationally effective platform for reinforcing solubility principles and functional group identification in a precollege chemistry curriculum.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.5c00395.

Instructor's notes, student handout, and hazards (PDF)

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Notes

The authors declare no competing financial interest.

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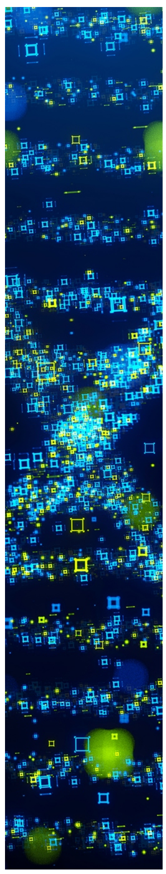

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