Liquid CO₂ Extraction of Limonene from Orange Rind
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Synopsis:
An environmentally friendly extraction of a natural essential oil from a citrus fruit using liquid CO₂.

Chemical Concepts
Extraction, natural products, physical properties, phases, phase change

Green Lessons
Prevention of solvent waste, use of safer solvents

Estimated Lab Time
60 minutes

Material List (per student):
<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>Orange (~1/2 per student)</td>
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<tr>
<td>Cheese grater</td>
<td></td>
</tr>
<tr>
<td>Wire coil</td>
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<tr>
<td>Crushed dry ice (~100 g)</td>
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<tr>
<td>15 mL polypropylene centrifuge tube with plug seal cap (Corning #430052 or #430766)</td>
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<tr>
<td>Transparent plastic cylinder</td>
<td></td>
</tr>
<tr>
<td>Warm tap water</td>
<td></td>
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<tr>
<td>Spatula</td>
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</table>

Safety Precautions:
Due to the safety issues involved with the rapid increase of pressure during this procedure, it is important to read and understand the entire procedure before beginning!

Discussion:
Hypothetical Scenario
Lecher Consulting Enterprises has recently been contacted by Jack Cain, a citrus fruit distributor located in Tampa, Florida. Jack’s passion in life is oranges. His life-long fascination with the yummy fruit led him to observe that the oil from the peel of the orange was a very effective degreasing agent. Believing that profit follows passion, Jack is developing an all natural multi-purpose household cleaner made from orange peels. He has he has dubbed his product Orange Power!. Jack’s plan is to isolate orange oil from the discarded orange peels from the orange juice manufacturing industry. This oil will be the primary ingredient in his cleaner. Jack has sought Lecher Consulting Enterprises’ expertise in green extraction technologies. Jack is looking for a rapid, environmentally benign method of isolating large amounts of this oil. Our charge is to identify the key component of the essential oil from oranges, to propose a method of extraction, and to demonstrate the method on a small scale. Jack also needs us to calculate the amount of orange peel needed to generate 1000 kg of orange oil.

Background
Essential oils are organic compounds that are extracted from natural sources and used in many products such as flavorings, fragrances, and cleaning products. Traditionally essential oils have been extracted through the use of steam distillation or organic solvent extraction. During the past two decades, technical advances have been made in the industrial use of supercritical and liquid carbon dioxide in place of organic solvents.

A major benefit of using carbon dioxide as a solvent is its accessible phase changes. Unlike other gases, relatively low temperatures and pressures can be used to form liquid and supercritical CO₂. Figure 1, the temperature-pressure diagram for carbon dioxide, indicates the three phases, the triple point, and the critical point. Solid CO₂ sublimes (goes directly from a solid to a gas) at normal atmospheric pressure (1 atm = 1.01 bar). The triple point of CO₂, where solid, liquid, and gas phases coexist in equilibrium, is achieved at 5.2 bar and -56.6 °C. At this point, dry ice melts, forming liquid carbon dioxide. If the temperature and pressure are increased to the critical point (73.8 bar and 31.0°C), the CO₂ exists as a supercritical fluid and has no distinct liquid.

By definition, supercritical fluids have no distinct liquid or vapor phase but retain properties of each. ScCO₂ is especially beneficial when used as a solvent in selective extraction processes. The gas-like properties, such as very
low surface tension and viscosity, allow the solvent to penetrate into the substrate, while the liquid-like properties solubilize compounds and remove them from the substrate. Small changes in pressure or temperature alter the bulk density of the fluid leading to increased or decreased solubility of various compounds. In this way, the use of supercritical fluids can allow for control of separations of materials. Through manipulation of temperature and pressure conditions within accessible ranges, both the phase and properties of CO$_2$ can be easily controlled.

![Temperature / Pressure Diagram for CO$_2$](image)

**Figure 1.** Temperature / Pressure Diagram for CO$_2$

Large-scale CO$_2$ processing has had commercial success in many separation and extraction processes. The tunable solubility properties, low toxicity, and ease of removal of CO$_2$ have led to well established CO$_2$ technology for the extraction of various food products, inducing essential oils and hops, and for the decaffeination of coffee and tea. Additionally, a closed loop system can be used to compress the gas in order to use the supercritical fluid or liquid in processing, depressurize the solvent for separation of dissolved compounds, and recompress the CO$_2$ to begin the cycle again. CO$_2$ extraction processes can also be run at relatively constant pressure when liquid–liquid extraction against water is used for product recovery. These loop systems allow for easy recovery and recycling of the pure solvent.

CO$_2$ can also be used for bench top extraction processes. As shown in the above phase diagram, CO$_2$ is a liquid under relatively mild temperatures and pressures, in the ranges of -56.6 to 31.0 °C and 5.2 to 73.8 bar. Solid CO$_2$ (dry ice) sublimes at atmospheric pressure and temperatures above -78°C. If the CO$_2$ is sealed in a vessel during sublimation, the internal pressure in the vessel increases. After the temperature and pressure have increased sufficiently, liquid carbon dioxide forms.

**Green Considerations**

CO$_2$ is useful as a green alternative solvent because it provides environmental and safety advantages: it is nonflammable, essentially nontoxic, readily available, and environmentally benign. Processing with CO$_2$ also poses minimal hazard in the event of unintentional release or residual solvent in the product. Although CO$_2$ can act as a greenhouse gas, is captured from the atmosphere to be used as a solvent it. Since it is not created anew, there is no net CO$_2$ increase.

**Safety Precautions**

*Dry ice:* Use gloves when handling dry ice. Contact can cause skin damage.

**WARNING:** Due to the high pressure generated during this experiment, there is some risk of vessel rupture and / or flying projectiles. Wear eye protection!! Do not use any glass during this experiment. Under no circumstances should any vessels other than the instructor-provided tubes be used. Safety procedures have been implemented to minimize the danger, but it is essential to follow these procedures to protect yourself and other students. Read all safety notes and entire procedure before beginning lab.
Experimental Procedure

Preparation of the Orange Rind
1. Grate the outside of an orange peel (colored part) with the smallest grating surface of the grater.

2. Obtain 2.5 to 3.0 grams of orange rind.

Preparation of the Extraction Vessel
3. Record the mass of a 15 mL centrifuge tube by weighing it in a small tared beaker.

4. Slide a coiled copper wire into the tube. All wire should be inside the tube. Have the instructor cut off any extra wire. Record the mass of the tube and wire.

5. Add approximately 2.5 grams of grated orange rind to your tube.
   Note: do not pack tightly. Calculate the exact mass of orange peel added to the tube.

Preparation of the Extraction Environment
6. Fill a 250 mL plastic graduated cylinder one half to two thirds full of warm (40-50° C) tap water.

   Note: Do not heat the water in the cylinder or add hot water later in the procedure. Any sudden increase in temperature of surrounding water when the centrifuge tube is under pressure can cause the cap to blow off suddenly and violently.

   Note: Move items that should not get wet away from the cylinder because splashing may occur if the cap shoots off the tube.

   [Diagram of centrifuge tube and dry ice]

   Figure 2.

Extraction

NOTE: Due to the safety issues involved with the rapid increase of pressure during this procedure, it is important to read and understand the entire extraction procedure (#7-13) before beginning!

7. Fill the rest of the centrifuge tube with crushed dry ice. Remember to wear gloves! Dry ice may be scooped up with the tube, added with a scoop, or poured into the tube from a beaker. Tap the tip of tube on the bench and add more dry ice until the tube is full. Twist cap on tightly until it stops turning. If cap does not stop turning when tightened, remove the cap before proceeding. Without a complete seal, the cap is likely to shoot off. Replace the cap with a new one before placing in water. If this does not provide a seal, replace the tube.

8. Immediately after capping, drop centrifuge tube, tapered end down, into the water in the cylinder (see Figure 2.E). Pressure will begin to build in the tube and gas will escape slowly from the region where the tube and the cap meet. The plastic cylinder functions as a secondary container and protects you from possible injury. If the tube shatters or the cap shoots off, any projectiles will be directed straight up. Do not place anything (including your face) above the cylinder. Watch the extraction from the side, not the top, of the cylinder.
9. After ca. 15 seconds, liquid CO$_2$ should appear. If no liquid has appeared after ca. 1 minute, there is not a sufficient seal. Remove tube from cylinder, tighten cap, and put back in water. If repeated trials do not produce liquid, the cap or tube may need to be replaced. Liquid should boil and gas should escape for 2 – 3 minutes.

NOTE: During this time, it is sometimes helpful to slowly rotate the cylinder on its base to prevent the centrifuge tube from freezing to the side of the cylinder. **Never remove the tube from the plastic cylinder when the CO$_2$ is liquid.** Tubes may rupture due to pressure and therefore must always remain in secondary containment.

10. As the liquid boils, it should pass through the peel and move to the bottom of the tube. If it cannot reach the bottom of the tube, the oil will deposit in the region of the tube containing the orange peel. This does not allow for isolation of the product.

11. After the liquid has evaporated and gas is no longer escaping, remove the tube from the cylinder with tweezers and open the cap. **Open centrifuge tubes slowly and only after the gas has escaped.** Opening tubes that are under pressure could result in the ejection of the cap.

12. If necessary, rearrange the solid orange peel before the second extraction. A piece of wire can be used to break up the solid mass and create a channel to the bottom for liquid CO$_2$. Repeat the extraction by refilling the tube with dry ice, resealing the cap, and putting the tube back in the water.

13. Product (approximately 0.1 mL pale yellow oil) should be in the tip of the tube when the extraction is complete. Carefully remove the solid and the trap by pulling the wire handle. Use tweezers if necessary. If any solid remains in the tube, remove it with a spatula or wire. **NOTE:** Keep tube upright to avoid product loss.

**Characterization and Verification of Purity**

14. Determine the mass of your product. Be sure to dry the outside of the tube with a paper towel.

15. Obtain an IR spectrum if possible and compare to known compounds.

**Calculations**

16. Calculate percentage recovery based upon the yield of the product compared to the mass of rind used.

**Disposal**

17. When you are satisfied with your data and calculations, place your centrifuge tube in the container labeled as Limonene.

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1. What are some of the uses of essential oils?

2. Why are supercritical fluids beneficial when used as a solvent in selective extraction processes?

3. Recreate the table below in your lab notebook and enter information from your lab.

<table>
<thead>
<tr>
<th>Mass of rind used</th>
<th>Mass of empty tube</th>
<th>Mass of tube with oil</th>
<th>Mass of oil</th>
<th>Percent Yield</th>
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